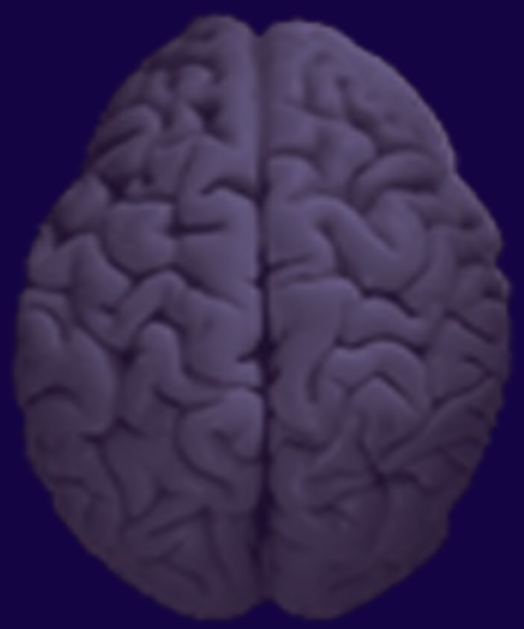

Ph.D. Thesis

SHERIN ANTONY

MAY 2010

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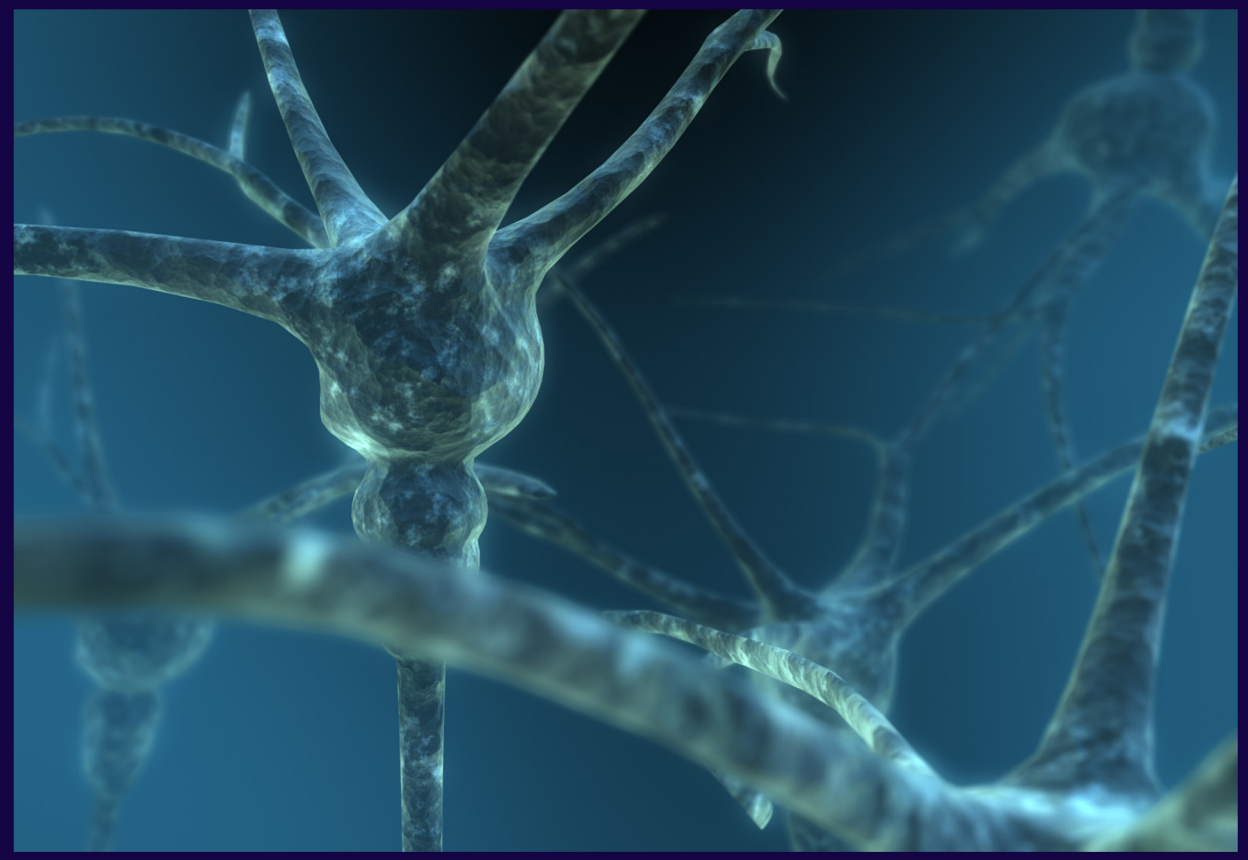

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MUSCARINIC M1, M3, NICOTINIC, $GABA_A$ AND $GABA_B$ RECEPTOR SUBTYPES GENE EXPRESSION IN INSULIN INDUCED HYPOGLYCEMIC RAT BRAIN REGIONS: FUNCTIONAL REGULATION THROUGH PHOSPHOLIPASE C AND CREB PROTEIN

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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CERTIFICATE

This is to certify that the thesis entitled **"Muscarinic M1, M3, Nicotinic, $GABA_A$ AND $GABA_B$ Receptor Subtypes Gene Expression in Insulin Induced Hypoglycemic Rat Brain Regions: Functional Regulation Through Phospholipase C and CREB Protein"** is a bonafide record of the research work carried out by **Mrs. Sherin Antony,** under my guidance and supervision in the Department of Biotechnology, Cochin University of Science and Technology and that no part thereof has been presented for the award of any other degree.

Cochin – 682 022

(C. S. Paulose)

May 26, 2010

DECLARATION

I hereby declare that the thesis entitled **"Muscarinic M1, M3, Nicotinic, $GABA_A$ and $GABA_B$ Receptor Subtypes Gene Expression in Insulin Induced Hypoglycemic Rat Brain Regions: Functional Regulation through Phospholipase C and CREB Protein"** is the authentic record of research work carried out by me for my doctoral degree, under the supervision and guidance of Dr. C. S. Paulose, Professor & Head, Department of Biotechnology, Director, Centre for Neuroscience, Cochin University of Science and Technology and that no part thereof has previously formed the basis for the award of any degree or diploma, associateship or other similar titles or recognition.

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Dedicated to all those who…

Inspired… Guided… and accompanied me during

The course of my Life.

ABBREVIATIONS

5-HIAA	5 Hydroxy indole – 3 acetic acid
5- HT	5 Hydroxy tryptamine
ACh	Acetylcholine
AChE	Acetylcholine esterase
AD	Alzheimers disease
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
B_{max}	Maximal binding
BSA	Bovine serum albumin
cAMP	Cylic adenosine monophosphate
ChAT	Choline acetyltransferase
CNS	Central Nervous System
Ct	Crossing threshold
DAMP	Deoxy acetyl methyl piperidine
DEPC	Di ethyl pyro carbonate
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
EPI	Epinephrine
EPSCs	Excitatory postsynaptic current

EPSP	Excitatory postsynaptic potential
FITC	Florescent isothiocyanate
GABA	Gamma amino butyric acid
GAD	Glutamate Decarboxylase
GLUT3	Glucose transporter type 3
GPCR	G protein-coupled receptor
HBSS	Hang's balanced salt solution
IGF	Insulin-like growth factor
INS	Insulin
K_d	Dissociation constant
L-DOPA	L-3,4 Dihydroxy phenyl alanine
LGIC	Ligand Gated Ion Channel
LTD	Long term depression
mRNA	Messenger ribonucleic acid
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NE	Norepinephrine
P	Level of significance
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline Triton X- 100
PCR	Polymerase Chain Reaction

PFC	Prefrontal cortex
PLC	Phospholipase C
QNB	Quinuclidinylbenzilate
RNA	Ribonucleic acid
SEM	Standard error of mean
Ser	Serine
SOD	Superoxide dismutase
STZ	Streptozotocin
Thr	Threonine
Tyr	Tyrosine
VDR	Vitamin D receptor
VICC	Voltage - insensitive calcium channels

CONTENTS

Introduction

Homeostasis of blood and cellular glucose is an important factor of body functioning as a whole and the nervous system in particular. Glucose is the principal source for energy production in the brain and undisturbed glucose metabolism is pivotally significant for normal function of this organ. Brain is dependent on a continuous supply of glucose diffusing from the blood into the interstitial tissue within the central nervous system and into neurons themselves. Glucose metabolism and energy homeostasis of the body are regulated by the nerve system and special glucose sensory neurons with action potential depending on the glucose level in the extracellular medium (Levin et al, 2004). The glucose excitable neurons elevate their activity with an increase in the external glucose concentration and the glucose suppressible neurons are activated with a decrease in its level. These specialized neurons use glucose and products of its intracellular metabolism for regulation of their activity and release of a neurotransmitter (Yang *et al.*, 2004).

Diabetes mellitus is a common metabolic disorder resulting from defects in insulin secretion, insulin action, or both (Feldman, 1997). Hyperglycemia - high blood glucose level is associated with diabetes. Hypoglycemia - low level of blood glucose, is a relatively common episode primarily affecting diabetic patients receiving treatment with insulin or other hypoglycemic drugs and patients suffering from insulinoma (Cryer, 2004). The neurological consequences of diabetes mellitus in the central nervous system (CNS) are now receiving greater attention. Cognitive deficits, along with morphological and neurochemical alterations illustrate that the neurological complications of diabetes are not limited to peripheral neuropathies (Biessels *et al.*, 1994). The central complications of hyperglycemia also include the potentiation of neuronal damage observed following hypoxic/ischemic events, as well as stroke (McCall, 1992). Glucose

utilization is decreased in the brain during diabetes (McCall, 1992), providing a potential mechanism for increased vulnerability to acute pathological events. Neuroendocrine dysfunction is also observed in diabetes (Mooradian, 1988).

Severe hypoglycemia is a serious complication of insulin therapy in diabetic patients exceeding insulin administration and hypoglycemic episodes are frequent in many people with type 1 diabetes mellitus and advanced type 2 diabetes mellitus (Cryer, 2004). Furthermore, the ability to sense a reduction in blood glucose levels and the counterregulatory mechanisms responsible for its correction are impaired in patients with diabetes, which make them susceptible of suffering from hypoglycemia (Becker & Ryan, 2000; Jones & Davis, 2003; Cryer, 2006). Diabetes mellitus and its most common treatment side effect, hypoglycemia, have multiple effects on the central nervous system. Transient neurological deficits associated with hypoglycemia is generalized with confusion, motor restlessness, hypotonia and generalized seizures (Lahat *et al.*, 1995). The brain's activities rely heavily on glucose for energy (Laughlin, 2004). The metabolization of glucose from the bloodstream allows each brain region to carry out its given functions (McNay *et al.*, 2001). Regulation of glucose at the biochemical level affects every area of the brain and has impact from cellular to behavioral brain function. Intense glycemic control with low target ranges invariably carries a risk of inadvertent hypoglycemic episodes. Several studies have reported a potentially higher incidence of hypoglycemia in patients under strict glycemic control (Van den Berghe *et al.,* 2005; Krinsley & Grover, 2007; Thomas *et al.,* 2007). Hypoglycemia impose alterations upon both the central (CNS) and peripheral (PNS) nervous systems. Hypoglycemia lead to brain damage and long-term cognitive impairment (Wieloch, 1985; Gazit *et al.*, 2003). The hypoglycemic counter regulatory mechanisms are blunted irreversibly by disease duration or by acute episodes of prior stress (Ertl & Davis, 2004). Hypoglycemia affects all aspects of life, including employment, driving, recreational activities involving exercise and travel. Measures should be taken in

all of these situations to avoid the potentially dangerous side-effect of insulin therapy (Frier, 2008).

Studies suggest that acute or chronic hypoglycemia lead to neurological dysfunction and injury. Hypoglycemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients. Prolonged insulin-induced hypoglycemia causes widespread loss of neurons and permanent brain damage with irreversible coma. As in brain injury associated with ischemia and neurodegenerative conditions, altered neurotransmitter action appears to play a role in hypoglycemic brain injury. Pathological studies in humans and animals show that hypoglycemia-induced neuronal death occurs preferentially in the hippocampus, superficial layers of the cortex and striatum (Auer, 2004). Because of the extensive neuronal loss, one of the neurological consequence associated with hypoglycemia is cognitive decline. According to clinical studies, significant learning and memory deficits correlate with the frequency of hypoglycemia not only in patients with type 1 diabetes, but also in the relatively younger group among the population with type 2 diabetes (Dey *et al.*, 1997). Acute neuroglycopenia causes rapid deterioration of cognitive function in humans with and without diabetes. Numerous clinical studies suggest that intensive insulin treatment of type 1 diabetes is associated with an increased frequency of hypoglycemic coma (Rovet & Ehrlich, 1999; Hannonen *et al.*, 2003) and cognitive impairment (Wredling *et al.*, 1990; Langan *et al.*, 1991). Hypoglycemic episodes in diabetic patients induce cognitive impairment in children (Naguib *et al.*, 2009) and adults (Akyol *et al.*, 2003; Carroll *et al.*, 2003; Roberts *et al.*, 2008) and in rodent models of hypoglycemia and type 1 diabetes hippocampal damage has been associated with impairment in learning and memory tests (Suh *et al.*, 2003; Alvarez *et a*l., 2009). During moderate hypoglycemia, the patient experiences impaired motor function, confusion and inappropriate behaviour but is still aware enough to take action, whereas severe hypoglycemia lead to disabling neurologic

impairment, coma or seizure (Cryer, 2002). Patients with recurrent severe hypoglycemia are exposed to convulsions or seizures which are mistakenly diagnosed as epileptic attacks (Frier, 2000).

The CNS neurotransmitters play an important role in the regulation of glucose homeostasis. They exert their function through receptors present in both neuronal and non-neuronal cell surface that trigger second messenger signaling pathways (Julius *et al.,* 1989). Neurotransmitters have been reported to show significant alterations during hyperglycemia resulting in altered functions causing neuronal degeneration (Bhardwaj *et al.,* 1999). Chronic hyperglycemia during diabetes mellitus is a major initiator of diabetic micro-vascular complications like retinopathy, neuropathy and nephropathy (Sheetz & King, 2002). Impairment of dopaminergic and glutamatergic function is reported in brain regions of hypoglycemic and hyperglycemic rats (Robinson *et al.,* 2009, Joseph *et al.,* 2007, 2008, 2010) thereby contributing to neuronal damage. Severe hypoglycemia is frequently associated with seizures. Brain regions are prone to develop seizures and seizure-induced damage. Repeated hypoglycemic episodes have frequent memory problems, suggesting impaired hippocampal function (Kirchner *et al*., 2006).

Acetylcholine (ACh) is an excitatory neurotransmitter in both the PNS and CNS which functions as a neuromodulator. In the peripheral nervous system, acetylcholine activates muscles and is a major neurotransmitter in the autonomic nervous system. In the central nervous system, acetylcholine and the associated neurons form a neurotransmitter system, the cholinergic system, which tends to cause excitatory actions. ACh is involved with synaptic plasticity, specifically in learning and short-term memory. Acetylcholine released by cholinergic neurons from presynaptic neurons into the synaptic cleft acts as a ligand for Nicotinic Acetylcholine receptors, which are ligand gated Na+ ion channels. Ligand binding opens the channel causing depolarization and increases the probability of an action potential firing, occcuring once the threshold is reached (Connors & Long, 2004).

The function of acetylcholine is mediated through nicotinic and muscarinic acetylcholine receptors (AChRs). Central mAChRs contribute to the regulation of GABAergic transmission and the activation of mAChRs enhanced GABA release in the rat globus pallidus (Kayadjanian *et al*., 1997) and substantia gelatinosa is reported (Baba *et al*., 1998). The muscarinic AChRs (mAChRs) are mediators of multiple cellular functions. mAChRs are expressed throughout the entire central nervous system of vertebrates (Levey, 1993) and are thought to be involved in many brain functions such as learning and memory. They are frequently found at presynaptic sites and their activation results in the modulation of transmitter release. The activation of neuronal nicotinic AChRs (nAChRs) results in fast excitatory synaptic transmission or the enhancement of the release of transmitters including glutamate, GABA and ACh (De Filippi *et a*l., 2001). Studies have shown that cholinergic transmission is more sensitive to hypoxia and to low glucose concentrations than is axonal conduction (Dolivo *et al*., 1974).

GABA, the most important inhibitory neurotransmitter in the mammalian central nervous system, acts through 2 classes of receptors; $GABA_A$ receptors are ligand-operated ion channels and $GABA_B$ are the G-protein-coupled metabotropic receptors. Impairment of GABAergic transmission by genetic mutations or application of GABA receptor antagonists are reported to induced seizures. Dysfunction of the GABAergic system has a fundamental role in the propagation of seizures. Indeed mutant mice lacking the enzyme glutamate decarboxylase (GAD) or certain subunits of $GABA_A$ receptors are prone to spontaneous seizures.

GABAergic neurons release GABA, which is one of two neuroinhibitors in the CNS, the other being Glycine. GABA has a homologous function to ACh, gating anion channels that allow Cl^- ions to enter the post synaptic neuron. Cl^- causes hyperpolarization within the neuron, decreasing the probability of an action potential firing as the voltage becomes negative. Despite our advances in the treatment of diabetes, hypoglycemic episodes are often the limiting factor in

achieving optimal blood sugar control. Recent therapeutic strategies aimed at closely controlling elevated glucose levels in diabetic individuals put them at risk for experiencing episodes of hypoglycemia. Reports suggest that if intensive insulin therapy is to be used, great effort must be taken to avoid hypoglycemia (Bilotta *et al.*, 2008). Acute and recurrent hypoglycemia cause transient or persistent alteration of cognitive functions and result in seizures or coma. The pathogenesis of hypoglycemia induced cognitive and functional deficit is largely unknown, but mechanisms that could result in damage to cells of the CNS include deregulation in neurotransmitter signaling. To understand the effects of hypoglycemia on the cells of the CNS, it is essential to characterize the response of CNS cells to reduced glycemic levels, to determine the extent of CNS cell injury induced by hypoglycemia and to identify the mechanisms involved in hypoglycemia induced cell or tissue damage in brain. Identification of neuronal damage caused by neurotransmitter variations provides a better understanding of the neurochemical mechanisms responsible for hypoglycemia associated deficit in cognitive and behavioural response. The reports so far stated did not attempt to emphasis the functional role of muscarinic, nicotinic and GABAergic receptor subtypes in hypoglycemic and hyperglycemic brain.

Phospholipase C (PLC) is a key enzyme in phosphatidyl inositol turnover and generates two second messengers, inositol 1,4,5-bisphosphate (IP3) and diacylglycerol (DAG) from phosphatidyl inositol 4,5-bisphosphate [PI(4,5)P2] in response to activation of receptors by hormones, neurotransmitters, growth factors, and other molecules. IP3 induces calcium mobilization and DAG induces activation of protein kinase C. PI(4,5)P2 is a substrate for PLC, and PI 3- kinase. In addition, PI(4,5)P2 directly regulates a variety of cellular functions, including cytoskeletal reorganization, exocytosis and channel activity. Therefore, strict regulation of PI(4,5)P2 levels by PLC or other converting enzymes is necessary for homeostasis (Fukami, 2002). Disruption of the cyclic AMP response element binding (CREB) protein expression and its activity is associated with

hypoglycemia and reduced expression of gluconeogenic enzymes (Herzig et al, 2001). Insulin administration to rodents is the most common experimental model of hypoglycemia (Auer *et al*., 1985). The development of hypoglycemia is associated with a decrease in the glucose inflow to the brain, which cause convulsions, coma (Shah *et al*., 1984) and even death. Therefore, studies on damages of the central nerve system under conditions of hypoglycemia are very important for clinical applications.

In the present study, a detailed investigation on the alterations of muscarinic M1, M3, α7 nicotinic acetylcholine receptor (α7 nAchR), GABA receptors and its subtypes; $GABA_{A\alpha1}$ and $GABA_B$ in the brain regions of streptozotocin induced diabetic and insulin induced hypoglycemic rats were carried out. Gene expression of acetylcholine esterase (AChE), choline acetyltransferase (ChAT), GAD, GLUT3, Insulin receptor, superoxide dismutase (SOD), Bax protein, Phospholipase C and CREB in hypoglycemic and hyperglycemic rat brain were studied. Muscarinic M1, M3 receptors, AChE, ChAT, $GABA_{A\alpha1}$, $GABA_B$, GAD, Insulin receptor, SOD, Bax protein and Phospholipase C expression in pancreas was also carried out. The molecular studies on the CNS and PNS damage will elucidate the therapeutic role in the corrective measures of the damage to the brain during hypoglycemia and hyperglycemia.

OBJECTIVES OF THE PRESENT STUDY

1. To measure the circulating insulin level in streptozotocin induced diabetic and insulin induced hypoglycemic rats.

2. To study the behavioural changes in diabetic and hypoglycemic rats using Y-maze and grid walk.

3. To study the total muscarinic, muscarinic M1 and muscarinic M3 receptor subtypes binding parameters in cerebral cortex, cerebellum, brainstem, corpus striatum, hippocampus and pancreas of diabetic and hypoglycemic rats.

4. To study the GABA binding parameters in cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus and pancreas of diabetic and hypoglycemic rats.

5. To study the expression of AChE, ChAT, muscarinic M1, muscarinic M3, α7 nAchR, $GABA_{A\alpha1}$, $GABA_B$, GAD, insulin receptor gene expression in the cerebral cortex, cerebellum, brainstem, corpus striatum, hippocampus of diabetic and hypoglycemic rats using Real Time PCR.

6. To study the gene expression status of GLUT3, superoxide dismutase, Bax, phospholipase C, CREB in the cerebral cortex, cerebellum, brainstem, corpus striatum and hippocampus of diabetic and hypoglycemic rats using Real Time PCR.

7. To study the expression of acetylcholine esterase, choline acetyltransferase, muscarinic M1, muscarinic M3, $GABA_{A\alpha1}$, $GABA_{B}$, GAD, insulin receptor, superoxide dismutase, Bax protein and phospholipase C in the pancreas of diabetic and hypoglycemic rats using Real Time PCR.

8. To study the localisation and expression status of muscarinic M1, muscarinic M3, α7 nicotinic acetylcholine receptor and $GABA_{A\alpha1}$ receptors in the brain slices of cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus of diabetic and hypoglycemic rats using specific antibodies in confocal microscope.

9. Immunocytochemical studies to analyse the localisation and expression status of muscarinic M1, muscarinic M3 and $GABA_{A\alpha1}$ receptors in the pancreas of diabetic and hypoglycemic rats using specific antibodies in confocal microscope.

Literature Review

Diabetes mellitus is a major global health problem that affects more than 246 million people in the world, but by 2025, that number is estimated to reach 380 million (Sicree et al., 2006; Zimmet *et al.,* 2001). It is an increasingly prevalent metabolic disorder in humans and is characterised by hyperglycemia (Kumar & Clarke, 2002**;** Dunne *et al.,* 2004). The symptoms of diabetes mellitus results from abnormal glucose metabolism. The lack of insulin activity results in failure of transfer of glucose from the plasma into the cells. This situation is so called "starvation in the midst of plenty". The pancreatic hormones have an important role in the regulation of glucose metabolism. The secretion of insulin by ß-cells of the endocrine pancreas is regulated by glucose and other circulating nutrients. It is also modulated by several hormones and neurotransmitters, among which acetylcholine and GABA plays a prominent role. Hypoglycemia is a serious complication of diabetes and insulin therapy. Hypoglycemic brain injury occurs most frequently in patients attempting tight glucose control (Auer, 2004; Davis *et al*., 1998).

Increased incidence of hypoglycemia occurs when attempts are made to institute tight glycemic control using currently available regimens of subcutaneous insulin administration in diabetic patients (Cryer, 1994). Insulin and sulfonylurea therapy for diabetes mellitus carries the risk of hypoglycemic brain injury and this risk is a major impediment to optimal glucose regulation in diabetic patients (Davis *et al*., 1998). Tight blood glucose control reduces the risk of diabetes complications but also increases the risk of hypoglycemic episodes. Symptomatic hypoglycemia occurs frequently in insulin-treated patients and 36% of patients were found in one study to have experienced hypoglycemic coma in their lifetime (Pramming *et al*., 1991). Upto 10% of patients practicing conventional insulin therapy and 25% of those practicing intensive therapy suffer at least one episode of severe, temporarily disabling hypoglycaemia, often with seizure or coma, in a

given year (Cryer, 1994) and hypoglycemia causes recurrent and even persistent psychological morbidity in many diabetic patients. Speculation that an adaptation in the CNS exist in patients with diabetes, depending upon antecedent glycemia, appeared nearly a decade ago (Cryer, 2003). Amiel *et al*., (1988) observed that lower glucose concentrations were required to initiate epinephrine secretion following a period of intensified diabetes management with its attendant increase in hypoglycemia. Similar hormonal defects are induced in patients with diabetes (Hepburn *et al*., 1991; Dagogo *et al*., 1993) and nondiabetics (Davis & Shamoon, 1991; Heller & Cryer, 1991; Veneman *et al*., 1993), after an episode of hypoglycemia.

Glucose is the only fuel that neuronal tissue use for energy under normal circumstances (Sokoloff, 1981). Chronic changes in the antecedent level of glycemia (either sustained hyperglycemia or hypoglycemia) induce alterations in brain glucose metabolism in rodents (Boyle *et al*., 1994). Sensory and cognitive impairments have been documented in diabetic humans and animals, but the pathophysiology of diabetes in the central nervous system is poorly understood. It seems that glucose metabolism and energy homeostasis of the body are also regulated by the nerve system and special glucose sensory neurons with action potential depending on the glucose level in the extracellular medium (Levin *et al.*, 2004). These specialized neurons use glucose and products of its intracellular metabolism for regulation of their activity and release of a neurotransmitter (Yang *et al.*, 2004).

Depending upon its severity, hypoglycemia cause irritability, impaired concentration, focal neurological deficits, seizures, comma and with profound hypoglycemia, neuronal death (Auer 2004; McCrimmon & Sherwin, 1999; Ben-Ami *et al*., 1999). Symptoms of hypoglycemia result primarily from a lowered glucose level in the brain and its effects on the central and autonomic nervous systems (Charles & Goh, 2005). Declining glucose levels in the brain stimulate the autonomic nervous system, causing epinephrine and norepinephrine to be released

from the adrenal medulla. Norepinephrine and acetylcholine from the sympathetic nervous system is also involved in glucose control. Symptoms occur as these hormones and neurotransmitters simultaneously stimulate α-cells in the pancreas to release glucagon, which consequently induces new glucose production in the liver (Cryer 2002 a, b, 2003). In this homeostatic mechanism, rising blood glucose levels shut down the neoglucogenesis activities of autonomic nervous system (McAulay *et al.*, 2001, Charles & Goh, 2005). Recent studies indicate that neuronal NADPH oxidase is the primary source of neuronal oxidative stress after hypoglycemia and the rate of superoxide production is influenced by the blood glucose concentration achieved in the immediate posthypoglycemic period. Restoring blood glucose to 1–2 mM during the first hour after hypoglycemia resulted in less superoxide production and less neuronal death than restoration to higher glucose levels (>5 mM).

Hypoglycemia and brain

Hypoglycemia and glucose deprivation as its extreme expression are very interesting, because in the nervous system glucose is not only the main source of energy necessary for its functioning, but also a substance capable of preventing oxidative damage and reducing the damage to mitochondria caused by neurotoxicity (Delgado *et al.*, 2000). A major concern of diabetic patients is that repeated episodes of hypoglycemia results in neuronal loss because of impaired fuel supply (McNay & Cotero, 2010). The incidence of severe hypoglycemia in patients with diabetes treated by intensive insulin therapy is two to six times higher as in conventionally treated patients with diabetes. Hypoglycemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients (Sang *et al.*, 2005). Disorders in the transport and metabolism of glucose are an important signal for triggering the apoptotic cascade (Moley & Mueckler, 2000). Glucose deprivation leads to rapid suppression of synaptic transmission (Sakurai *et al.*, 2002; Gee *et al.*, 2010). In particular, recurrent hypoglycemic

episodes during the night represent a relevant risk for the patient, because they are often not realized and lead to a deterioration in the awareness for subsequent hypoglycemic episodes. Recent data show that recurrent hypoglycemia not only affects neuroendocrine counter regulation but also autonomic and neuroglucopenic symptoms (Minna *et al*., 2005; Kale *et al*., 2006). Hyperglycemia and hypoinsulinemia could increase the neuronal damage produced by pathological events such as hypoxia, hypoglycemia (Messier & Gagnon, 1996).

Hypoglycemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients. The progress of neuronal dysfunction and damage during energy deprivation is a complex process that includes presynaptic and postsynaptic mechanisms (Auer & Siesjo, 1988; Martin *et al*., 1994). As in brain injury associated with ischaemia and neurodegenerative conditions, altered neurotransmitter action appears to play a role in hypoglycemic brain injury (Aral *et al*., 1998; Auer & Seisjo, 1993). Two main events have been described when energy levels are reduced: an increased release of excitatory amino acids and a reduced concentration of intracellular ATP, which leads to diminished Na^+/K^+-ATPase activity (Benveniste *et al*., 1984; Hansen, 1985; Lees, 1991; Roettger & Lipton, 1996). Children and adults exposed to hypoglycemia develop long-term impairment of cognitive function (Hawdon, 1999; Karp, 1989; Ryan *et al*., 1985; Vannucci & Vannucci, 2001) and are at risk of epilepsy (Kaufman, 1998). Prolonged insulin-induced hypoglycemia causes widespread loss of neurons and permanent brain damage with irreversible coma. Severe hypoglycemia constitutes a medical emergency, involving seizures, coma and death. Studies suggest that suppressing seizures during hypoglycemia decrease subsequent neuronal damage and dysfunction (Abdelmalik *et al.*, 2007). The only treatment for hypoglycemia is blood glucose repletion and there is no currently available intervention for preventing the neuronal death that develops after hypoglycemia is corrected.

Hypoglycemic coma induces a purely neuronal lesion of neo cortex and the hippocampus in rat brain (Wieloch *et al.*, 1984). CT studies show that hypoglycemia predominantly affects cerebral gray matter in the brain. Analysis of regional cerebral blood flow (CBF) differences identified neuronal activation during hypoglycemia in bilateral medial prefrontal cortex (Auer & Siesjo, 1993). Hypoglycemic neuronal death is most pronounced in specific neuron populations: neurons in the hippocampal CA1, subiculum and dentate granule cell layer; cortical layers 2 and 3 of cerebral cortex and the dorsolateral striatum (Auer *et al*., 1989; Auer & Siesjo, 1993). The hippocampal neurons in particular are important for learning and memory. Patients who survive hypoglycemic coma are left with significant cognitive impairment (Kalimo & Olsson, 1980; Patrick & Campbell, 1990).

Glucose homeostasis and pancreas

The endocrine cells of pancreas are arranged into small islands of cells called the islets of Langerhans. The interactive function of both the exocrine and the endocrine parts are particularly important for the normal functioning of the body. The endocrine cells produce indispensable hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide, which are crucial to the optimum functioning of body metabolism. The pancreas is well innervated by autonomic nerves rich in different types of neuropeptides including vasoactive intestinal polypeptide and neuropeptide Y; galanin, Calcitonin-gene-related-peptide, cholecystokinin and leucine-enkephalin (Adeghate *et al.,* 2001). In addition to the presence of neuropeptides, neurotransmitters such as serotonin, GABA or neurotransmitter-regulating enzymes such as tyrosine hydroxylase and dopamine β hydroxylase have been identified in the pancreas (Adeghate & Donath 1991; Adeghate & Ponery 2001; Adeghate & Ponery 2002). The endocrine pancreas is richly innervated, but the abundance and organisation of these innervations are highly variable between species (Kobayashi & Fujita, 1969).

Most of the nerve fibers enter the pancreas along the arteries (Miller, 1981; Woods & Porte, 1974). Unmyelinated nerve fibers are found in the neighborhood of all islet cell types at the periphery and within the islet. At some distance from the islets, glial Schwann cells often form a thin sheet around nerve fibers on their travel toward and within the islet. In the vicinity of islet cells, however, it is not rare to see some nerve fibers lacking this glial protection and coming close to or ending blindly 20–30 nm from the endocrine cells (Bock, 1986; Fujita & Kobayashi, 1979; Legg, 1967; Radke & Stach, 1986; Shorr & Bloom, 1970; Watari, 1968).

The preganglionic fibers of the parasympathetic limb originate from perikarya located in the dorsal motor nucleus of the vagus (Berthoud *et al*., 1990; Berthoud & Powley, 1991; Chen *et al*., 1996) and possibly also in the nucleus ambiguus (Luiten *et al*., 1986) which are both under the control of the hypothalamus. They are organized in well separated branches traveling within the vagus nerves (cranial nerve X) and through the hepatic, gastric (Berthoud *et al*., 1990; Berthoud & Powley, 1991) and possibly celiac branches of the vagus (Kinami *et al*., 1997). They reach intrapancreatic ganglia that are dispersed in the exocrine tissue. These ganglia send unmyelinated postganglionic fibers toward the islets. Preganglionic vagal fibers release acetylcholine that binds to nicotinic receptors on intraganglionic neurons. Postganglionic vagal fibers release several neurotransmitters: acetylcholine, Vasoactive Intestinal Peptide (VIP), gastrin-releasing peptide (GRP), nitric oxide (NO) and pituitary adenylate cyclase-activating polypeptide (PACAP) (Havel *et al*., 1997, Ahren *et al*., 1999; Love & Szebeni, 1999; Wang *et al*., 1999; Ahren, 2000; Myojin *et al*., 2000). Cholinergic terminals are found in the neighborhood of all islet cell types at the periphery and within the islet (Van der Zee *et al*., 1992). The importance of the cholinergic innervation of the endocrine pancreas is attested by the presence of a 10-fold higher activity of choline acetyltransferase and acetylcholine esterase (the enzymes involved in the synthesis and the degradation of acetylcholine

respectively) in the islets than in the surrounding exocrine tissue (Godfrey & Matschinsky, 1975). Cholinergic synapses with endocrine cells have been observed in some species (Golding & Pow, 1990).

Understanding the organisation of the pancreatic innervations permits correct interpretation of some experiments using different cholinergic antagonists. The stimulation of insulin release occurring upon electrical stimulation of vagal nerves in the dog is abolished by both nicotinic and muscarinic antagonists (Ahren & Taborsky Jr, 1986). In the perfused rat pancreas, nicotine produces an increase of insulin secretion that is blocked by atropine (Miller, 1980). These observations can be explained by the presence of nicotinic receptors on pancreatic ganglia and nerves (Stagner & Samols, 1986; Karlsson & Ahren, 1998; Kirchgessner & Liu, 1998) and muscarinic receptors on ß-cells.

The overall effect of a parasympathetic stimulation is an increase of insulin secretion because postganglionic fibers contain various neurotransmitters in addition to the classic neurotransmitter acetylcholine. It is important to keep in mind that parasympathetic neurotransmission is the sum of various biological effects. VIP and PACAP stimulate insulin secretion by increasing cAMP levels (Ahren, 2000). They act on the same family of receptors and exert their action by two mechanisms, directly by stimulating ß-cells through the PLC-PKC pathway (Ahren, 2000) and indirectly by activating intrapancreatic postganglionic nerves that stimulate insulin secretion (Karlsson & Ahren, 1998).

The stimulatory role of ACh in insulin secretion is well established. Studies from our laboratory have reported the regulatory role of mAChRs in glucose induced insulin secretion (Renuka *et al*., 2004, 2006). Muscarinic M1 receptor subtype antagonist, pirenzepine inhibits cholinergic mediated insulin secretion confirming the role of this receptor subtype in insulin synthesis and secretion (Iismaa *et al*., 2000). Most of the studies with ACh suggest muscarinic M3 receptor as the predominant cholinergic receptor subtype expressed by pancreatic ß -cells and in pancreatic insulin and glucagon release (Gilon &

Henquin, 2001; Duttaroy *et al*., 2004). The presence of GABA and functional $GABA_A$ and $GABA_B$ receptors in pancreatic endocrine cells and their ability to modulate secretion of insulin and glucagon is well studied (Brice *et al*., 2002). Over-expression of glutamate decarboxylase (GAD65), the enzyme which regulate conversion of glutamate to GABA, in mouse ß -cells leads to an inhibition of insulin release and glucose intolerance *in vivo* (Shi *et al*., 2000). Although these manoeuvres, which are likely to lead to a decrease in intracellular glutamate concentrations, are entirely compatible with an important role of glutamate in the b-cell (Maechler & Wollheim, 2000) it should be stressed that the product of glutamate breakdown, GABA itself act as an inhibitor of insulin secretion.

Brain neurotransmitters and diabetes

Diabetes mellitus is a metabolic disorder that either arrives during the early years of growth (Juvenille diabetes) or later in life called as maturity onset diabetes. It is observed as the body's inability to effectively regulate the sugar balance which leads to severe complications such as hyperglycemia, obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporesis and coma leading to death. Pancreatic damage resulting in the dysfunction of α and β cells causes disordered glucose homeostasis.

Hyperglycemia during diabetes has been reported to cause degenerative changes in neurons of the central nervous system (Garris, 1990; Lackovic, *et al.*, 1990; Bhattacharya & Saraswathi, 1991). Previous studies demonstrated adrenergic, serotonergic and dopaminergic receptor function alterations in the brain of diabetic rats (Abraham & Paulose, 1999; Padayatti & Paulose, 1999; Paulose *et al*., 1999, Eswar *et al*., 2007). The concentration of 5-HT, DA and NE increased in the brain regions of diabetic rats and accumulation of these monoamines is produced by inhibition of monoamine oxidase activity (Salkovic, *et al.,* 1992). Norepinephrine has been reported to increase in several brain regions during diabetes. Ohtani *et al*., (1997) have reported a significant decrease

in extracellular concentrations of NE, 5HT and their metabolites in the ventro medial hypothalamus (VMH). Epinephrine (EPI) levels were significantly increased in the striatum, hippocampus and hypothalamus of diabetic rats and these changes were reversed to normal by insulin treatment (Ramakrishan & Namasivayam, 1995). Diabetes is reported to cause a high level of degeneration in neurons in different regions of the brain. Streptozotocin -induced diabetes and acute deficiency of insulin is reported to result in increased concentrations of EPI in the supra chiasmatic nucleus. It is also reported that β-adrenergic receptor populations were decreased in diabetes (Garris, 1995). 5-HT content in the brain is reported to be decreased during diabetes (Chu *et al*., 1986; Sumiyoshi *et al*., 1997; Jackson & Paulose, 1999). Sandrini *et al* (1997) reported reduced concentration of serotonin in the cerebral cortex and in the brain-stem of the rat during diabetes. Garris (1990) reported chronically elevated levels of NE in the brain regions of amydgala, hypothalamus and medulla of diabetic mice. This was proposed to be associated with the expression of the gene causing diabetes mellitus. Hyperglycemia is reported to alter the noradrenergic and cholinergic nerve components (Akria, *et al*., 1994) with decrease in the Na^+ K^+ ATPase activity in different brain regions (Gurcharan & Sukwinder,, 1994). NE, DA and 5-HIAA are reported to be increased in the heart and adrenal gland of STZ induced diabetic rats. In the adrenal gland there was an initial reduction followed by a steady increase in the concentration of NE and EPI (Cao & Morrison, 2001). Studies of Gireesh *et al.,* (2008a) showed that there is a decrease in total muscarinic and muscarinic M1 receptors during diabetes in the cerebral cortex. A decreased muscarinic M1 receptor gene expression in the hypothalamus, brainstem and pancreatic islets of diabetic rats was also demonstrated by Gireesh *et al.,* (2008b). Changes in neurotransmitter concentration and in receptor binding have been described in rat brain regions (Bitar *et al.*, 1986). Diabetes is reported to cause increased glutamate content and glutamate receptor activation in the brain regions is reported by Joseph *et al* (2007, 2008). Glutamate excitotoxicity causing increase

Ca^{2+} mediating neuronal dysfunction contributes to hyperglycemia induced cell death (Joseph *et al.*, 2010).

Brain neurotransmitters and hypoglycemia

Glucose in brain, supplies energy essential for maintenance of the nervous system. During hypoglycemia, energy dependent mechanisms for restoring normal transmembrane gradients of Na^{+} and Ca^{2+} cannot operate because of the depletion of ATP and phosphocreatine associated with hypoglycemia. Excess Ca^{2+} influx activates cellular phospholipases and proteases, alters mitochondrial metabolism, triggers free radical formation, changes patterns of synaptic transmission, and eventually result in selective neuronal necrosis (Jane *et al*., 1999). Deficiency in glucose that results from hypoglycemic insults triggers neuronal injuries. Balance in ion homeostasis is disturbed, which in turn results in membrane depolarization and massive release of neurotransmitters, including glutamate. The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA-kainate (Choi, 1988). In addition, neurons impaired of energy metabolism appear to be highly sensitive to excitotoxicity (Monyer *et al*., 1992; Cebers *et al*., 1998).

Pyruvate derived from glucose is the major precursor of the acetyl group of acetylcholine. Inhibition of pyruvate oxidation results in reduced ACh synthesis both *in vitro* and *in vivo.* Incorporation of [^{14}C] choline into ACh in brain *in vivo* is decreased in rats with insulin-induced hypoglycemia. Hypoglycemia results in decreased synthesis of the neurotransmitter pool of ACh are supported by the observation that administration of the CNS cholinesterase inhibitor physostigmine to hypoglycemic animals delays the onset of seizures and coma (Gibson & Blass, 1976).

Similar findings of an adverse effect of hypoglycemia on the synthesis of the amino acid neurotransmitters GABA and glutamate have also been reported. Utilization of amino acids such as glutamate and glutamine as alternative energy

substrates in moderate to severe hypoglycaemia results in accumulation of aspartate and ammonia in the brain. Hypoglycemia also produces a transient but substantial increase in extracellular concentrations of glutamate, GABA and dopamine, as measured using *in vivo* cerebral microdialysis (Butterworth, 1999). Studies reported that modulation of the GABAergic system in the ventromedial hypothalamus (VMH) alters both glucagon and sympathoadrenal, but not corticosterone, responses to hypoglycemia. GABAergic inhibitory tone within the VMH modulates glucose counter regulatory responses (Owen *et al.*, 2006). Alterations of neurotransmission mediated by ACh, Glu, GABA and/or DA contribute to the neurological signs and symptoms that characterize moderate hypoglycemia.

Cholinergic regulation of glucose homeostasis

Acetylcholine

Cholinergic system plays an important role in physiological and behavioural functions mediated by acetylcholine. Acetylcholine acts by binding to specific membrane receptors and is divided into muscarinic and nicotinic receptors. ACh consists of choline; which is taken up by the cholinergic cells through a sodium-dependent choline uptake system and an acetyl group provided by acetyl-Coenzyme A (intracellularly produced by oxidative metabolism in mitochondria). The synthesis of ACh takes place in the axonal terminals and is catalyzed by the cytosolic enzyme choline acetyltransferase (ChAT) (Van Der Zee *et al.*, 1999). Acetylcholine not only acts as a chemical signal at the neuromuscular junction, it plays a role in neural network formation. Acetylcholine, as a neurotransmitter mediates muscle contraction and glandular secretions *via* an extensive array of peripheral nerves, and in its capacity as a neuromodulator mediates consciousness by ultimately regulating the tone of activity patterns throughout the entire cerebral cortex.

Cholinergic Innervation

Cholinergic neurons form extensive networks with each other and with glutamatergic and GABAergic neurons. Cholinergic neurons also interconnect with other neuromodulator systems, such as networks using dopamine, norepinephrine, serotonin and histamine, to modulate sensory information relayed by glutamate synapses. Neuroplasticity is essential to the continuous updating these neural networks and some cholinergic axons are particularly plastic (Farris *et al.*, 1995). At both central and peripheral sites, cholinergic receptor activation leads to cytoskeletal responses. Centrally, muscarinic acetylcholine receptors, which utilize G-protein mechanisms, far out number nicotinic acetylcholine receptors, which are ionotropic in nature. In the periphery, nicotinic acetylcholine receptors predominate. Despite marked differences in receptor mechanisms, the binding of acetylcholine to either muscarinic or nicotinic acetylcholine receptors triggers cytoskeletal protein interactions with other cytoskeletal proteins at central and peripheral sites (Woolf, 2006).

Cholinergic neurons comprise less than one percent of neurons in the nervous system, yet they appear to be involved in the most intriguing and enigmatic of neural functions, ranging from gross observable movement to consciousness. Cholinergic systems orchestrate activity across groups of muscle cells and in conjunction monoamines - dopamine, norepinephrine, serotonin and histamine neuronal assemblies in cortical fields. The end result is a unified action, either behavioral or cognitive, which because of inherent neuroplasticity can be honed through the learning process to become purposeful action (Woolf & Butcher, 2010).

With a few exceptions, cholinergic neurons assume a ventral or basal location. This ventral or basal location is consistent with the motor and “cognitive action” functions of cholinergic neurons. Mesopontine cholinergic neurons are located in the laterodorsal tegmental nucleus and the pedunculopontine tegmental

nucleus, although the boundaries of the pedunculopontine nucleus are unclear resulting in some cholinergic neurons invading surrounding regions. These cholinergic neurons innervate the spinal cord, brainstem, thalamus, hypothalamus, basal forebrain and medial frontal cortex (Woolf & Butcher, 1986; Rye *et al.*, 1987). Mesopontine cholinergic neurons are intermingled with separate populations of glutamatergic and GABAergic cells (Wang & Morales, 2009). Mesopontine cholinergic neurons to the thalamus and basal forebrain play roles in higher cognitive functions, such as gating sensory input, attention and mediating level of consciousness. Cholinergic and GABAergic circuits in the mesopontine region have been shown to inhibit the startle response, a response that is necessary for gating sensory inputs (Bosch & Schmid, 2008). Some studies link mesopontine cholinergic cells with attention, but not all studies are in agreement (Rostron *et al.*, 2009). In one such study, lesions of the pedunculopontine tegmental nucleus produced an increase in errors and latency times on a serial reaction time task testing attention (Inglis *et al*., 2001).

Brain sites capable of producing coma and neurotransmitter correlates of coma point to a role for cholinergic mesopontine neurons in consciousness. Damage to mesopontine areas containing cholinergic neurons produces coma (Parvizi & Damasio, 2003). Many anesthetics block central cholinergic receptors and overdose can lead to central anticholinergic syndrome and coma (Moos, 2007). The role of cholinergic systems in consciousness is additionally illustrated by the pharmacology of anesthetic drugs that markedly diminish conscious awareness. Many known anesthetic compounds target nicotinic acetylcholine receptors and $GABA_A$ receptors (among other receptors). Muscarinic acetylcholine receptors and $GABA_B$ receptors represent potential targets for future anesthetics (Sanders *et al.*, 2008; Van Dort *et al.*, 2008). The cholinergic basal forebrain plays a role in selective attention, learning, memory, perception and consciousness (Sarter *et al.*, 2003). Specific regions of the basal forebrain play particular roles, even though each region takes a part in multiple functions and

there is overlap in function among the regions. Spatial learning, contextual learning, associative learning, and reorganization of sensory fields are some examples. Cholinergic septohippocampal pathway appears to contribute to its role in memory.

Muscarinic receptors

Muscarinic receptors are a family of G protein-coupled receptors that have a primary role in central cholinergic neurotransmission. Specific agonists, which activate postsynaptic muscarinic receptors, stimulate cholinergic signaling (Valentin *et al*., 2006). These receptors are widely distributed throughout the body and subserve numerous vital functions in both the brain and autonomic nervous system (Hassal *et al*., 1993). In the brain, muscarinic receptors participate in many important functions such as learning, memory and the control of posture. In the periphery, among other effects, muscarinic receptors mediate smooth muscle contraction, glandular secretion and modulation of cardiac rate and force. In the CNS there is evidence that muscarinic receptors are involved in motor control, temperature regulation, cardiovascular regulation and memory. Interest in the classification of muscarinic receptors involved in functions at different locations has been heightened by the potential therapeutic application of selective agents in areas such as AD, Parkinson's disease, asthma, analgesia and disorders of intestinal motility, cardiac and urinary bladder function (Caulfield & Birdsall, 1998).

Classification of Muscarinic receptors

Muscarinic receptors are G protein coupled receptors (Hulme *et al*., 1990). Molecular cloning studies have revealed the existence of five molecularly distinct mammalian muscarinic receptor proteins (Bonner, 1989). Muscarinic receptors, widely distributed throughout the central and peripheral nervous system have critical functions in learning and memory, attention and motor activity (Weiner *et*

al., 1990; Levey, 1993). Their effects depend on the receptor subtypes involved. Cholinergic stimulation of pancreatic β-cells increases insulin secretion. These are mediated by muscarinic cholinergic, rather than nicotinic receptors (Ahren *et al*., 1990; Stubbe & Steffens, 1993) and are dependent on extracellular glucose concentration (Henquin & Nenquin, 1988). Acetylcholine stimulated insulin secretion coupling is mediated by complex mechanisms of signal transduction. It has been proposed that acetylcholine activates phospholipid turnover and thereby increases the intracellular Ca^{2+} level.

The five muscarinic receptor (mAChR) subtypes are designated as M1 - M5. The odd-numbered receptors (M1, M3, and M5) couple to Gq/11 and thus activate PLC, which initiates the phosphatidyl inositol trisphosphate cascade. This leads to the dissociation of phosphatidyl 4, 5- bisphosphates (PIP2) into two components, *i.e*. IP_3 and DAG. IP_3 mediates Ca^{2+} release from the intracellular pool (endoplasmic reticulum), whereas DAG is responsible for activation of protein kinase C. On the other hand, PIP2 is required for the activation of several membrane protein, such as the "M current" channel and Na^{+}/Ca^{2+} exchanger and muscarinic receptor- dependent depletion of PIP2 inhibits the function of these proteins (Meyer *et al*., 2001; Fuster *et al*., 2004; Suh & Hille, 2005; Winks *et al*., 2005). The M1, M2 and M4 subtypes of mAchRs are the predominant receptors in the CNS. These receptors activate a multitude of signaling pathways important for modulating neuronal excitability, synaptic plasticity and feedback regulation of acetylcholine release (Volpicelli & Levey, 2004). The activation of mAChRs has been reported to suppress GABA release in the rat subfornical organ (Xu *et al*., 2001). Presynaptic M3 receptors inhibited excitatory and inhibitory transmission to rat subthalamic neurons (Shen & Johnson, 2000). M1 and probably M3 receptors inhibited GABA release in neurons of the rat lateral amygdala, nucleus accumbens, and striatum (Sugita *et al*., 1991).

Muscarinic M1 receptor

Muscarinic M1 receptors are predominantly expressed in the forebrain, including the cerebral cortex, hippocampus and corpus striatum, where this sub-type contributes by 50-60% to the total of the muscarinic receptors (Hamilton *et al*., 1997; Gerber *et al*., 2001; Miyakawa *et al*., 2001). The muscarinic M1 receptor subtype, which is also expressed in peripheral tissues, has been implicated in stress adaptive cardiovascular reflexes and central blood pressure control. Studies have shown that central administration of the muscarinic M1 specific antagonist pirenzepine lowered the blood pressure (Brezenoff & Xiao, 1986; Buccafusco, 1996). A putative overexpression of the M1 subtype in selected brain areas of spontaneously hypertensive rats has been reported (Scheucher *et al*., 1991). Muscarinic agonist depolarisation of rat isolated superior cervical ganglion is mediated through M1 receptors (Brown *et al*., 1980). Muscarinic M1 is one of the predominant muscarinic receptor subtypes expressed in pancreatic islets (Gilon & Henquin, 2001). Studies in pancreatic islets revealed that activation of muscarinic receptors is pertusis toxin insensitive and Gq mediated. Muscarinic M1 receptor number was decreased in the brainstem during pancreatic regeneration without any change in the affinity (Renuka *et al*., 2006).

Muscarinic M2 receptor

Muscarinic receptor activation in guinea pig heart produces a reduction in force of contraction and a decrease in the rate of beating. These effects are probably the consequence of inhibition of voltage-gated Ca^{2+} channels and activation of inwardly rectifying K^{+} channels, respectively. Extensive studies with many antagonists have defined this response as being mediated by the muscarinic M2 receptor (Caulfield, 1993). Muscarinic M2 receptors mediate both negative and positive ionotropic responses in the left atrium of the reserpinized rat, latter effect being insensitive to pertusis toxin (Kenakin & Boselli, 1990). Central cholinergic transmission is activated by inhibition of the presynaptic muscarinic M2 acetylcholine autoreceptor using selective antagonists. The presynaptic

muscarinic M2 autoreceptor negatively influences the release of acetylcholine in several brain regions, including the striatum, hippocampus, and cerebral cortex (Kitaichi *et al*., 1999; Zhank *et al*., 2002). A direct consequence of brain muscarinic M2 autoreceptor inhibition is an elevation of acetylcholine release in the synaptic cleft. Methoctramine and other muscarinic M2 receptor antagonists have been shown to enhance the release of acetylcholine in different brain structures (Stillman *et al.*, 1996).

Muscarinic M3 receptor

Muscarinic M3 receptors are broadly expressed in the brain, although the expression level is not high, compared to those of the muscarinic M1 and M2 receptors (Levey, 1993). Muscarinic M3 receptor is widely distributed in the peripheral autonomic organs with the highest expression found in the exocrine glands (Kashihara *et al*., 1992; Matsui *et al*., 2000). Expression of the muscarinic M3 receptor in the rat pancreatic islets and insulin secreting cell lines has been established (Lismaa *et al.*, 2000). Muscarinic M3 receptor also triggers direct contractions of smooth muscle; however, it only represents a minor fraction of total muscarinic receptor population in smooth muscle. It is expressed in relatively low density throughout the brain. Studies using knock out mice for muscarinic M3 receptors gave evidences for the primary importance of these receptors in the peripheral cholinergic system. In urinary bladder, pupillary muscles and intestinal smooth muscles the cholinergic contractions are mediated predominately through muscarinic M3 receptors (Matsui *et al*., 2000).

Muscarinic M4 receptor

Muscarinic M4 receptor is known to be abundantly expressed in the striatum (Levey, 1993). Muscarinic M4 receptors act as inhibitory muscarinic autoreceptors in the mouse (Zhang & Warren, 2002). The neuroblastoma-glioma hybrid cell line NG108–15 expresses M4 mRNA and M4 receptors can be

detected readily in radioligand binding assays (Lazareno *et al*., 1990). Inhibition of adenylyl cyclase activity by muscarinic agonists in rat corpus striatum is mediated by M4 receptors (Caulfield, 1993; Olianas *et al*., 1996).

Muscarinic M5 receptor

The muscarinic M5 receptor was the last muscarinic acetylcholine receptor cloned. Localisation studies have revealed that the M5R is abundantly expressed in dopamine-containing neurons of the substantia nigra par compacta, an area of the midbrain providing dopaminergic innervation to the striatum. Concordantly, oxotremorine-mediated dopamine release in the striatum was markedly decreased in M5R-deficient mice. More intriguingly, in M5R-deficient mice, acetylcholine induced dilation of cerebral arteries and arterioles was greatly attenuated (Yamada *et al*., 2001), suggesting that the M5 receptor is suitable target for the treatment of cerebrovascular ischemia. Muscarinic M5 receptor subtype is expressed at low levels in the brain (Hulme *et al*., 1990; Hosey, 1992).

Studies of the muscarinic M5 receptor have been hampered both by the lack of selective ligands and of tissues or cell lines that endogenously express the native receptor protein. Immunoprecipitation and RT-PCR studies have shown that the M5 receptor is expressed at very low densities in the mammalian brain. However, *in situ* hybridisation studies have demonstrated that M5 transcripts are highly concentrated in the basal ganglia and are the only muscarinic receptor transcripts expressed on dopaminergic neurons in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) (Reever *et al*., 1997). Another potentially useful system is the eosinophilic leukemia cell line (EoL-1) where M5 receptors are induced on differentiation with interferon-γ (Mita *et al*., 1996).

Signal transduction by muscarinic activation

Gq-protein-coupled receptors (GqPCRs) are widely distributed in the CNS and play fundamental roles in a variety of neuronal processes. Their activation results in phosphatidyl inositol 4,5-bisphosphate (PIP2) hydrolysis and Ca^{2+} release from intracellular stores *via* the PLC-inositol 1,4,5-trisphosphate (IP_3) signaling pathway. Because early GqPCR signaling events occur at the plasma membrane of neurons, they are influenced by changes in membrane potential (Billups *et al*., 2006). Muscarinic receptors, which are G protein coupled, stimulate signaling by first binding to G protein complex ($\alpha\beta\gamma$) which provides specificity for coupling to an appropriate effector. The α subunit interacts with an effector protein or ion channel to stimulate or inhibit release of intracellular second messengers. Mutation analysis showed that the G protein is primarily but not exclusively acts through interaction with the third cytoplasmic loop. It is suggested that the short sequences, N terminal 16-21 and C terminal 19 amino acids of the loop play a key role in determining the specificity (Wess *et al*., 1989).

Cyclic adenosine monophosphate

Adenylate cyclase is either positively or negatively regulated by G protein coupled receptors resulting in an increase or decrease in the generation of the second messenger, Cyclic adenosine monophosphate (cAMP). The stimulation of muscarinic M2 and M4 receptors endogenously expressed in cell lines, results in the inhibition of adenylate cyclase. G protein reconstitution experiments have shown that muscarinic M2 receptors inhibit adenylate cyclase through Gi and possibly through the pertusis toxin insensitive Gz. In neuroblastoma SK-N-SH cells which express endogenous muscarinic M3 receptors stimulate adenylate cyclase activity (Baumgold & Fishman, 1988). The muscarinic M1 receptor which ectopically expressed at physiological levels in A9L cells, was shown to stimulate adenylate cyclase through an IP_3 and Ca^{2+} dependent mechanism (Felder *et al*., 2000). In contrast, M1 receptors stimulate adenylate cyclase in CHO cells

predominantly through an IP_3 and Ca^{2+} independent mechanism that also contained a small Ca^{2+} dependent component (Gurwitz *et al.*, 1994).

Phospholipase C

The family of PLC enzymes has been grouped into three classes, β, γ and δ (Rhee & Choi, 1992). PLC serves as the primary effector for the muscarinic M1 receptor that is coupled through Gq α subunits (Berstein *et al*., 1992). Muscarinic M1, M3 and M5 receptors stimulate the production of IP_3, independent of direct PLCβ and G protein interaction (Gusovsky *et al*., 1993). This alternate route for the generation of IP_3 involves the tyrosine kinase dependent phosphorylation of PLCγ, a mechanism normally stimulated by growth factors and their receptors (Meisenhelder *et al.*, 1989). Expression studies revealed that the cloned muscarinic M2 receptor stimulates PLC through a pertusis toxin-sensitive G protein although with lower efficiency than muscarinic M1 or M3 receptors (Ashkenazi *et al*., 1987). Inhibition of PLC by an endogenously expressed muscarinic M2 receptor has been reported in FRTL5 cells suggesting that negative regulation occur in some cells (Bizzarri *et al*., 1990).

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Nicotinic Receptors

The nicotinic acetylcholine receptor (nAChR), a key player in neuronal communication, converts neurotransmitter binding into membrane electrical depolarization. Nicotinic acetylcholine receptors that contain α7 subunits are prevalent in the mammalian brain and have received special attention because of their linkage to cognitive functions (Adams & Freedman, 1997; Levin & Simon, 1998). This protein combines binding sites for the neurotransmitter acetylcholine and a cationic transmembrane ion channel. It mediates synaptic transmission at the junction between nerve and muscle cells and various types of nAChR are expressed in the brain. It is involved in several neurological pathologies. The α7 nicotinic receptor, also known as the α7 receptor, is the predominant type of

nicotinic acetylcholine receptor in the brain, consisting entirely of α7 subunits (Rang *et al.*, 2003). As with other nicotinic acetylcholine receptors, functional α7 receptors are pentameric i.e., (α7)5 stoichiometry. It is located in the brain, where activation yields post- and presynaptic excitation (Rang *et al.*, 2003), mainly by increased Ca^{2+} permeability.

Neuronal nicotinic cholinergic receptors are crucial to acetylcholine neurotransmission in both the CNS and autonomic nervous system. However, in the CNS, these receptors are more often associated with modulation of release of several neurotransmitters including dopamine, norepinephrine, GABA and glutamate (Alkondon *et al*., 1999; Girod & Role, 2001). In the CNS, nicotinic acetylcholine receptors mediate the release of glutamate (De Filippi *et al*., 2001; Rossi *et al*., 2003; Reno *et al*., 2004) and norepinephrine (O Leary & Leslie, 2003). Thus, these receptors significantly influence the activity within the CNS circuitry and deregulation of this activity could contribute to disorders involving the CNS. Abnormalities of nicotinic acetylcholine receptor function in the hippocampus lead to cognitive and memory impairments (Green *et al*., 2005; Levin *et al*., 2002) and sensory gating deficits (Adler *et al*., 1998). Nicotinic acetylcholine receptors are involved with neuroplastic responses, such as dendritic growth in cholinoceptive neurons (Torrao *et al.*, 2003).

GABA regulation of glucose homeostasis

GABA is a major inhibitory neurotransmitter in the mammalian central nervous system (CNS) and is synthesized from glutamic acid by glutamic acid decarboxylase (GAD) (Gerber & Hare , 1979). There is significant ongoing work aimed at understanding the specific roles of synaptic and extrasynaptic GABA in regulating neural activity in both normal and pathological conditions. Precise GABAergic synaptic signaling is critical to the accurate transmission of information within neural circuits and even slight disruptions can produce hypersynchronous activity (Chagnac-Amitai & Connors, 1989). Moreover,

changes in ambient GABA can alter tonic inhibition and thus the overall synaptic tone of a brain region (Farrant & Nusser, 2005). There is an extensive literature showing that seizures can be provoked by blocking GABA synthesis with 3-mercaptoproprionic acid (MPA) *in vivo* (Mares *et al*., 1993). These studies were demonstrating the involvement of GABA in the prevention of the overstimulation of neuronal networks.

GABA Receptors

GABA mediates its actions *via* three distinct receptors: the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors (Bormann, 2000). In addition to its CNS functions, the GABAergic system is also present in peripheral tissues, including the gastrointestinal tract (Gilon *et al*., 1990, 1991; Harty *et al*., 1991; Krantis *et al*., 1994). Ionotropic GABA receptors are the most important Cl− channels in the central nervous system CNS and their expression has also been found in peripheral organs. These receptors mediate a fast inhibitory neurotransmission in the CNS (Akinci & Schofield, 1999). Ionotropic GABA receptors can be categorized into $GABA_A$ and $GABA_C$ receptors based on their subunit compositions and pharmacological properties (Bormann, 2000).

$GABA_B$ receptors mediate slow prolonged inhibition in the brain by activating postsynaptic G protein-coupled inwardly rectifying K+ channels (GIRKs) and inactivating presynaptic voltage-gated Ca2+ channels. $GABA_B$ receptors also inhibit adenylate cyclase, leading to diminished activity of PKA signaling pathways (Bowery, 2006). Structurally $GABA_B$ receptors are members of the class C family of G-protein coupled receptors (GPCR) and are encoded in vertebrates by two genes - $GABA_B$ receptor-1 ($GABA_BR1$) and $GABA_BR2$ respectively (Couve *et al*., 2002; Bettler *et al*., 2004).

$GABA_A$ Receptors

$GABA_A$ receptors are pentameric in structure, with the five subunits arranged like spokes of a wheel around a central Cl^- selective pore (Barnard, 2001). Nineteen GABA receptor subunits have been cloned from rats, which include α1–6, β1–3, γ1–3, ρ1–3, δ, θ, ε, and π (Whiting *et al*., 1999). The 19 subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, ρ1–2) are encoded by 19 distinct genes. Each subunit has four transmembrane segments, with both the amino and carboxy termini located extracellularly. These extracellular segments form the recognition sites (two per channel) for GABA and also, in some channel types, the recognition site (one per channel) for benzodiazepine-like allosteric modulators. The subunit composition determines both the biophysical properties of the receptor–channel complex and its pharmacology, most notably the sensitivity to benzodiazepines (BDZ) (Rudolph & Mohler 2004; Johnston, 2005). A typical benzodiazepine-sensitive $GABA_A$ receptor consists of two α1, α2, α3, or α5 subunits, two β2 or β3 subunits (or one each) and a γ2 subunit.

Classically, $GABA_A$ receptors have been recognized as mediating phasic inhibition through the generation of fast, transient, rapidly desensitizing currents (IPSCs) in postsynaptic neurons in response to synaptically released GABA. It has been recognized that $GABA_A$ receptors also contribute to tonic (extrasynaptic) inhibition, representing the Cl^- conductance activated at nonsynaptic sites in response to background concentrations of GABA (Farrant & Nusser, 2005). Phasic and tonic inhibitions are mediated by $GABA_A$ receptors with different subunit composition, GABA affinities and rates of desensitization. The most notable difference in subunit composition is that the receptors mediating tonic inhibition contain the δ subunit, rather than the γ subunit characteristic of synaptic $GABA_A$ receptors (Nusser *et al.*, 1998). Receptors containing α4, α5, or α6 are commonly found nonsynaptically. Pharmacologically, the most notable difference is that receptors with α4, α6, or δ subunits are not potentiated by benzodiazepines or by nonbenzodiazepine benzodiazepine receptor agonists (such as zolpidem),

whereas those with α1, α2, α3, α5, or γ2 subunits are benzodiazepine sensitive. The benzodiazepine-sensitive α subunits (α1, α2, α3, α5) differ from the insensitive ones (α4, α6) in possessing a histidine residue at position 101. Activation of $GABA_AR$, a Cl^- ion channel, results in membrane hyperpolarization as a consequence of an inward Cl^- flux (Kittler & Moss, 2003). In the CNS, $GABA_ARs$ are subject to modulation by their subunit composition, localization, number and phosphorylation states and variance of GABA concentration in the synaptic cleft (Chebib & Johnston, 1999; Mody & Pearce, 2004).

$GABA_B$ Receptors

The $GABA_B$ receptor is part of the class C of GPCRs that also includes the mGlu, the Ca^{2+}-sensing and the sweet and umami taste receptors among others (Pin *et al.,* 2003). These receptors are dimers, either homodimers linked by a disulphide bond mGlu and Ca^{2+}-sensing receptors or heterodimers made of two similar, but distinct subunits, the $GABA_B$ and taste receptors. Indeed, the $GABA_B$ receptor was the first GPCR to be identified that requires two distinct subunits to function: the $GABA_{B1}$ and $GABA_{B2}$ subunits (Jones *et al*., 1998; White *et al*., 1998; Kaupmann *et al*., 2003). Although the $GABA_{B1}$ subunit was soon shown to bind all known $GABA_B$ ligands, both agonists and antagonists, this protein did not form a functional $GABA_B$ receptor when expressed alone (Kaupmann *et al*., 1997). Only when $GABA_{B1}$ was co-expressed with the homologous $GABA_{B2}$ subunit was a functional $GABA_B$ receptor observed, either in cell lines or in cultured neurons. The $GABA_B$ dimeric entity was confirmed in native tissue (Kaupmann *et al*., 1998). Indeed, both $GABA_{B1}$ and $GABA_{B2}$ mRNAs are co-localized in most brain regions. Second, both proteins are found in the same neurons, even in the same subcellular compartments as observed at the electron microscopic level. Moreover, co-immunoprecipitation of $GABA_{B1}$ with a $GABA_{B2}$ antibody could be demonstrated from brain membranes. Eventually, mice lacking either $GABA_{B1}$ or $GABA_{B2}$ share very similar phenotypes and none of the known

$GABA_B$-mediated responses could be measured in either mice (Prosser *et al*., 2001; Schuler *et al*., 2001).

$GABA_B$ receptors play an important role in maintaining excitatory–inhibitory balance in brain and alterations can lead to seizures (Hossein *et al.*, 2008). $GABA_B$ receptors, the metabotropic receptors for GABA, are G protein-coupled receptors (GPCR) which regulate neuronal excitability both pre and postsynaptically. The action of GABA at presynaptic $GABA_B$ receptors is to reduce Ca^{2+} influx and thus inhibit neurotransmitter release (Takahashi *et al*., 1998). These receptors exist on GABAergic terminals (autoreceptors), or on terminals arising from cells containing other neurotransmitters, such as glutamate (heteroreceptors). Postsynaptically, $GABA_B$ receptors are responsible for the generation of the late inhibitory postsynaptic potential (IPSP), *via* the opening of K^+ channels and inhibit adenylate cyclase (Bettler *et al*., 1998). Abnormality in either of these functions could have consequences for the generation and/or prevention of epileptic seizures. Multiple laboratories have demonstrated altered expression of $GABA_BR1$ and $GABA_BR2$ in animal models for seizure disorders (Princivalle *et al*., 2003; Straessle *et al*., 2003). Han *et al*. (2006) found that as a result of multiple seizures, there was a long-term decrease in $GABA_BR1$ (15 days) and $GABA_BR2$ (30 days) expression in rat hippocampus. Taken together, these animal studies suggest that the changes in the number $GABA_B$ receptors lead to epilepsy, due to changes in transmitter release (presynaptic) and inhibition (postsynaptic). Accordingly, GABA neurons have been alternately proposed to be highly vulnerable or relatively invulnerable after insults known to cause epilepsy (Ribak *et al*., 1979; Sloviter *et al.*, 1987) and GABA-mediated inhibition is reportedly decreased, in animal models of epilepsy (Dalby *et al.*, 2001).

$GABA_C$ Receptors

$GABA_C$ receptors, which are a subfamily of $GABA_A$ receptors, are members of the Cys-loop superfamily of ligand-gated ion channels (LGICs), an

important group of receptors involved in rapid synaptic transmission and whose malfunction can result in a variety of neurological disorders; hence, understanding their mechanism of action is of considerable pharmacological interest. $GABA_C$ receptors are mostly located in retinal neurons where they play a role in retinal signaling involved in diseases such as macromolecular degeneration (Bormann, 2000). The receptors are activated by the binding of GABA, the main inhibitory neurotransmitter in the central nervous system. $GABA_C$ receptors have distinct pharmacological properties from $GABA_A$ receptors, e.g., they are not inhibited by bicuculline, the classic $GABA_A$ receptor antagonist (Barnard *et al*., 1998; Chebib *et al*., 2000). Like all the LGICs belonging to the Cys-loop superfamily, $GABA_C$ receptors are composed of five subunits arranged in a pentagonal array around a central ion-permeant pore. Each subunit has an extracellular N-terminal domain (ECD), a transmembrane domain composed of four α-helices, and an intracellular domain. Three subunits (ρ_{1-3}) have been identified; these can all form functional homomeric or heteromeric receptors (Enz, 2001).

Similar to $GABA_A$ receptors, they possess a high permeability to Cl^-, but in contrast to $GABA_A$ channels, they are insensitive to bicuculline, barbiturates and benzodiazepines (Polenzani *et al.,* 1991). The activity of $GABA_C$ receptors is regulated by extracellular agents, such as Zn^{2+}, H^+, Ca^{2+} (Ouyang *et al.*, 2007; Kaneda *et al.*, 1997) and also by intracellular factors, such as Ca^{2+}, phosphatases and kinases (Feigenspan & Bormann 1994b; Kusama *et al.*, 1995). Protein phosphorylation is postulated to be an important physiological mechanism for regulating GABA mediated synaptic inhibition (Moss & Smart, 1996).

Glutamic Acid Decarboxylase (GAD)

GABA the main inhibitory neurotransmitter in the brain is synthesized by GAD. GAD exists in two isoforms termed GAD65 and GAD67 due to their molecular weights of 65 and 67 kDa, respectively. These enzymes are the products of two independently regulated genes sharing 65% sequence homology in rats

(Erlander *et al*., 1991; Bu *et al*., 1992). Most GABAergic interneurons express both subtypes of GAD (Houser & Esclapez 1994) which are simultaneously detectable in the rat brain as early as embryonic day 17 (Dupuy & Houser, 1996). GAD67 is found in axonal regions as well as in neuronal cell bodies, whereas GAD65 is mainly associated with synaptic terminals (Kaufman *et al*., 1991). Therefore it has been suggested that GAD67 mostly provides a pool of GABA for general metabolic activity while GABA synthesized by GAD65 is likely to be more involved in synaptic transmission (Martin & Rimvall, 1993). Mice lacking GAD65 are vital and do not exhibit changes in their brain GABA content though they have an increased susceptibility to seizures (Asada *et al*., 1996; Kash *et al*., 1997). During embryogenesis the mRNA coding for GAD67 is regulated by alternative splicing (Bond *et al*., 1990; Szabo *et al*., 1994). At least two additional transcripts exist, I-80 and I-86 (summarized as EGAD), distinguished by insertions of 80 or 86 base pair (bp) in GAD67 mRNA, respectively. The two inserts are identical with exception of the 6 bp at the 3′-end of the larger fragment containing an overlapping stop-start codon. The complete coding region of embryonic GAD messages comprises 1,860 (80 bp insert) and 1,866 bp (86 bp insert). Both embryonic transcripts code for a short enzymatically inactive GAD protein of 25 kDa (GAD25) which corresponds to the amino-terminal regulatory region of GAD67 and therefore has putative regulatory functions. Termination-reinitiation at the stop-start codon of I-80 additionally produces an enzymatically active protein of 44 kDa (GAD44) corresponding to the carboxy-terminal catalytic domain of GAD67 that contains the pyridoxal phosphate cofactor binding site (Szabo *et al*., 1994).

The early finding that baby food deficient in vitamin B6, a cofactor of the GABA-synthesizing enzyme GAD caused vitamin-reversible seizures provided one of the first clues that seizures might be caused by reduced synthesis of GABA (Bankier *et al*., 1983). Accordingly, GABA neurons have been alternately proposed to be highly vulnerable or relatively invulnerable after insults known to

cause epilepsy (Ribak *et al*., 1979; Sloviter, 1987) and GABA-mediated inhibition is reportedly altered, in a variety of constantly compared but greatly dissimilar animal models (Dalby & Mody, 2001). The use of GAD immunocytochemistry in kindled and control tissue was used to allow direct anatomic confirmation for measuring changes in GAD-immunoreactivity (GAD-IR) which would represent GABA synthesis for release by the recurrent inhibitory system of the fascia dentata. Immediately after the last kindled seizure, optically detected GAD-IR puncta densities were significantly reduced in stratum granulosum at 3 or 7 days after the last kindled seizure (Babb *et al*., 1989). GAD65-/- mice develop spontaneous seizures that result in increased mortality. Seizures can be precipitated by fear or mild stress. Seizure susceptibility is dramatically increased in GAD65 mice backcrossed into a second genetic background, the nonobese diabetic strain of mice enabling electroencephalogram analysis of the seizures. The generally higher basal brain GABA levels in this backcross are significantly decreased by the GAD65 mutation, suggesting that the relative contribution of GABA synthesized by GAD65 to total brain GABA levels is genetically determined (Kash *et al*., 1997).

Glutamate decarboxylase, the primary enzyme that is involved in the synthesis of GABA, has been identified as an early target antigen of the T-lymphocyte mediated destruction of pancreatic β-cells causing insulin-dependent diabetes mellitus (Baekkeskov *et al.*, 1990). GABA through its receptors has been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic α–cells and δ-cells respectively (Gaskins, 1995). It is present in the cytoplasm and in synaptic-like microvesicles (Reetz, 1991) and is co-released with insulin from β-cells in response to glucose. The released GABA inhibits islet α -and β -cell hormonal secretion in a paracrine manner. During diabetes the destruction of β -cells will lead to decrease in GABA release resulting in the enhancement of glucagon secretion from α-cells leading to hyperglycemia. In patients with diabetes, an oral glucose load induced a paradoxical rise in glucagon secretion,

which could be normalized with optimal administration of insulin, suggesting that the dysfunctional regulation of pancreatic α–cells in diabetes is related to insulin deficiency and an anomalous internal environment of the islets (Greenbaum *et al*., 1991; Hamaguchi *et al*., 1991). However, this defect is undefined because of an inadequate understanding of the mechanisms underlying suppression of glucagon by insulin in response to hyperglycemia. Secretion of glucagon from α– cells is regulated by various factors, including glucose, zinc, and the chemical transmitter gammaaminobutyric acid (GABA) (Pipeleers *et al*., 1985; Ishihara *et al*., 2003). The role of GABA and the A type GABA receptor ($GABA_AR$) in the regulation of glucagon release has been demonstrated (Braun *et al*., 2004; Rorsman *et al*., 1989). Pancreatic β -cells contain high concentrations of GABA and GAD (Taniguchi *et al*., 1979). GABA is localized in ‘‘synaptic’’- like microvesicles within β - cells that are distinct from the insulincontaining large dense core vesicles, suggesting that exocytosis of pancreatic GABA is similar to the process found in neurons (Reetz *et al*., 1991; Sorenson *et al*., 1991). Functional $GABA_ARs$ are expressed in the α cells (Hales & Tyndale, 1994). It has been proposed that, during hyperglycemia, GABA is co-released with insulin from the b cells and acts on $GABA_ARs$ on the α cells to reduce their secretion of glucagon (Rorsman *et al*., 1989).

The brain GABAergic mechanisms also play an important role in glucose homeostasis. Inhibition of central $GABA_A$ receptors increases plasma glucose concentration (Lang, 1995). $GABA_A$ receptors in brainstem have a regulatory role in pancreatic regeneration (Kaimal *et al*., 2007). It is known that in ischemia, the released glutamate activates glutamate receptors, particularly of the NMDA type, increases the intracellular concentration of Ca^{2+}, and triggers a long-lasting potentiation of NMDA-receptor-gated currents (Szatkowski & Attwell, 1994). The massive amount of GABA released simultaneously in the hippocampus is an important protective mechanism against the excessive release of excitatory amino acids, counteracting the harmful effects that lead to neuronal death. The release of

GABA limit excitation and prevent this excitation from reaching neurotoxic levels. Activation of $GABA_A$ receptors increases $C1^-$ conductance, inducing hyperpolarization and reducing cell excitability (Sivilotti & Nistri, 1991). Enhanced GABA release increases extracellular GABA levels, thus contributing to the maintenance of homeostasis in the hippocampus upon impending hyperexcitation. Moreover, hippocampal GABAergic neurons are more resistant than excitatory aminoacid neurons to transient ischemia (Matsumoto *et al.*, 1991). The augmentation of inhibitory mechanisms has important neuroprotective effects. To date, drugs that enhance GABAergic systems provide significant neuronal protection when used before or after insults. Thus, any impairment in the GABAergic mechanism in the CNS and/or in the PNS is important in the pathogenesis of hypoglycemia and diabetes.

Insulin and Insulin receptors in the brain

Two decades ago both insulin and its receptors were discovered in the brain (Havrankova *et al.*, 1978). Moreover, contrary to old assumptions, it is now known that insulin is actively transported across the blood–brain barrier and it is produced locally in the brain (Schwartz *et al.,* 1998). Concentrations of insulin receptors in the brain are particularly high in neurons, with abundant insulin receptor protein in both cell bodies and synapses (Zhao *et al*., 1999).

These findings have raised questions about the physiological role of insulin in the brain. Some suggest that, as in peripheral tissues, insulin mainly acts by mediating cerebral glucose uptake (Hoyer, 1998), but this opinion is not shared by others. Insulin and insulin receptors appear to play a modulatory role in certain behaviours, such as feeding behaviour, learning and memory (Wickelgren, 1998; Kumagai , 1999). For example, after training in a water maze, insulin receptor mRNA levels were increased in the hippocampus of rats, in parallel with accumulation of insulin receptor protein. Moreover, intracerebroventricular

administration of insulin facilitated retention of a passive-avoidance task in rats (Park *et al*., 2000).

The complexity of the mechanisms underlying these behavioural findings is only now starting to be appreciated (Fernandes *et al*., 1999). When applied to brain slices, insulin inhibits the spontaneous firing rate of hippocampal pyramidal neurons and the frequency of AMPA-receptor mediated miniature EPSCs of cerebellar Purkinje neurons. In addition, insulin attenuates the amplitude of AMPA-receptor-mediated currents in cerebellar Purkinje neurons (Palovcik *et al*., 1984), through the stimulation of clathrin-dependent receptor internalisation, a phenomenon that appears to have links with cerebellar LTD (Wang *et al*., 2000). These same authors have reported no effect of insulin on NMDA-receptor-mediated currents in cerebellar Purkinje neurons. Conversely, in hippocampal slices insulin has been shown to increase NMDA-receptor mediated EPSPs (Liu *et al*., 1995). These different findings are possibly due to variations in insulin signalling in different brain regions. Insulin thus appears to play a modulatory role in synaptic transmission in the brain. However, studies of its involvement in behaviour and synaptic transmission have so far mainly examined its effects after local (for example, intracerebroventricular) administration or *ex vivo*. The challenge for future studies will be to determine whether systemic insulin also has neuromodulatory effects under physiological conditions and to dissociate these effects from the associated effects of insulin on peripheral and central glucose homeostasis.

Alterations of neuronal glucose transport during diabetes and hypoglycemia

Under physiological conditions, maintenance of normal cerebral functions depends almost entirely on the availability of glucose for the supply of ATP (Anderson & Swanson, 2000). Since the brain cannot store the significant carbohydrates, a steady glucose supply is required from the blood. Thus glucose transport into the brain is critical for the maintenance of brain metabolism.

Clinical and experimental studies have revealed that altered glucose status is an important factor controlling learning and memory processes (Messier & Gagnon, 1996). Although under basal conditions the rate of glucose transport is not the rate-limiting step for glycolysis in the central nervous system hypoglycemia or hyperglycemia is known to change the glucose transport system in the brain (Devivo, *et al*., 1991) suggesting that there should be glucose-regulatable mechanisms associated with the transport of glucose. The brain is dependent on a steady supply of glucose (Erecinska & Silver, 1994).

Neuronal glucose uptake relies on the glucose transporter isoforms GLUT1 (Maher *et al*., 1994), GLUT3 (Olson & Pessin, 1996), and GLUT8 (Piroli *et al*., 2002; Sankar *et al*., 2002) on the plasma membrane. Due to its relative abundance in the brain, GLUT3 is often considered the neuronal glucose transporter (Maher *et al*., 1994). GLUT3 is a major facultative glucose transporter expressed in neurons, though there is evidence for expression in neurons of GLUT1, GLUT2, GLUT4, GLUT5 and GLUT8 (Choeiri *et al*., 2002). Classically, neurons are considered to be insulin insensitive, that is, glucose uptake is not significantly increased in response to insulin stimulation (Heidenreich *et al*., 1989) as is regularly observed in muscle and fat cells (Watson & Pessin, 2001). Insulin treatment dramatically increased the translocation of GLUT 3 to the plasma membrane, and insulin pre-treatment potentiated a KCl stimulated glucose uptake (Uemura & Greenlee, 2006).

Although neurons have insulin receptors (Schulingkamp *et al*., 2000), they do not respond to insulin alone by increasing glucose uptake. In insulin-sensitive cells, insulin increases glucose uptake by promoting two critical processes: translocation of vesicles containing the glucose transporter isoform GLUT4 to the plasma membrane and their fusion with the plasma membrane (Czech & Corvera, 1999; Olson & Pessin, 1996. In neurons, fusion of GLUT3 with the plasma membrane is induced by membrane depolarization, resulting in a marked increase in glucose uptake (Uemura & Greenlee, 2001). It is well known that nerve cells in

the hippocampus, striatum, piriform and neocortical regions are susceptible to ischaemic injury and stress during glucose deprivation (Thilmann *et al.*, 1986). GLUT3 is probably also a stress-induced protein, which may protect the damaged nerve cell from energy depletion. This indicates that glucose starvation effectively activates the transcriptional rate of the GLUT3 gene in vulnerable neurons (Nagamatsu *et al.*, 1993). Many studies have been done describing changes subcellular localization of glucose transporters in response to a variety of different stimuli including insulin, IGF-1, and glucose deprivation (Fladeby *et al*., 2003; Shin *et al*., 2004; Watson and Pessin, 2001).

Oxidative stress and neuronal damage during diabetes and hypoglycemia

Oxidative stress is known to be present in different pathological conditions in the CNS such as ischemia and various neurodegenerative diseases (Margaill *et al*., 2005; Halliwell, 2006). The deleterious actions of diabetes and stress increase oxidative stress in the brain, leading to increases in neuronal vulnerability. The presence of oxidative stress during hypoglycemia has been recently suggested (Patocková *et al*., 2003; Singh *et al*., 2004; Suh *et al*., 2007), although its temporality and regional distribution in brain have not been explored in detail. Oxidative stress has been suggested as a mechanism contributing to neuronal death induced by hypoglycemia and an early production of reactive species (RS) during the hypoglycemic episode has been observed. Early activation of calcium-dependent ROS producing pathways is involved in neuronal death associated with glucose deprivation (Paramo *et al.*, 2010). Recent studies show that oxidative stress develops mainly after the isoelectric period during the glucose perfusion phase and that its presence is related to the subsequent death of neurons (Suh *et al*., 2007). Diabetes has been reported to increase superoxide dismutase (SOD) activity within the brain which in turn makes the brain more vulnerable to damage (Kadekaro *et al.*, 1988). During prolonged periods of stress, exhaustion of neuronal defense mechanisms, such as anti-oxidant enzymes, reported to increase

neuronal vulnerability to the point where neuronal adaptation shifts from neuronal plasticity towards neuronal damage (Reagan *et al.*, 2000).

In the central nervous system, programmed cell death or apoptosis is considered to be an important phenomenon that is related to neuron vulnerability to stress condition. Bax is a protein, identified as regulating molecules for programmed cell death. A possible relationship between the localization and expression of Bax protein and the cell vulnerability in central nervous system is reported (Hara *et al.*, 2004).

The cAMP responsive element binding protein (CREB)

The cAMP responsive element binding protein (CREB) is a nuclear protein that modulates the transcription of genes with cAMP responsive elements in their promoters. Increases in the concentration of either Ca^{2+} or cAMP trigger the phosphorylation and activation of CREB. This transcription factor is a component of intracellular signaling events that regulate a wide range of biological functions, from spermatogenesis to circadian rhythms and memory. Byrne (1993) reported the role of CREB in cellular events underlying long-term but not short-term memory. Genetic and pharmacological studies in mice and rats demonstrate that CREB is required for a variety of complex forms of memory, including spatial and social learning, thus indicating that CREB may be a universal modulator of processes required for memory formation (Silva, 1998).

The present study was carried out to elucidate hypoglycemic and hyperglycemic effect on brain cholinergic and GABAergic receptors functional regulation, alterations in insulin receptor, GLUT3, pro-apoptotic protein - Bax and the changes at second messenger level by analyzing the expressional changes in second messenger enzyme; phospholipase C and changes at transcription level using transcription factor, CREB in the brain regions and pancreas of experimental rats. Studies on the functional regulation of ACh and GABA through their receptor subtypes during hyperglycemia and hypoglycemia will lead to a better

understanding of the cognitive and memory dysfunction due to neuronal damage in the brain.

Materials and Methods

CHEMICALS USED IN THE STUDY AND THEIR SOURCES

Biochemicals

Gamma amino butyric acid (GABA), pirenzepine, atropine, 4-DAMP mustard (4-deoxy acetyl methyl piperidine mustard), ethylene diamine tetra acetic acid - EDTA, HEPES - [n′ (2-hydroxy ethyl)] piperazine-n′-[2-ethanesulfonic acid], Streptozotocin, citric acid, Tris HCl, foetal calf serum (heat inactivated), D-glucose, calcium chloride, collagenase type XI, bovine serum albumin fraction V and RPMI-1640 medium were purchased from Sigma Chemical Co., St. Louis, USA). All other reagents were of analytical grade purchased locally.

Radiochemicals

Quinuclidinylbenzilate, L-[Benzilic-4,4'-^{3}H]-[4-^{3}H] (Sp. Activity 42 Ci/mmol), 4-DAMP, [N-methyl-^{3}H] (Sp. Activity 83 Ci/mmol) and 4-amino-n-[2, 3-^{3}H] butyric acid (Specific activity-84.0 Ci/mmol) was purchased from NEN life sciences products Inc., Boston, U.S.A.

Radioimmunoassay kit for insulin was purchased from Baba Atomic Research Centre (BARC), Mumbai, India.

Molecular Biology Chemicals

Tri-reagent kit was purchased from Sigma chemicals Co., St. Louis, USA. ABI PRISM High capacity cDNA Archive kit, primers and Taqman probes for Real Time- PCR were purchased from Applied Biosystems, Foster City, CA, USA.

Confocal Dyes

Rat primary antibody for muscarinic M1 (Cat. No. 087k1395), M3 (Cat. No. 126k1205), α7 nicotinic acetylcholine receptor (Cat. No. 018k4811) and $GABA_{A\alpha1}$ (Cat. No. 077k4838) and FITC coated secondary antibody (Cat. No. No-AP307R) were purchased from Sigma Aldrich and Chemicon, USA.

ANIMALS

Adult male Wistar rats of 180-240g body weight purchased from Amrita Institue of Medical Sciences, Cochin and Kerala Agriculture Unviersity, Mannuthy were used for all experiments. They were housed in separate cages under 12 hour light and 12 hour dark periods and were maintained on standard food pellets and water *ad libitum*.

DIABETES INDUCTION AND HYPOGLYCEMIA

Experimental rats were divided into the following groups as i) Control ii) Diabetic iii) Insulin induced hypoglycemia in diabetic rats (D+IIH) and iv) Insulin induced hypoglycemia in control rats (C+IIH). Each group consisted of 6-8 animals**.**

Diabetes was induced by a single intrafemoral dose, 55 mg/kg body weight, of STZ prepared in citrate buffer, p^H 4.5 (Arison *et al*., 1967; Hohenegger & Rudas, 1971). The D+IIH group received daily 2 doses of 10 Unit/Kg body weight of regular human insulin (Actrapid) and C+IIH received daily 2 doses of 1.5 Unit/Kg body weight of regular human insulin (Flanagan *et al*., 2003). D+IIH and C+IIH group had daily two episodes of insulin-induced hypoglycemia for 10 days. Control rats were injected with citrate buffer.

DETERMINATION OF BLOOD GLUCOSE

The diabetic state of animals was assessed by measuring blood glucose concentrations at 72 hours after streptozotocin treatment. The rats with a blood sugar level above 250 mg/dl were selected as diabetic rats.

SACRIFICE AND TISSUE PREPARATION

The animals were then sacrificed on 15^{th} day by decapitation. The cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus were dissected out quickly over ice according to the procedure of Glowinski & Iversen, (1966) and the pancreas was dissected quickly over ice. Hippocampus was dissected according to the procedure of Heffner *et al*., (1980). The blood samples were collected and plasma was separated by centrifugation. The tissue samples and plasma were kept at -80° C until assay. All animal care and procedures were in accordance with Institutional and National Institute of Health guidelines.

ESTIMATION OF BLOOD GLUCOSE

Blood glucose was estimated using Glucose estimation kit (Merck). The spectrophotometric method using glucose oxidase-peroxidase reactions is as follows:

Principle: Glucose oxidase (GOD) catalyses the oxidation of glucose in accordance with the following equation:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{(GOD)}} \text{Gluconic acid} + H_2O_2.$$

$$H_2O_2 + \text{Phenol} \quad \text{4-aminoantipyrene} \xrightarrow{\text{(Peroxidase)}} \text{Coloured complex} + H_2O$$

The hydrogen peroxide formed reacts with 4-aminoantipyrine and 4-hydroxybenzoic acid in the presence of peroxidase (POD) to form N-(-4-

antipyryl)-p-benzo quinoneimine. The addition of mutarotase accelerates the reactions. The amount of dye formed is proportional to the glucose concentration. The absorbance was read at 510nm in spectrophotometer (Shimadzu UV-1700 pharmaspec).

ESTIMATION OF CIRCULATING INSULIN BY RADIOIMMUNOASSAY

Principle of the assay

The assay was done according to the procedure of BARC radioimmunoassay kit. The radioimmunoassay method is based on the competition of unlabelled insulin in the standard or samples and [^{125}I] insulin for the limited binding sites on a specific antibody. At the end of incubation, the antibody bound and free insulin were separated by the second antibody-polyethylene glycol (PEG) aided separation method. Measuring the radioactivity associated with bound fraction of sample and standards quantitates insulin concentration of samples.

Assay Protocol

Standards, ranging from 0 to 200 μU/ml, insulin free serum and insulin antiserum (50μl each) were added together and the volume was made up to 250μl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. They were incubated overnight at 2°C. Then [^{125}I] insulin (50μl) was added and incubated at room temperature for 3 hours. The second antibody was added (50μl) along with 500μl of PEG. The tubes were then vortexed and incubated for 20 minutes and they were centrifuged at 1500 x g for 20 minutes. The supernatant was aspirated out and the radioactivity in the pellet was determined in a gamma counter.

A standard curve was plotted with %B/Bo on the Y-axis and insulin concentration/ml on the X-axis of a log-logit graph. %B/Bo was calculated as:

$$\frac{\text{Corrected average count of standard or sample}}{\text{Corrected average count of zero standard}} \times 100$$

Insulin concentration in the samples was determined from the standard curve plotted using MultiCalc™ software (Wallac, Finland).

BEHAVIOURAL STUDIES

Y-Maze Test

The Y-maze was made of grey wood, covered with black paper and consisted of three arms with an angle of 120 degrees between each of the arms. Each arm was 8 cm width ×30 cm length ×15 cm height. The three identical arms were randomly designated: Start arm, in which the rat started to explore (always open); Novel arm, which was blocked at the 1st trial, but open at the 2nd trial; and the other arm (always open). The maze was placed in a separate room with enough light. The floor of the maze was covered with saw dust, which was mixed after each individual trial in order to eliminate olfactory stimuli. Visual cues were placed on the walls of the maze.

The Y-maze test consisted of two trials separated by an inter-trial interval (ITI). The first trial (training) was of 10 minutes duration and allowed the rat to explore only two arms (start arm and the other arm) of the maze, with the third arm (novel arm) blocked. After a 1 hour ITI (Ma *et al*., 2007), the second trial (retention) was conducted, during which all three arms were accessible and novelty *vs* familiarity was analyzed through comparing behaviour in all three arms. For the second trial, the rat was placed back in the maze in the same starting arm, with free access to all three arms for 5 minutes. The time spent in each arm

was analyzed. Data was expressed as percentage of performance in all three arms during the five minutes of test (Akwa *et al*., 2001, Jobin, *et al*., 2010).

Grid Walk Test

Deficits in descending motor control were examined by assessing the ability to navigate across a 1 m long runway with irregularly assigned gaps (0.5–5 cm) between round metal bars. Crossing this runway requires that animals accurately place their limbs on the bars. In baseline training and postoperative testing, every animal had to cross the grid for at least three times. The number of footfalls (errors) was counted in each crossing for 3 minute and a mean error rate was calculated (Z'Graggen *et al.*, 1998).

MUSCARINIC AND GABA RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS

Binding studies in the Brain regions

Total muscarinic, muscarinic M1 and M3 receptor binding studies

[^{3}H] QNB and [^{3}H] DAMP binding assay in cerebral cortex, cerebellum, brain stem, hippocampus, corpus striatum and pancreas were done according to the modified procedure of Yamamura & Snyder (1981). Brain tissues were homogenised in a polytron homogeniser with 20 volumes of cold 50mM Tris-HCl buffer, p^H 7.4, containing 1mM EDTA. The supernatant was then centrifuged at 30,000xg for 30 minutes and the pellets were resuspended in appropriate volume of the buffer.

Total muscarinic and muscarinic M1 receptor binding parameter assays were done using 0.1-2.5nM of [^{3}H] QNB and M3 receptor using 0.01-5nM of [^{3}H] DAMP in the incubation buffer, p^H 7.4 in a total incubation volume of 250µl. The non-specific binding was determined using 100µM atropine for total muscarinic, pirenzepine for muscarinic M1 and 4-DAMP mustard for muscarinic

M3 receptor. Total incubation volume of 250 µl contains 200-250µg protein concentrations. Tubes were incubated at 22°C for 60 minutes and filtered rapidly through GF/C filters (Whatman). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris-HCl buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter. The non-specific binding determined showed 10% in all our experiments.

Total GABA receptor binding studies.

[^{3}H] GABA binding to GABA receptor was assayed in Triton X-100 treated synaptic membranes according to the modified procedure of Kurioka *et al* (1981). Crude synaptic membranes were prepared using sodium free 10mM Tris buffer p^H 7.4. Each assay tube contained a protein concentration of 100µg. In saturation binding experiments, 5-40 nM of [^{3}H] GABA was incubated with and without excess of unlabelled 100µM GABA for 20 min at 5°C and terminated by centrifugation at 35000xg for 20 min. [^{3}H] GABA in the pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting non-specific binding from the total.

Protein determination

Protein was measured by the method of Lowry *et al* (1951) using bovine serum albumin as standard. The intensity of the purple blue colour formed was proportional to the amount of protein which was read in a spectrophotometer at 660nm.

ANALYSIS OF THE RECEPTOR BINDING DATA

Linear regression analysis for Scatchard plots

The data were analysed according to Scatchard (1949). The specific binding was determined by subtracting non-specific binding from the total. The binding parameters, maximal binding (B_{max}) and equilibrium dissociation constant (K_d), were derived by linear regression analysis by plotting the specific binding of the radioligand on X-axis and bound/free on Y-axis. The maximal binding is a measure of the total number of receptors present in the tissue and the equilibrium dissociation constant is the measure of the affinity of the receptors for the radioligand. The K_d is inversely related to receptor affinity.

GENE EXPRESSION STUDIES IN BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL RATS

Isolation of RNA

RNA was isolated from the brain regions and pancreas of control and experimental rats using the Tri reagent from Sigma Chemicals Co., St. Louis, U.S.A. 25-50 mg tissue homogenates were made in 0.5 ml Tri Reagent. The homogenate was kept in room temperature for 5 minutes. 100 µl of chloroform was added to the homogenate, mixed vigorously for 15 seconds kept in the RT for 15 minutes and was centrifuged at 12,000xg for 15 minutes at 4°C. The upper aqueous phase was transferred to a fresh tube and 250µl of isopropanol was added and the tubes were allowed to stand at room temperature for 10 minutes. They were centrifuged at 12,000xg for 10 minutes at 4°C. RNA precipitated as a pellet on the sides and bottom of the tube. RNA pellet was washed with 500µl of 75% ethanol, vortexed and centrifuged at 12,000xg for 5 minutes at 4°C. The pellets were semi dried and dissolved in minimum volume of DEPC-treated water. 2 µl of RNA was made up to 1 ml and absorbance was measured at 260nm and 280nm.

For pure RNA preparation the ratio of absorbance at 260/280 was ≥ 1.7. The concentration of RNA was calculated as one absorbance $_{260}$ = 42μg.

REAL-TIME POLYMERASE CHAIN REACTION

cDNA synthesis

Total cDNA synthesis was performed using ABI PRISM cDNA arhive kit in 0.2ml microfuge tubes. The reaction mixture of 20 μl contained 0.2μg total RNA, 10 X RT buffer, 25 X dNTP mixture, 10 X random primers, MultiScribe RT (50U/μl) and RNase free water. The cDNA synthesis reactions were carried out at 25 °C for 10 minutes and 37 °C for 2 hours using an Eppendorf Personal Cycler. The primers and probes were purchased from Applied Biosystems, Foster City, CA, USA designed using Primer Express software version (3.0).

Real-time PCR assays

Real Time PCR assays were performed in 96-well plates in an ABI 7300 Real Time PCR instrument (Applied Biosystems). To detect gene expression, a TaqMan quantitative 2-step reverse transcriptase "polymerase chain reaction (RT-PCR) was used to quantify mRNA levels. First-strand cDNA was synthesized with use of a TaqMan RT reagent kit, as recommended by the manufacturer. PCR analyses were conducted with gene-specific primers and fluorescently labeled TaqMan probe, designed by Applied Biosystems. All reagents were purchased from Applied Biosystems. The probes for specific gene of interest were labeled with FAM at the 5' end and a quencher (Minor Groove Binding Protein - MGB) at the 3' end. The Real-Time data were analyzed with Sequence Detection Systems software version 1.7. All reactions were performed in duplicate.

The TaqMan reaction mixture of 20 μl contained 25 ng of total RNA-derived cDNAs, 200 nM each of the forward primer, reverse primer and PCR analyses were conducted with gene-specific primers and fluorescently labelled

Taqman probes of muscarinic M1, M3, α7 nicotinic acetylcholine receptor, GAD, $GABA_{A\alpha1}$, $GABA_B$, insulin receptor, AChE, ChAT, GLUT3, superoxide dismutase, Bax, phospholipase C and CREB. Endogenous control, β-actin was labeled with a reporter dye, VIC. 12.5 µl of TaqMan 2X Universal PCR Master Mix was taken and the volume was made up with RNAse free water. Each run contained both negative (no template) and positive controls.

The thermocycling profile conditions were as follows:

50ºC -- 2 minutes ---- Activation

95ºC -- 10 minutes ---- Initial Denaturation

95ºC -- 15 seconds ---- Denaturation 40 cycles

50ºC -- 30 seconds --- Annealing

60ºC -- 1 minutes --- Final Extension

Fluorescence signals measured during amplification were considered positive if the fluorescence intensity was 20-fold greater than the standard deviation of the baseline fluorescence. The ΔΔCT method of relative quantification was used to determine the fold change in expression. This was done by first normalizing the resulting threshold cycle (CT) values of the target mRNAs to the CT values of the internal control β- actin in the same samples ($\Delta CT = CT_{Target} - CT_{\beta\text{- actin}}$). It was further normalized with the control ($\Delta\Delta CT = \Delta CT - CT_{Control}$). The fold change in expression was then obtained ($2^{-\Delta\Delta}C$ T).

IMMUNOHISTOCHEMISTRY OF MUSCARINIC M1, M3, α7 NICOTINIC ACETYLCHOLINE AND $GABA_{A\alpha1}$ RECEPTORS IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS USING CONFOCAL MICROSCOPE

Control and experimental rats were anesthetized with ether. The rats were transcardially perfused with PBS, p^H, 7.4, followed by 4% paraformaldehyde in PBS (Chen *et al*., 2007). After perfusion the brains were dissected and immersion fixed in 4% paraformaldehyde for 1 hr and then equilibrated with 30% sucrose solution in 0.1 M PBS, pH- 7.0. 10 µm sections were cut using Cryostat (Leica, CM1510 S). The sections were treated with PBST (PBS in 0.01% Triton X-100) for 20 min. Brain slices were incubated overnight at 4°C with either rat primary antibody for muscarinic M1, M3, α7 nicotinic acetylcholine receptor and $GABA_{A\alpha1}$ receptor, diluted in PBST at 1: 500 dilution (polyclonal or monoclonal). After overnight incubation, the brain slices were rinsed with PBST and then incubated with appropriate secondary antibody of FITC. The sections were observed and photographed using confocal imaging system (Leica SP 5). The specificity of the immunohistochemical procedure is validated by negative controls to ensure that the labelling method accurately, identified the antibody bound to the specific muscarinic M1, M3 and α7 nicotinic acetylcholine receptor in the brain regions. Expressions of muscarinic M1, M3 and α7 nicotinic acetylcholine receptor were analysed using pixel intensity method. The given mean pixel value is the net value which is deducted from the negative control pixel value (Peeyush *et al.,* 2010).

IMMUNOCYTOCHEMISTRY OF MUSCARINIC M1, M3 AND $GABA_{A\alpha1}$ RECEPTOR EXPRESSION IN THE PANCREAS OF CONTROL AND EXPERIMENTAL RATS USING CONFOCAL MICROSCOPE

Pancreatic islets were isolated from control and experimental rats by standard collagenase digestion procedures using aseptic techniques (Howell &

Taylor, 1968). The islets were isolated in HEPES-buffered sodium free Hanks Balanced Salt Solution (HBSS) (Pipeleers *et al.,* 1985) with the following composition: 137mM Choline chloride, 5.4mM KCl, 1.8mM $CaCl_2$, 0.8mM $MgSO_4$, 1mM KH_2PO_4, 14.3mM $KHCO_3$ and 10mM HEPES**.** The pancreas was aseptically transferred to a sterile glass vial containing 2.0ml collagenase type XI solution (1.5 mg/ml in HBSS), pH 7.4. The collagenase digestion was carried out for 15 minutes at 37°C in an environmental shaker with vigorous shaking (300rpm/minute). The tissue digest was filtered through 500 μm nylon screen and the filtrate was washed with three successive centrifugations and resuspensions in cold HBSS. The pancreatic islet preparation having a viability of >90% was assessed by Trypan Blue. The islets were seeded in culture wells and allowed to adhere to the plate. The islets were rinsed with PBS and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH- 7.0., for 30 minutes on ice. After fixation, the islets were washed thrice with blocking buffer containing 0.1 M phosphate buffer, pH- 7.0., 0.1% Triton X-100 and 10% BSA. Then the islets were incubated with primary antibody for muscarinic M1, muscarinic M3 and GABA receptors, diluted in PBST at 1: 1000 dilution), prepared in blocking buffer with 1% serum and incubated overnight at 4°C. After the incubation, the islets were washed thrice with blocking buffer. Then the islets were incubated with secondary antibody tagged with FITC (No: AB7130F, Chemicon, diluted in PBST at 1: 1000 dilution) diluted in blocking buffer with 1% serum and incubated at room temperature in dark for two hours. After incubation the islets were rinsed with blocking buffer and were observed and photographed using confocal imaging system (Leica SP 5). The specificity of the immunocytochemical procedure was validated by negative controls to ensure that the labelling method accurately identifies the antibody bound to the specific muscarinic M1, muscarinic M3 and GABA receptors in the pancreatic islets. Expressions of muscarinic M1, muscarinic M3 and GABA receptors were analysed using pixel intensity method.

The given mean pixel value is the net value which is deducted from the negative control pixel value.

STATISTICS

Statistical evaluations were done by ANOVA using InStat (Ver.2.04a) computer programme. Linear regression Scatchard plots were made using SIGMA PLOT (Ver 2.03). Sigma Plot software (version 2.0, Jandel GmbH, Erkrath, Germany). Relative Quantification Software was used for analyzing Real-Time PCR results.

Results

BODY WEIGHT AND BLOOD GLUCOSE LEVEL

Streptozotocin induced diabetic rats showed a significant ($p<0.001$) decrease in body weight after 10 days compared to control. Insulin induced hypoglycemia in diabetes (D + IIH) and control (C + IIH) rats showed no significant change in the body weight compared to their respective control (Figure-1; Table-1).

Diabetic rats showed a significant ($p<0.001$) increase in blood glucose compared to control. There was a significant ($p<0.001$) decrease in blood glucose level of D + IIH and C + IIH rats compared to their respective diabetic and control rats (Figure-2; Table-2). Insulin administration to diabetic rats decreased blood glucose level significantly ($p<0.001$) below 50mg/dL after 3 hours and in control rats after 1 hour. The decreased glucose level reversed to diabetic level after 5 hours and control level after 2 hours respectively (Figure-3; Table-3).

CIRCULATING INSULIN LEVEL

Diabetic rats showed a significant ($p<0.001$) decrease in circulating insulin level compared to control. There was a significant ($p<0.001$) increase in circulating insulin level of D + IIH and C + IIH rats compared to their respective diabetic and control rats. C + IIH rats showed a significant ($p<0.01$) increase in the insulin level compared to D + IIH (Figure-4; Table-4).

BEHAVIOURAL STUDIES

Behavioural response of control and experimental rats on Y maze test

Y maze test showed that the number of visits and time spent in the novel arm decreased significantly ($p<0.001$) in the diabetic group compared to control. D + IIH and C + IIH rats showed a significant ($p<0.01$) decrease in number of visits to the novel arm compared to diabetic rats (Figure-5; Table-5).

Behavioural response of control and experimental rats on grid walk test

There was significant increase ($p<0.001$) in the number of foot falls in diabetic rats compared to control. D + IIH and C + IIH rats showed a significant ($p<0.001$) increase in number of foot falls compared to diabetic rats (Figure-6; Table-6).

MUSCARINIC, NICOTINIC AND GABA RECEPTORS, GLUT3, INSULIN RECEPTOR, SOD, BAX, PHOSPHOLIPASE C AND CREB EXPRESSION IN THE BRAIN REGIONS AND PANCREAS OF EXPERIMENTAL RATS

CEREBRAL CORTEX

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine, in the cerebral cortex of control and experimental rats

The total muscarinic receptor assay using the specific ligand, [^{3}H] QNB and muscarinic general antagonist atropine showed that B_{max} ($p<0.001$) and K_d ($p<0.01$) decreased significantly in diabetic, D + IIH and C + IIH rats compared to control. D +

IIH and C + IIH rats showed a significant ($p<0.001$) decrease in B_{max} compared to diabetic group. C + IIH rats showed a significant ($p<0.001$) decrease in B_{max} compared to D + IIH rats (Figure-7; Table-7).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine, in the cerebral cortex of control and experimental rats.

Binding analysis of muscarinic M1 receptor using [^{3}H] QNB and M1 subtype specific antagonist pirenzepine, showed that the B_{max} ($p<0.001$) and K_d ($p<0.01$) decreased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant decrease in B_{max} ($p<0.001$) and K_d ($p<0.01$) compared to diabetic group. C + IIH rats showed a significant ($p<0.001$) decrease in B_{max} compared to D + IIH (Figure-8; Table-8).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor, antagonist, 4-DAMP mustard in the cerebral cortex of control and experimental rats.

Binding analysis of muscarinic M3 receptors using [^{3}H] DAMP and M3 subtype specific antagonist, 4-DAMP mustard showed a significant increase in B_{max} ($p<0.001$) and decrease in K_d ($p<0.01$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant increase in B_{max} ($p<0.001$) and decrease in K_d ($p<0.01$) compared to diabetic group. C + IIH rats showed a significant ($p<0.001$) increase in B_{max} and decrease in K_d ($p<0.01$) compared to D + IIH (Figure-9; Table-9).

GABA receptor analysis

Scatchard analysis of [^{3}H] GABA binding against GABA in the cerebral cortex of control and experimental rats.

Binding analysis of GABA receptors were done using [^{3}H] GABA and unlabelled GABA. The B_{max} decreased significantly ($p<0.01$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant decrease in B_{max} ($p<0.01$) compared to diabetic group. C + IIH rats showed a significant ($p<0.01$) decrease in B_{max} compared to D + IIH rats. K_d did not show any significant change in all experimental rats (Figure-10; Table-10).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of AChE

Gene expression of AChE mRNA showed significant up regulation ($p<0.001$) in the cerebral cortex of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant increase ($p<0.001$) compared to diabetic rats. A significant ($p<0.001$) increased expression of acetylcholine esterase mRNA in C + IIH rats was observed compared to the D + IIH rats (Figure-11; Table-11).

Real Time-PCR analysis of ChAT

Gene expression of cholineacetyl transferase (ChAT) mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats (Figure-12; Table-12).

Real Time-PCR analysis of muscarinic M1 receptor

Gene expression of muscarinic M1 receptor mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats. C + IIH group showed a significant decrease ($p<0.001$) compared to D + IIH (Figure-13; Table-13).

Real Time-PCR analysis of muscarinic M3 receptor

Muscarinic M3 receptor mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant increased ($p<0.001$) expression compared to diabetic rats. In C + IIH group, muscarinic M3 receptor mRNA significantly ($p<0.001$) up regulated compared to D + IIH (Figure-14; Table-14).

Real Time-PCR analysis of α7 nAChR

α7 nAChR mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. In C + IIH group, α7 nAChR mRNA significantly ($p<0.001$) up regulated compared to D + IIH (Figure-15; Table-15).

Real Time PCR analysis of Glutamate Decarboxylase (GAD)

Gene expression of GAD mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats (Figure-16; Table-16).

Real Time PCR analysis of $GABA_{A\alpha1}$ receptor

Gene expression of $GABA_{A\alpha1}$ receptor mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats. C + IIH group showed a significant decrease ($p<0.001$) compared to D + IIH. (Figure-17; Table-17).

Real Time PCR analysis of $GABA_B$ receptor

Gene expression of $GABA_B$ receptor mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.01$) compared to diabetic rats (Figure-18; Table-18).

Real Time PCR analysis of GLUT3

GLUT3 mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. In C + IIH group, GLUT3 mRNA significantly ($p<0.001$) up regulated compared to D + IIH. (Figure-19; Table-19).

Real Time PCR analysis of insulin receptor

Insulin receptor mRNA expression showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. In C + IIH group, insulin receptor mRNA significantly ($p<0.01$) up regulated compared to D + IIH (Figure-20; Table-20).

Real Time PCR analysis of SOD

Gene expression of SOD mRNA showed significant down regulation ($p<0.001$) in the cerebral cortex of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.01$) compared to diabetic rats (Figure-21; Table-21).

Real Time PCR analysis of Bax

Bax mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. In C + IIH group, bax mRNA significantly ($p<0.01$) up regulated compared to D + IIH (Figure-22; Table-22).

Real Time PCR analysis of phospholipase C

Gene expression phospholipase C mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant downregulated ($p<0.001$) expression compared to diabetic rats. In C + IIH group, phospholipase C mRNA significantly ($p<0.01$) downregulated compared to D + IIH (Figure-23; Table-23).

Real Time PCR analysis of CREB

Gene expression CREB mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control (Figure-24; Table-24).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the cerebral cortex of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the cerebral cortex showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. D + IIH and C + IIH group showed a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.01$) decrease in mean pixel value compared to D + IIH rats (Table-25, Figure-25).

Muscarinic M3 receptor antibody staining in the cerebral cortex of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the cerebral cortex showed a significant increase ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. In D + IIH and C + IIH, there was a significantly increased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.01$) increase in mean pixel value compared to D + IIH rats (Table-26, Figure-26).

α7 nACh receptor antibody staining in the cerebral cortex of control and experimental rats

α7 nACh subunit antibody staining in the cerebral cortex showed a significant increase ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. In D + IIH and C + IIH, there was a significantly increased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.01$) increase in mean pixel value compared to D + IIH rats (Table-27, Figure-27).

$GABA_{A\alpha1}$ receptor antibody staining in the cerebral cortex of control and experimental rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the cerebral cortex showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. D + IIH and C + IIH group showed a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.01$) decrease in mean pixel value compared to D + IIH rats (Table-28, Figure-28).

CEREBELLUM

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine, in the cerebellum of control and experimental rats

The total muscarinic receptor binding assay using the specific ligand, [^{3}H] QNB and muscarinic general antagonist, atropine, showed that B_{max} ($p<0.001$) increased significantly in diabetic, D + IIH and C + IIH rats compared to control rats. D + IIH and C + IIH rats showed a significant ($p<0.01$) increase in B_{max} compared to diabetic group. K_d value increased significantly ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control rats. D + IIH and C + IIH rats showed a significant ($p<0.01$) increase in K_d compared to diabetic group (Figure-29; Table-29).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine, in the cerebellum of control and experimental rats.

Muscarinic M1 receptor binding assay using the specific ligand, [^{3}H] QNB and muscarinic M1 subtype specific antagonist, pirenzepine, showed that B_{max} ($p<0.001$) increased significantly in diabetic, D + IIH and C + IIH rats compared to control rats. K_d value of diabetic rats did not show any significant change compared to control. D + IIH and C + IIH rats showed a significant ($p<0.001$) increase in B_{max} and K_d compared to diabetic group. C + IIH rats showed a significant increase in B_{max} ($p<0.01$) compared to D + IIH rats (Figure-30; Table-30).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor, antagonist, 4-DAMP mustard in the cerebellum of control and experimental rats.

Binding analysis of muscarinic M3 receptors using [^{3}H] DAMP and M3 subtype specific antagonist, 4-DAMP mustard showed a significant increase in B_{max} ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant increase in B_{max} ($p<0.001$) compared to diabetic group. K_d value did not show any significant change in experimental rats compared to control (Figure-31; Table-31).

GABA receptor analysis

Scatchard analysis of [^{3}H] GABA binding against GABA in the cerebellum of control and experimental rats.

Binding analysis of GABA receptors were done using [^{3}H] GABA and unlabelled GABA showed that B_{max} ($p<0.001$) and K_d ($p<0.01$) decreased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant decrease in B_{max} ($p<0.001$) and K_d ($p<0.01$)

compared to diabetic group. C + IIH rats showed a significant decrease in B_{max} ($p<0.01$) and K_d ($p<0.01$) compared to D + IIH rats (Figure-32; Table-32).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of AChE

Gene expression of AChE mRNA showed significant up regulation ($p<0.001$) in the cerebellum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant increase ($p<0.001$) compared to diabetic rats. A significant ($p<0.001$) increased expression of AChE mRNA in C + IIH rats was observed compared to the D + IIH (Figure-33; Table-33).

Real Time-PCR analysis of ChAT

Gene expression of ChAT mRNA showed significant down regulation ($p<0.001$) in the cerebellum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats (Figure-34; Table-34).

Real Time-PCR analysis of muscarinic M1 receptor

Gene expression of muscarinic M1 receptor mRNA showed significant up regulation ($p<0.001$) in the cerebellum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant increased expression ($p<0.001$) compared to diabetic rats (Figure-35; Table-35).

Real Time-PCR analysis of muscarinic M3 receptor

Muscarinic M3 receptor mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group

showed a significant ($p<0.01$) up regulation compared to diabetic rats (Figure-36; Table-36).

Real Time-PCR analysis of α7 nAChR

α7 nAChR mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.01$) expression compared to diabetic rats. In C + IIH group, α7 nAChR mRNA significantly ($p<0.01$) up regulated compared to D + IIH (Figure-37; Table-37).

Real Time PCR analysis of GAD

Gene expression of GAD mRNA showed significant down regulation ($p<0.001$) in the cerebellum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats (Figure-38; Table-38).

Real Time PCR analysis of $GABA_{A\alpha1}$ receptor

Gene expression of $GABA_{A\alpha1}$ receptor mRNA showed significant down regulation ($p<0.001$) in the cerebellum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats. C + IIH group showed a significant decrease ($p<0.01$) compared to D + IIH (Figure-39; Table-39).

Real Time PCR analysis of $GABA_B$ receptor

Gene expression of $GABA_B$ receptor mRNA showed significant down regulation ($p<0.001$) in the cerebellum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$)

compared to diabetic rats. C + IIH group showed a significant decrease ($p<0.01$) compared to D + IIH (Figure-40; Table-40).

Real Time PCR analysis of GLUT3

GLUT3 mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats (Figure-41; Table-41).

Real Time PCR analysis of insulin receptor

Insulin receptor mRNA expression showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed significant ($p<0.001$) increased expression compared to diabetic rats. C + IIH group showed significantly ($p<0.001$) decreased expression compared to D + IIH (Figure-42; Table-42).

Real Time PCR analysis of SOD

Gene expression of SOD mRNA showed significant down regulation ($p<0.001$) in the cerebellum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.01$) compared to diabetic rats. C + IIH group showed a significant decrease ($p<0.01$) compared to D + IIH (Figure-43; Table-43).

Real Time PCR analysis of Bax

Bax mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. In C + IIH group, Bax

mRNA significantly ($p<0.01$) up regulated compared to D + IIH (Figure-44; Table-44).

Real Time PCR analysis of phospholipase C

Gene expression phospholipase C mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant dow nregulated ($p<0.001$) expression compared to diabetic rats (Figure-45; Table-45).

Real Time PCR analysis of CREB

Gene expression of CREB mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant down regulated ($p<0.001$) expression compared to diabetic rats. C + IIH group showed significantly ($p<0.01$) down regulated expression compared to D + IIH (Figure-46; Table-46).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the cerebellum of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the cerebellum showed a significant increase ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. D + IIH and C + IIH group showed a significantly increased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.001$) decrease in mean pixel value compared to D + IIH (Table-47, Figure-47).

Muscarinic M3 receptor antibody staining in the cerebellum of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the cerebellum showed a significant increase ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control. In D + IIH and C + IIH, there was a significantly increased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.01$) increase in mean pixel value compared to D + IIH (Table-48, Figure-48).

α7 nACh antibody staining in the cerebellum of control and experimental rats

α7 nACh subunit antibody staining in the cerebellum showed a significant increase ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control. In D + IIH and C + IIH, there was a significantly increased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.01$) increase in mean pixel value compared to D + IIH rats (Table-49, Figure-49).

$GABA_{A\alpha1}$ receptor antibody staining in the cerebellum of control and experimental rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the cerebellum showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) in mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.001$) decrease in mean pixel value compared to D + IIH (Table-50, Figure-50).

BRAIN STEM

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine, in the brain stem of control and experimental rats

The total muscarinic receptor binding assay using the specific ligand, [^{3}H] QNB and muscarinic general antagonist, atropine showed that B_{max} ($p<0.001$) increased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant increase in B_{max} ($p<0.001$) and K_d ($p<0.01$) compared to diabetic group. C + IIH rats showed a significant ($p<0.01$) increase in B_{max} and K_d compared to D + IIH (Figure-51; Table-51).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the brain stem of control and experimental rats.

Binding analysis of muscarinic M1 receptor using [^{3}H] QNB and M1 subtype specific antagonist, pirenzepine showed that the B_{max} ($p<0.001$) decreased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant decrease in B_{max} ($p<0.001$) compared to diabetic group. C + IIH rats showed a significant ($p<0.01$) decrease in B_{max} compared to D + IIH. K_d did not show any significant change in experimental groups compared to control (Figure-52; Table-52).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor, antagonist, 4-DAMP mustard, in the brain stem of control and experimental rats.

Binding analysis of muscarinic M3 receptors using [^{3}H] DAMP and M3 subtype specific antagonist, 4-DAMP mustard, showed that B_{max} ($p<0.001$) increased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant increase in B_{max} ($p<0.001$) and K_d ($p<0.01$) compared to diabetic group. C + IIH rats showed a significant increase in B_{max} ($p<0.01$) and K_d ($p<0.01$) compared to D + IIH (Figure-53; Table-53).

GABA receptor analysis

Scatchard analysis of [^{3}H] GABA binding against GABA in the brain stem of control and experimental rats.

Binding analysis of GABA receptors done using [^{3}H] GABA and unlabelled GABA showed a significant ($p<0.001$) decrease in B_{max} in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant decrease in B_{max} ($p<0.001$) compared to diabetic group. C + IIH rats showed a significant ($p<0.001$) decrease in B_{max} compared to D + IIH rats. There was no significant change in K_d in all experimental rats compared to control (Figure-54; Table-54).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of AChE

Gene expression of AChE mRNA showed significant up regulation ($p<0.001$) in the brain stem of diabetic, D + IIH and C + IIH rats compared to control. D + IIH

and C + IIH group did not show any significant change compared to diabetic rats. (Figure-55; Table-55).

Real Time-PCR analysis of ChAT

Gene expression of ChAT mRNA showed significant up regulation ($p<0.001$) in the brain stem of diabetic rats compared to control. D + IIH and C + IIH group showed a significant down regulation ($p<0.001$) compared to control and diabetic rats. C + IIH group showed a significant down regulation ($p<0.01$) compared to D + IIH (Figure-56; Table-56).

Real Time-PCR analysis of muscarinic M1 receptor

Gene expression of muscarinic M1 receptor mRNA showed significant down regulation ($p<0.001$) in the brain stem of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats. C + IIH group showed a significant ($p<0.001$) downregulated expression compared to D + IIH rats (Figure-57; Table-57).

Real Time-PCR analysis of muscarinic M3 receptor

Muscarinic M3 receptor mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant increased ($p<0.001$) expression compared to diabetic rats. In C + IIH group, muscarinic M3 receptor mRNA significantly ($p<0.01$) up regulated compared to D + IIH (Figure-58; Table-58).

Real Time-PCR analysis of α7 nACh

α7 nAChR mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. In C + IIH

group, α7 nAChR mRNA significantly ($p<0.01$) up regulated compared to D + IIH (Figure-59; Table-59).

Real Time PCR analysis of GAD

Gene expression of GAD mRNA showed significant down regulation ($p<0.001$) in the brain stem of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats. In C + IIH group, GAD mRNA significantly ($p<0.01$) down regulated compared to D + IIH (Figure-60; Table-60).

Real Time PCR analysis of $GABA_{A\alpha1}$ receptor

Gene expression of $GABA_{A\alpha1}$ receptor mRNA showed significant up regulation ($p<0.001$) in the brain stem of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulation ($p<0.001$) compared to diabetic rats (Figure-61; Table-61).

Real Time PCR analysis of $GABA_B$ receptor

Gene expression of $GABA_B$ receptor mRNA showed significant down regulation ($p<0.001$) in the brain stem of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group did not show any significant change compared to diabetic rats (Figure-62; Table-62).

Real Time PCR analysis of GLUT3

GLUT3 mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. In C + IIH

group, GLUT3 mRNA significantly ($p<0.001$) up regulated compared to D + IIH (Figure-63; Table-63).

Real Time PCR analysis of insulin receptor

Insulin receptor mRNA expression in brain stem showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant ($p<0.01$) down regulation compared to diabetic rats. C + IIH group showed a significant ($p<0.01$) down regulation compared to D + IIH rats (Figure-64; Table-64).

Real Time PCR analysis of SOD

Gene expression of SOD mRNA showed significant up regulation ($p<0.001$) in the brain stem of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant down regulation ($p<0.01$) compared to diabetic rats. C + IIH group showed a significant ($p<0.01$) down regulation compared to D + IIH rats (Figure-65; Table-65).

Real Time PCR analysis of Bax

Bax mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH rats showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. C + IIH group showed a significant ($p<0.01$) downregulated bax mRNA expression compared to diabetic and D + IIH rats (Figure-66; Table-66).

Real Time PCR analysis of phospholipase C

Gene expression phospholipase C mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant downregulated ($p<0.001$) expression compared to

diabetic rats. In C + IIH group, phospholipase C mRNA significantly ($p<0.01$) downregulated compared to D + IIH (Figure-67; Table-67).

Real Time PCR analysis of CREB

Gene expression CREB mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant downregulated ($p<0.001$) expression compared to diabetic rats (Figure-68; Table-68).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the brain stem of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the brain stem showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. D + IIH and C + IIH group showed a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats (Table-69, Figure-69).

Muscarinic M3 receptor antibody staining in the brain stem of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the brain stem showed a significant increase ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control. In D + IIH and C + IIH, there was a significantly increased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.001$) increase in mean pixel value compared to D + IIH s (Table-70, Figure-70).

α7 nACh antibody staining in the brain stem of control and experimental rats

α7 nACh receptor subunit antibody staining in the brain stem showed a significant increase ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. In D + IIH and C + IIH, there was a significantly increased ($p<0.001$) mean pixel value compared to diabetic rats (Table-71, Figure-71).

$GABA_{A\alpha1}$ receptor antibody staining in the brain stem of control and experimental rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the brain stem showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.01$) decrease in mean pixel value compared to D + IIH (Table-72, Figure-72).

CORPUS STRIATUM

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the corpus striatum of control and experimental rats

The total muscarinic receptor binding assay using the specific ligand, [^{3}H] QNB and muscarinic general antagonist, atropine showed that B_{max} ($p<0.01$) decreased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant decrease in B_{max} ($p<0.01$) compared to diabetic group. K_d in D + IIH rats showed a significant ($p<0.01$) decrease compared to control and diabetic (Figure-73; Table-73).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the corpus striatum of control and experimental rats.

Binding analysis of muscarinic M1 receptor using [^{3}H] QNB and M1 subtype specific antagonist, pirenzepine showed that the B_{max} ($p<0.001$) increased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant increase in B_{max} ($p<0.001$) compared to diabetic group. C + IIH rats showed a significant ($p<0.01$) decrease in B_{max} compared to D + IIH. K_d did not show any significant change in experimental groups compared to control (Figure-74; Table-74).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor, antagonist, 4-DAMP mustard, in the corpus striatum of control and experimental rats

Binding analysis of muscarinic M3 receptors using [^{3}H] DAMP and M3 subtype specific antagonist, 4-DAMP mustard, showed that B_{max} ($p<0.001$) decreased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant decrease in B_{max} ($p<0.001$) and K_d ($p<0.01$) compared to diabetic group. C + IIH rats showed a significant decrease in B_{max} ($p<0.01$) and increase in K_d ($p<0.01$) compared to D + IIH (Figure-75; Table-75).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of AChE

Gene expression of AChE mRNA showed significant up regulation ($p<0.001$) in the corpus striatum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group did not show any significant change compared to diabetic (Figure-76; Table-76).

Real Time-PCR analysis of ChAT

Gene expression of ChAT mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group did not show any significant change compared to diabetic (Figure-77; Table-77).

Real Time-PCR analysis of muscarinic M1 receptor

Gene expression of muscarinic M1 receptor mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.01$) compared to diabetic rats. C + IIH group showed a significant ($p<0.01$) down regulated expression compared to D + IIH rats (Figure-78; Table-78).

Real Time-PCR analysis of muscarinic M3 receptor

Muscarinic M3 receptor mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decreased ($p<0.001$) expression compared to diabetic rats. In C + IIH group, muscarinic M3 receptor mRNA significant ($p<0.01$) increased compared to D + IIH (Figure-79; Table-79).

Real Time-PCR analysis of α7 nAChR

α7 nAChR mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats (Figure-80; Table-80).

Real Time PCR analysis of GAD

Gene expression of GAD mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group did not show any significant change compared to diabetic rats (Figure-81; Table-81).

Real Time PCR analysis of $GABA_{A\alpha1}$ receptor

Gene expression of $GABA_{A\alpha1}$ receptor mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant down regulation ($p<0.001$) compared to diabetic rats. C + IIH group showed a significant ($p<0.001$) increased expression compared to D + IIH (Figure-82; Table-82).

Real Time PCR analysis of $GABA_B$ receptor

Gene expression of $GABA_B$ receptor mRNA showed significant down regulation ($p<0.001$) in the corpus striatum of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed significant down regulation ($p<0.01$) compared to diabetic rats. C + IIH group showed a significant ($p<0.001$) decreased expression compared to D + IIH (Figure-83; Table-83).

Real Time PCR analysis of GLUT3

GLUT3 mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. In C + IIH group, GLUT3 mRNA significantly ($p<0.01$) up regulated compared to D + IIH (Figure-84; Table-84).

Real Time PCR analysis of insulin receptor

Insulin receptor mRNA expression in corpus striatum showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant ($p<0.01$) down regulation compared to diabetic rats. C + IIH group showed a significant ($p<0.001$) decreased expression compared to D + IIH (Figure-85; Table-85).

Real Time PCR analysis of SOD

Gene expression of SOD mRNA showed significant up regulation ($p<0.001$) in the corpus striatum of diabetic compared to control. D + IIH and C + IIH group showed a significant down regulation ($p<0.001$) compared to diabetic rats. C + IIH group showed a significant ($p<0.01$) down regulation compared to D + IIH rats (Figure-86; Table-86).

Real Time PCR analysis of Bax

Bax mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH rats showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. C + IIH group showed a significant ($p<0.01$) up regulated Bax mRNA expression compared to D + IIH rats (Figure-87; Table-87).

Real Time PCR analysis of phospholipase C

Gene expression phospholipase C mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group did not show any significant change compared to diabetic rats (Figure-88; Table-88).

Real Time PCR analysis of CREB

Gene expression of CREB mRNA showed significant up regulation ($p<0.001$) in the corpus striatum of diabetic compared to control. D + IIH and C + IIH group showed a significant down regulation ($p<0.001$) compared to diabetic rats. C + IIH group showed a significant ($p<0.001$) down regulation compared to D + IIH rats (Figure-89; Table-89).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the corpus striatum of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the corpus striatum showed a significant increase ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significantly increased ($p<0.001$) mean pixel value compared to diabetic rats (Table-90, Figure-90).

Muscarinic M3 receptor antibody staining in the corpus striatum of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the corpus striatum showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH

and C + IIH rats compared to control. In D + IIH and C + IIH, there was a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.001$) decrease in mean pixel value compared to D + IIH (Table-91, Figure-91).

α7 nACh receptor antibody staining in the corpus striatum of control and experimental rats

α7 nACh receptor subunit antibody staining in the corpus striatum showed a significant increase ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control. In D + IIH and C + IIH, there was a significantly increased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.001$) increase in mean pixel value compared to D + IIH (Table-92, Figure-92).

$GABA_{A\alpha1}$ receptor antibody staining in the corpus striatum of control and experimental rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the corpus striatum showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.01$) decrease in mean pixel value compared to D + IIH (Table-93, Figure-93).

HIPPOCAMPUS

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine, in the hippocampus of control and experimental rats

The total muscarinic receptor assay using the specific ligand, [^{3}H] QNB and muscarinic general antagonist, atropine, showed that B_{max} ($p<0.001$) decreased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant ($p<0.001$) decrease in B_{max} compared to diabetic group. K_d of C + IIH rats showed a significant decrease in ($p<0.001$) compared to control, ($p<0.01$) when compared to diabetic and D + IIH (Figure-94; Table-94).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine, in the hippocampus of control and experimental rats.

Binding analysis of muscarinic M1 receptor using [^{3}H] QNB and M1 subtype specific antagonist, pirenzepine, showed that the B_{max} ($p<0.001$) and K_d ($p<0.001$) decreased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant decrease in B_{max} ($p<0.001$) and K_d ($p<0.01$) when compared to diabetic group. C + IIH rats showed a significant ($p<0.01$) decrease in B_{max} compared to D + IIH (Figure-95; Table-95).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor, antagonist, 4-DAMP mustard, in the hippocampus of control and experimental rats.

Binding analysis of muscarinic M3 receptors using [^{3}H] DAMP and M3 subtype specific antagonist, 4-DAMP mustard, showed a significant increase in B_{max} ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant increase in B_{max} ($p<0.001$) compared to diabetic group. C + IIH rats showed a significant ($p<0.001$) increase in B_{max} compared to D + IIH. K_d did not show any significant change in experimental groups compared to control (Figure-96; Table-96).

GABA receptor analysis

Scatchard analysis of [^{3}H] GABA binding against GABA in the hippocampus of control and experimental rats.

Binding analysis of GABA receptors were done using [^{3}H] GABA and unlabelled GABA showed that B_{max} ($p<0.001$) and K_d ($p<0.01$) significantly decreased in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH showed a significant ($p<0.01$) decrease in B_{max} and K_d compared to diabetic group. C + IIH rats showed a significant ($p<0.01$) decrease in B_{max} compared to D + IIH (Figure-97; Table-97).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of AChE

Gene expression of AChE mRNA showed significant up regulation ($p<0.001$) in the hippocampus of diabetic, D + IIH and C + IIH rats compared to control. D +

IIH and C + IIH group showed a significant increase ($p<0.001$) compared to diabetic rats. A significant ($p<0.001$) increased expression of AChE mRNA in C + IIH rats was observed compared to the D + IIH (Figure-98; Table-98).

Real Time-PCR analysis of ChAT

Gene expression of ChAT mRNA showed significant down regulation ($p<0.001$) in the hippocampus of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic. C + IIH group showed a significant decrease ($p<0.01$) compared to D + IIH (Figure-99; Table-99).

Real Time-PCR analysis of muscarinic M1 receptor

Gene expression of muscarinic M1 receptor mRNA showed significant down regulation ($p<0.001$) in the hippocampus of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats (Figure-100; Table-100).

Real Time-PCR analysis of muscarinic M3 receptor

Muscarinic M3 receptor mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant increased ($p<0.001$) expression compared to diabetic rats. In C + IIH group, muscarinic M3 receptor mRNA significantly ($p<0.001$) up regulated compared to D + IIH (Figure-101; Table-101).

Real Time-PCR analysis of α7 nAChR

α7 nAChR mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a

significant down regulated ($p<0.001$) expression compared to diabetic (Figure-102; Table-102).

Real Time PCR analysis of GAD

Gene expression of GAD mRNA showed significant down regulation ($p<0.001$) in the hippocampus of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decreased ($p<0.001$) expression compared to diabetic (Figure-103; Table-103).

Real Time PCR analysis of $GABA_{A\alpha1}$ receptor

Gene expression of $GABA_{A\alpha1}$ receptor mRNA showed significant down regulation ($p<0.001$) in the hippocampus of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats. C + IIH group showed a significant ($p<0.001$) down regulation compared to D + IIH (Figure-104; Table-104).

Real Time PCR analysis of $GABA_B$ receptor

Gene expression of $GABA_B$ receptor mRNA showed significant down regulation ($p<0.001$) in the hippocampus of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.01$) compared to diabetic rats. C + IIH group showed a significant decrease ($p<0.001$) compared to D + IIH (Figure-105; Table-105).

Real Time PCR analysis of GLUT3

GLUT3 mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. In C + IIH

group, GLUT3 mRNA significantly ($p<0.01$) up regulated compared to D + IIH (Figure-106; Table-106).

Real Time PCR analysis of insulin receptor

Insulin receptor mRNA expression showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. In C + IIH group, insulin receptor mRNA significantly ($p<0.01$) decreased compared to D + IIH (Figure-107; Table-107).

Real Time PCR analysis of SOD

Gene expression of SOD mRNA showed significant down regulation ($p<0.001$) in the hippocampus of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats (Figure-108; Table-108).

Real Time PCR analysis of Bax

Bax mRNA expression showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant up regulated ($p<0.001$) expression compared to diabetic rats (Figure-109; Table-109).

Real Time PCR analysis of phospholipase C

Gene expression of phospholipase C mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant downregulated ($p<0.001$) expression

compared to diabetic rats. In C + IIH group, phospholipase C mRNA significantly ($p<0.001$) down regulated compared to D + IIH (Figure-110; Table-110).

Real Time PCR analysis of CREB

Gene expression of CREB mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant down regulated ($p<0.001$) expression compared to diabetic rats (Figure-111; Table-111).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the hippocampus of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the hippocampus showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.001$) decrease in mean pixel value compared to D + IIH (Table-112, Figure-112).

Muscarinic M3 receptor antibody staining in the hippocampus of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the hippocampus showed a significant increase ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control. In D + IIH and C + IIH, there was a significantly increased ($p<0.001$) mean pixel value compared to diabetic rats (Table-113, Figure-113).

α7 nACh receptor antibody staining in the hippocampus of control and experimental rats

α7 nACh receptor subunit antibody staining in the hippocampus showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. In D + IIH and C + IIH, there was a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.01$) decrease in mean pixel value compared to D + IIH (Table-114, Figure-114).

$GABA_{A\alpha1}$ receptor antibody staining in the hippocampus of control and experimental rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the hippocampus showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats (Table-115, Figure-115).

PANCREAS

Total muscarinic receptor analysis

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine, in the pancreas of control and experimental rats

The total muscarinic receptor binding assay using the specific ligand, [^{3}H] QNB and muscarinic general antagonist, atropine, showed that B_{max} ($p<0.01$) and K_d ($p<0.01$) decreased significantly in diabetic, D + IIH and C + IIH rats compared to

control. D + IIH and C + IIH rats showed a significant decrease in B_{max} ($p<0.01$) and K_d ($p<0.01$) compared to diabetic group. C + IIH rats showed a significant ($p<0.01$) decrease in B_{max} compared to D + IIH (Figure-116; Table-116).

Muscarinic M1 receptor analysis

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine, in the pancreas of control and experimental rats.

Binding analysis of muscarinic M1 receptor using [^{3}H] QNB and M1 subtype specific antagonist, pirenzepine, showed that the B_{max} ($p<0.01$) decreased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant decrease in B_{max} ($p<0.01$) compared to diabetic group. C + IIH rats showed a significant ($p<0.01$) decrease in B_{max} ($p<0.01$) and increase in K_d ($p<0.01$) compared to D + IIH (Figure-117; Table-117).

Muscarinic M3 receptor analysis

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor, antagonist, 4-DAMP mustard, in the pancreas of control and experimental rats.

Binding analysis of muscarinic M3 receptors using [^{3}H] DAMP and M3 subtype specific antagonist, 4-DAMP mustard, showed that B_{max} ($p<0.01$) decreased significantly in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant decrease in B_{max} ($p<0.01$) compared to diabetic group. C + IIH rats showed a significant decrease in B_{max} ($p<0.01$) compared to D + IIH. K_d did not show any significant change in experimental groups compared to control (Figure-118; Table-118).

GABA receptor analysis

Scatchard analysis of [^{3}H] GABA binding against GABA in the pancreas of control and experimental rats.

Binding analysis of GABA receptors were done using [^{3}H] GABA and unlabelled GABA showed that B_{max} ($p<0.001$) significantly decreased in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH rats showed a significant ($p<0.01$) decrease in B_{max} compared to diabetic group. C + IIH rats showed a significant ($p<0.01$) decrease in B_{max} compared to D + IIH and K_d significantly increased ($p<0.01$) compared to control and diabetic (Figure-119; Table-119).

REAL TIME-PCR ANALYSIS

Real Time-PCR analysis of AChE

Gene expression of AChE mRNA showed significant up regulation ($p<0.001$) in the pancreas of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.001$) compared to diabetic rats (Figure-120; Table-120).

Real Time-PCR analysis of ChAT

Gene expression of ChAT mRNA showed significant down regulation ($p<0.001$) in the pancreas of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group did not show any significant change compared to diabetic (Figure-121; Table-121).

Real Time-PCR analysis of muscarinic M1 receptor

Gene expression of muscarinic M1 receptor mRNA showed significant down regulation ($p<0.001$) in the pancreas of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decrease ($p<0.01$) compared to diabetic rats (Figure-122; Table-122).

Real Time-PCR analysis of muscarinic M3 receptor

Muscarinic M3 receptor mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decreased ($p<0.001$) expression compared to diabetic rats (Figure-123; Table-123).

Real Time PCR analysis of GAD

Gene expression of GAD mRNA showed significant down regulation ($p<0.001$) in the pancreas of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant decreased ($p<0.001$) expression compared to diabetic rats (Figure-124; Table-124).

Real Time PCR analysis of $GABA_{A\alpha1}$ receptor

Gene expression of $GABA_{A\alpha1}$ receptor mRNA showed significant down regulation ($p<0.001$) in the pancreas of diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant down regulation ($p<0.001$) compared to diabetic rats (Figure-125; Table-125).

Real Time PCR analysis of $GABA_B$ receptor

Gene expression of $GABA_B$ receptor mRNA showed significant down regulation ($p<0.001$) in the pancreas of diabetic, D + IIH and C + IIH rats compared to

control. D + IIH and C + IIH group showed significant down regulation ($p<0.01$) compared to diabetic rats (Figure-126; Table-126).

Real Time PCR analysis of insulin receptor

Insulin receptor mRNA expression in pancreas showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant ($p<0.01$) down regulation compared to diabetic rats (Figure-127; Table-127).

Real Time PCR analysis of SOD

Gene expression of SOD mRNA showed significant up regulation ($p<0.001$) in the pancreas of diabetic compared to control. D + IIH and C + IIH group showed a significant up regulation ($p<0.001$) compared to diabetic rats. C + IIH group showed a significant ($p<0.01$) down regulation compared to D + IIH (Figure-128; Table-128).

Real Time PCR analysis of Bax

Bax mRNA showed significant up regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH rats showed a significant up regulated ($p<0.001$) expression compared to diabetic rats. C + IIH group showed a significant ($p<0.01$) down regulated Bax mRNA expression compared to D + IIH (Figure-129; Table-129).

Real Time PCR analysis of phospholipase C

Gene expression phospholipase C mRNA showed significant down regulation ($p<0.001$) in diabetic, D + IIH and C + IIH rats compared to control. D + IIH and C + IIH group showed a significant down regulation ($p<0.001$) compared to diabetic rats (Figure-130; Table-130).

CONFOCAL STUDIES

Muscarinic M1 receptor antibody staining in the pancreas of control and experimental rats

Muscarinic M1 receptor subunit antibody staining in the pancreas showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. D + IIH and C + IIH group showed a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH group showed a significant ($p<0.01$) down regulation compared to D + IIH rats (Figure -131; Table -131).

Muscarinic M3 receptor antibody staining in the pancreas of control and experimental rats

Muscarinic M3 receptor subunit antibody staining in the pancreas showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. In D + IIH and C + IIH, there was a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats (Figure-132; Table-132).

$GABA_{A\alpha1}$ receptor antibody staining in the pancreas of control and experimental rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the pancreas showed a significant decrease ($p<0.001$) in the mean pixel value in diabetic, D + IIH and C + IIH rats compared to control rats. D + IIH and C + IIH group showed a significantly decreased ($p<0.001$) mean pixel value compared to diabetic rats. C + IIH rats showed significant ($p<0.01$) decrease in mean pixel value compared to D + IIH (Figure-133; Table-133).

Discussion

Glucose is the principal source for energy production in the brain and undisturbed glucose metabolism is pivotally significant for normal function. Glucose metabolism and energy homeostasis of the body are regulated by the nerve system and special glucose sensory neurons with action potential depending on the glucose level in the extracellular medium (Levin *et al.*, 2004). The glucose excitable neurons elevate their activity with an increase in the external glucose concentration, and the glucose suppressible neurons are activated with a decrease in its level. These specialized neurons use glucose and products of its intracellular metabolism for regulation of their activity and release of neurotransmitters (Yang *et al.*, 2004). Diabetes mellitus is a common metabolic disorder resulting from defects in insulin secretion, insulin action or both (Feldman *et al.*, 1997). Hypoglycemia is a relatively common episode primarily affecting diabetic patients receiving treatment with insulin or other hypoglycemic drugs and patients suffering from insulinoma (Cryer, 2004). The metabolic stressor, hypoglycemia, is a potent threat to neurological function since the brain requires high substrate fuel input, yet maintains only limited energy reserves. Conventional therapeutic management of insulin dependent diabetes mellitus, defined as a regimen based upon one or two daily injections of an intermediate-acting insulin formulation, is highly correlated with iatrogenic hypoglycemia (Cryer & Polonsky, 1998). Diabetic patients utilizing such treatment regimens suffer on average one or two episodes of symptomatic hypoglycemia weekly. The most common cause of hypoglycemia is the use of exogenous insulin by individuals with type 1 diabetes. The incidence of recurrent hypoglycemia has increased with use of intensive insulin therapy, leading to hypoglycemia being the most feared consequence of such treatment (Amiel, 1998). The neurological consequences of diabetes mellitus in the central nervous system (CNS) are now receiving greater

attention. Cognitive deficits, along with morphological and neurochemical alterations illustrate that the neurological complications of diabetes are not limited to peripheral neuropathies (Biessels *et al.*, 1994). The central complications of hyperglycemia include the potentiation of neuronal damage observed following hypoglycemic events. Hypoglycemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients. As in brain injury associated with ischemia and neurodegenerative conditions, altered neurotransmitter action appears to play a role in hypoglycemic brain injury.

BLOOD GLUCOSE, CIRCULATING INSULIN LEVEL & BODY WEIGHT

Several experimental models have been described which provide information on the etiology of IDDM. Streptozotocin (STZ) is a toxic agent selective to pancreatic β-cells that induces IDDM by causing the β-cell destruction (Paik *et al*., 1980). The STZ diabetic rat serves as an excellent model to study the molecular, cellular and morphological changes in brain induced by stress during diabetes (Aragno *et al*., 2000). In the present study, STZ-induced rats were used as an experimental model for inducing diabetes, since they provide a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia (Low *et al*., 1997). Increased blood glucose and decreased body weight during diabetes is similar with previous reports as a result of the marked destruction of insulin secreting pancreatic islet β-cells by streptozotocin (Junod *et al.,* 1969). Hyperglycemia occurs as a result of impaired glucose transport across membranes and almost complete suppression of the conversion of glucose into fatty acids *via* acetyl-CoA. Hyperglycemic state during diabetes is due to the increased gluconeogenic pathway, which is physiologically less sensitive to the inhibition by insulin (Burcelin *et al*., 1995). Clinical and experimental studies have revealed that altered glucose status is an important factor controlling learning and memory processes (Messier & Gagnon, 1996).

The ability to sense a reduction in blood glucose levels and the counter regulatory mechanisms responsible of its correction are impaired in patients with type 1 diabetes, which make them susceptible of suffering from hypoglycemia (Becker & Ryan, 2000; Jones & Davis, 2003; Cryer, 2006). Antecedent hypoglycemia is a primary factor in the development of hypoglycemia-associated autonomic failure, a pathophysiological condition in diabetic patients that is characterized by diminished hypoglycemic awareness and impaired glucose counter regulation. During diabetes there is decrease in body weight as a result of altered metabolic function. There was a significant decrease in the circulating insulin level of diabetic rats when compared to control group.

Administration of 1.5 U/kg of regular insulin produced a fall in glucose level below 50mg/dL after 1 hour in C + IIH rats. The minimum required dose to produce irreversible severe hypoglycemia was 0.5 U/kg (Abdul-Ghani *et al*., 1989). In D + IIH rats, administration of 10 U/kg of insulin decreased the blood glucose level below 50mg/dL after 3 hours. Fluctuations in blood glucose are known to induce changes in neuronal function and therefore be used as a tool to investigate cognitive functioning (Deary., 1993). It is well recognized that the glucose level is the primary determinant of the hormonal and metabolic counter regulatory responses to insulin induced hypoglycemia. A single episode of very mild hypoglycemia (56 mg/dL) causes a reduction of neuroendocrine counter regulation that is readily discernible about 24 h later. A similar effect of a single hypoglycemic episode has been shown in healthy (Hvidberg *et al*., 1996) and diabetic (Dagogo *et al*., 1993) humans. The glycemic levels during antecedent hypoglycemia in those studies were (46–50 mg/dL). The hypoglycemic counter regulatory mechanisms are blunted irreversibly by disease duration or by acute episodes of prior stress (Ertl & Davis., 2004).

Recurrent episodes of hypoglycemia have been demonstrated to reduce subsequent endocrine counter regulation (Davis & Shamoon**,** 1991; Heller & Cryer, 1991; Widom & Simonson, 1992; Veneman *et al*., 1993; George *et al*.,

1995). As reported by McNay *et al* 2006 recurrently hypoglycemic rats required progressively less insulin to achieve hypoglycemia, presumably due to the loss of the counterregulatory response. Low blood glucose levels are associated with negative mood states, primarily self-reported ‘‘nervousness” (Boyle and Zrebiec, 2007). Moreover, patients with a history of severe hypoglycemia show significantly increased levels of anxiety (Wredling *et al.,* 1992) or ‘‘negative mood” (Gonder-Frederick *et al.,* 2008) relative to other patients with IDDM. The prolonged effects of even mild hypoglycemia on subsequent counter regulation underline the importance of scrupulously avoiding even mild hypoglycemic episodes in patients with diabetes. The body weights of D + IIH and C + IIH rats showed no significant change compared to control while they showed significant increased weight compared to diabetic. The initiation of insulin therapy in diabetes is usually associated with weight gain (Wing *et al.,* 1990).

In the study, both hypoglycemic groups showed increased circulating insulin level. Alterations in circulating insulin levels and insulin signaling pathways due to diabetes and its treatment directly affect the brain (Biessels *et al.,* 2004). Insulin affects multiple mechanisms related to neuronal activity. Although hyperinsulinemia does not affect whole-brain glucose use, hyperinsulinemia in rats changed glucose metabolism in the brain regions Hyperinsulinemia has been reported to antagonize the stimulatory effect of epinephrine on hepatic glucose production (Moan *et al*., 1995).

BEHAVIOURAL DEFICITS IN EXPERIMENTAL RATS

We evaluated the behavioural response of experimental rats in Y-maze test which is used to evaluate the spatial learning in different rat models (Murugesan, 2005). Also, motor performance of control and experimental rats on grid walk test were studied. Clinical and animal studies suggest that recurrent hypoglycemia leads to persistent behavioral and cognitive impairments (Wredling *et al.,* 1990; Gold *et al.,* 1993; Akyol *et al.,* 2003).

The Y-maze test is a classic model behavioral test, with a strong aversive component, utilized for evaluating learning and memory in rats and mice (Katz & Chudler, 1980; Woo *et al.,* 2008). Spatial memory and exploratory activity have an influence on behavioural tests including Y-maze performance. In this regard, the number of novel arm entries and time spent was significantly lower in hypoglycemic rats compared to diabetic and control group. Hypoglycemic rats showed performance deficit in Y maze which showed impairment in exploratory activity which is anxiety dependent behaviour. Intensity of derangement in hypoglycemic groups exacerbated compared to diabetic and control rats. GABA-mediated neurotransmission in the brain regions of the rat regulate anxiety (Shekhar *et al.,* 1990). Results show the impairment in spatial memory and exploratory activity in diabetic rats which is adversely influenced by recurrent hypoglycemia. This is in agreement with other studies which show that impairment in cognitive function have been observed in diabetic patients and also in animal models of diabetes (Brands *et al*., 2007). These impairments have been characterized mainly by moderate deficits in learning and memory, psychomotor slowing and reduced mental flexibility (Cukierman *et al*., 2005; Brands *et al*., 2007). The above data complement clinical and animal studies suggesting that recurrent hypoglycemia leads to persistent behavioral and cognitive impairments (Gold *et al.,* 1993; Akyol *et al.,* 2003).

There are also reports on the involvement of the cholinergic system abnormality in the impaired acquisition and/or retention of passive avoidance learning. Behavioral abnormalities consequent on an impairment of neuronal glucose metabolism is suggestive of cholinergic dysfunction (Jackson *et al*., 2000). Neurobehavioural derangements are reported to be associated with cholinergic system (Viberg *et al*., 2008). Increased Y maze deficit in hypoglycemic group shows the involvement of cholinergic system; muscarinic, nicotinic receptor subtype functional regulation in mediating behavioural impairment. Reduced function by CNS cholinergic systems has been found to be

associated with spatial and working memory tasks (Gioiosa *et al.,* 2008). Neurobehavioral alterations that manifest as deficits in locomotor responses are related to nicotinic receptors (Eriksson *et al*., 2000) and deficits in spatial learning and memory (Eppolito & Smith, 2006). Behavioural deficit suggests impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture and at the same time adjust their limb movements on the grid and is indicative of cerebellar dysfunction (Krishnakumar *et al.,* 2009). Spatial learning, which often involves training on a particular maze, has been shown to rely heavily on the septohippocampal pathway. Cholinergic neurons in the septohippocampal pathway are intermingled with GABAergic neurons, and damage to both cholinergic and GABAergic septohippocampal pathways contribute to spatial memory deficits (Pang *et al*., 2001).

The grid walk test in experimental rats demonstrated the impairment of the motor function and coordination in the hypoglycemic rats compared to diabetic and control rats. Increased number of foot slips in grid walk test suggests impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture. Motor skills and motor learning abilities are dependent on the integrity of the central cholinergic system (Thouvarecq *et al.,* 2001). Diabetes mellitus has been reported to be accompanied by a number of behavioural and hormonal abnormalities, including reduced locomotor activity (Marshall *et al*., 1976). Cholinergic system, particularly that of the neostriatum and of the septohippocampal formation, plays a role in locomotion and motor skills (Monmaur *et al.,* 1997) particularly in learning of motor abilities (Shapovalova, 1998). Previous reports showed impaired performance in hypoglycemic rats in rotarod test (Joseph *et al*., 2010).

Thus behavioral test conducted in experimental rats showed that hypoglycemia results in impaired motor and cognitive function thus leading to deficiency in exploratory activity and spatial memory related tasks which is attributed to impaired cholinergic and GABAergic neurotransmission.

CHOLINERGIC ENZYME ALTERATIONS IN BRAIN AND PANCREAS OF EXPERIMENTAL RATS.

Central cholinergic activity was studied in experimental rats using ChAT and AChE as markers. ChAT is the rate-limiting enzyme of Ach production, which is synthesized in cholinergic neuronal cell bodies and is often used in the studies of tissue localization and functional activity. Acetylcholine is the primary neurotransmitter of the cholinergic system and its activity is regulated by AChE. The termination of nerve impulse transmission is accomplished through the degradation of acetylcholine into choline and acetyl CoA by AChE (Weihua Xie *et al*., 2000). It is suggested that the changes in the plasma glucose or insulin levels be the stimuli that influence the activity of cholinergic neurons. Our results showed an increased expression of AChE in cerebral cortex, cerebellum, brainstem and hippocampus of hypoglycemic and diabetic rats when compared to control rats. In corpus striatum there was a decrease in the expression of AChE in both hypoglycemic and diabetic groups. The decrease in AChE activity has a negative correlation with the blood glucose level, suggested to be due to impaired glucose oxidation and glucose transport. These results are in accordance with Kuhad *et al* (2007), where a significant elevation in AChE activity was observed in cerebral cortex from STZ-induced diabetic rats. AChE activation leads to a fast ACh degradation and a subsequent down regulation of ACh receptors causing undesirable effects on cognitive functions (Appleyard *et al*., 1990).

ChAT shows a decreased expression in cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus in hypoglycemic rats compared to diabetic rats. It is suggested that the increase in AChE activity caused by recurrent hypoglycemia during diabetes leading to a reduction in the efficiency of cholinergic neurotransmission due to a decrease in acetylcholine levels in the synaptic cleft, thus contributing to the progressive cognitive impairment and other neurological dysfunctions seen in diabetic patients (Biessels *et al*., 1994). STZ

causes reduced cerebral energy metabolism leading to cognitive dysfunction by inhibiting the synthesis of adenosine triphosphate (ATP) and acetyl CoA which results in cholinergic deficiency supported by reduced ChAT activity in hippocampus (Prickaerts *et al*., 1999) and increased AChE activity in rat brain (Sonkusare *et al*., 2005). The enhancement of cholinergic activity by inhibition of AChE enzyme is the main stay of symptomatic treatment of dementia (Siddiqui & Levey, 1999).

Cholinergic neurons are widely distributed in the central nervous system.They have effects on postsynaptic neurons and induce long-term potentiation of synaptic transmission (LTP) and long term memory through a series of signal transduction mechanisms. The dysfunction or loss of cholinergic neuron is closely correlated with the decreasing ability of learning and memory formation. The reduction of ChAT is correlated with the severity of dementia and pathologic changes (Rodrigo *et al*., 2004). The altered expression of cholinergic enzymes, AChE and ChAT possibly relate to the reported delayed nerve transmission and impaired brain functions (Bartus *et al.*, 1982).

Cognitive decline in AD brain seems to be related to impaired cholinergic transmission that involves the synthesis of ACh by ChAT, release of ACh, binding to postsynaptic receptors and rapid withdrawal of ACh by cholinesterases (Ferna´ndez-Go´mez *et al*., 2009). Reduced activities of choline acetyl-transferase (Coyle *et al*., 1983; DeKosky *et al*., 2002) and acetylcholinesterase from cholinergic and non-cholinergic neurons (Kuhl *et al*., 1999; Blusztajn & Berse, 2000; Shinotoh *et al*., 2000) are associated with cognitive decline in neurodegenerative diseases. The altered AChE and ChAT expression causes specific alteration in ACh levels and mAChRs, leading to cholinergic dysfunction on diabetes which is exacerbated by its complication, hypoglycemia. Present study showed central cholinergic deficit associated with cognitive impairment during hypoglycemia in diabetes.

Pancreas

Insulin secretion from pancreatic islet β-cells is a tightly regulated process, under the close control of blood glucose concentrations and several hormones and neurotransmitters. Insulin secretion from the pancreatic islets are stimulated by parasympathetic nerves and inhibited by sympathetic nerves (Ahren, 2000). Acetylcholine mediates insulin release through vagal stimulation. ACh is released from cholinergic synapses on β-cells during the cephalic phase of digestion causing a transient increase in insulin secretion. Defects in glucose-triggered insulin secretion are ultimately responsible for the development of type II diabetes, a condition in which the total β-cell mass is essentially unaltered, but β-cells become progressively "glucose blind" and unable to meet the enhanced demand for insulin resulting in peripheral insulin resistance (Rutter, 2001). The pancreas is innervated by cholinergic fibers (Brunicardi *et al*., 1995) with ample amounts of choline acetyltransferase and acetylcholinesterase in islets (Godfrey & Matschinsky, 1975). Muscarinic M1 and M3 receptors are expressed on islet β -cells (Iismaa *et al*., 2000). It is well established that parasympathetic stimulation of the pancreas potentiates insulin secretion both before and after glucose absorption from the gut (Strubbe & Steffens, 1993) reinforcing the physiological importance of this system in glucose homeostasis.

During hypoglycemia, low circulating levels of glucose are detected by central and/or peripheral glucoreceptors. This information is relayed *via* central autonomic circuits that quickly correct this potentially dangerous condition causing the release of glucogenic hormones – glucagon from the α-cells of the pancreas and adrenaline from the adrenal medulla and inhibition of insulin secretion (Yamaguchi, 1992; Havel & Taborsky, 1994). The parasympathetic nervous system plays an important role in the glucagon release in response to insulin hypoglycemia in rats. The lack of glucagon response to insulin hypoglycemia reported in long-term diabetic rats could be due to deteriorated

parasympathetic nervous system and also could be corrected with carbachol (Patel, 1984).

In the present study, hypoglycemic group showed significantly increased AChE expression and decreased ChAT expression. Our findings showed the affect of hypoglycemia on cholinergic enzymes and cholinergic hypofunction in the pancreas during recurrent hypoglycemia. This is suggested to inhibit glucagon response to hypoglycemia and blunts the counter regulatory mechanism which exacerbates the neuronal damage induced by diabetes. Acute hypoglycemia is associated with stimulation of the pancreatic α-cells and a concurrent, prolonged suppression of insulin secretion by the β-cells. The islets receive a rich autonomic innervation and therefore are subjected to control by adrenergic and cholinergic mechanisms (Roger *et al.*, 1981). Recurrent hypoglycemia also leads to insulin insensitivity. Previous studies reported impaired glucagon secretion in diabetes (Unger & Orci, 1975) and insulin-induced hypoglycemia (Ishida *et al.*, 1993).

CENTRAL MUSCARINIC RECEPTOR ALTERATIONS

Muscarinic receptors are a family of G protein-coupled receptors that have a primary role in central cholinergic neurotransmission. Specific agonists, which activate postsynaptic muscarinic receptors, stimulate cholinergic signalling (Valentin *et al.*, 2006). It is known that different parts of the brain, particularly the hypothalamus and the brainstem are important centers involved in the monitoring of glucose status. The effect of the cholinergic receptors blocked by the muscarinic antagonist atropine showed the involvement of muscarinic receptors in the central cholinergic glucose homeostasis. The muscarinic M1, M2 and M4 subtypes of mAChRs are the predominant receptors in the CNS. These receptors activate a multitude of signaling pathways important for modulating neuronal excitability, synaptic plasticity and feedback regulation of ACh release (Volpicelli *et al.*, 2004). It is now widely accepted that processes such as sustained attention and working memory are regulated by central cholinergic systems (Sarter *et al.*,

2003). Cholinergic muscarinic receptor subtypes are localized in special parts of the body and are associated with numerous second messenger systems that mediate a variety of pre- and postsynaptic responses (Dutar & Nicoll, 1989). Central muscarinic M1 antagonism leads to cognitive dysfunction and other CNS-related adverse events. Muscarinic M1 and M2 knockout mice, both demonstrate cognitive defects (Tzavara *et al*., 2003). In the central nervous system, most of the cholinergic receptors are muscarinic. Muscarinic receptors exist pre- and postsynaptically. Presynaptic receptors are involved in the regulation of ACh release from the nerve terminals, while postsynaptic receptors are at the beginning of the cascade of molecular events that will lead to the biological response. The release of ACh from neurons in the central nervous system is modulated by muscarinic agonists (Polak & Meeuws, 1966).

Cerebral cortex

Cerebral mAChR are involved in many physiological brain functions, such as control of rapid eye movement, sleep (Velazquez-Moctezuma *et al.*, 1989), arousal (Albanus, 1970), learning and memory. Cerebral cortex participates in the memory, attention, perceptual awareness, thought, language and consciousness which are necessary for the normal life style. The muscarinic M1, M3 and M5 receptors are located predominantly on postsynaptic nerve terminals and are thought to be responsible for the role of the muscarinic cholinergic system in cognition and long term potentiation in the hippocampus and cortex (Bartus, 2000). Immunoprecipitation and immunofluorescence studies indicate that muscarinic M1 and M3 receptors are expressed in cortex (Levey, 1993).

Binding studies of total muscarinic and muscarinic M1 receptors revealed decreased receptor number in the cerebral cortex during diabetic condition and in hypoglycemic the expression was further decreased. Muscarinic M3 binding significantly increased in hypoglycemic rats compared to diabetic. Real Time-PCR analysis also revealed a down regulation of the muscarinic M1 receptor and

upregulation of muscarinic M3 mRNA level during hypoglycemic condition compared to diabetic. This is concordant with our receptor binding studies. Immunohistochemistry study using confocal microscope confirmed a similar expression pattern in localization of muscarinic M1, M3 receptors in the cerebral cortex of control and experimental rats. Highest levels of muscarinic M1 and M3 receptors are in cerebral cortex (Brann *et al*., 1988) which shows the importance of cholinergic neurotransmission in cerebral cortex; which in the present study showed altered by recurrent hypoglycemia. Muscarinic M3 receptors are implication in synaptic inhibition. Inhibition of excitatory transmission by M3 receptors has been reported (Sugita *et al.,* 1991; Hsu *et al.,* 1995; Auerbach & Segal, 1996). Alterations of mAChR were reported in several neurological and psychiatric diseases. Cholinergic deficits are accompanied by alterations of the mAChR in various cerebral regions. Previous reports showed that activation of muscarinic M1 cholinergic receptors produced an increase of glucose utilization (Hosey, 1992). The functional regulation of muscarinic receptors is a compensatory mechanism to facilitate insulin secretion and maintenance of normoglycemia in diabetic rats. Our findings showed cholinergic hypofunction mediated through muscarinic M1 and M3 receptors in the cerebral cortex which is suggested to depress the overall function mediated by cerebral cortex. The results showed the importance of maintaining euglycemia during diabetes treatment with insulin therapy.

Cerebellum

Cerebellum is a region of the brain that plays an important role in the integration of sensory perception, memory consolidation, coordination and motor control. In order to coordinate motor control, there are many neural pathways linking the cerebellum with the cerebral motor cortex and the spinocerebellar tract (Roberta & Peter, 2003). There is currently enough anatomical, physiological and theoretical evidence to support the hypothesis that cerebellum is the region of the

brain for learning, basal ganglia for reinforcement learning and cerebral cortex for unsupervised learning (Doya, 1999). There is increasing evidence that the cerebellum is involved in cognition, behaviour and emotion (Schmahmann & David, 2006). Cerebellar dysfuncton is associated with poor fine motor skills, hypotonia (Wassmer *et al*., 2003). Studies from our laboratory have demonstrated that cerebellum is susceptible to hypoglycemia (Joseph *et al.*, 2007, 2008).

Gene expression studies showed that the mRNA level of muscarinic M1 and M3 receptors in the cerebellum of hypoglycemic rats substantially increased compared to diabetic and control. Also, the binding parameters B_{max} of total muscarinic, muscarinic M1 and M3 receptors were increased in experimental rats. Immunohistochemical study using confocal microscope confirmed a similar expression pattern in localization of muscarinic M1, M3 receptors in the cerebellum of experimental rats. Our results showed that cholinergic transmission mediated through muscarinic receptors in the cerebellar circuitory is affected during diabetes and exacerbated by recurrent hypoglycemia increasing the susceptibility of cerebellar purkinje cells to neuronal damage. The cellular basis of motor learning has been mostly attributed to long term depression (LTD) at excitatory parallel fiber - purkinje cell synapses. LTD is induced when parallel fibers are activated in conjunction with a climbing fiber, the other excitatory input to Purkinje cells. The cerebellum has generally been suggested to be involved in the control and integration of motor processes, as well as cognitive functions. Synaptic ACh levels are known to be regulated by the activity of presynaptic muscarinic autoreceptors mediating inhibition of ACh release. In terms of the contribution of cholinergic cerebellar abnormalities to mental function, early reports of cerebellar abnormalities in autism (Courchesne, 1995) and of intellectual and behavioural abnormalities in patients with cerebellar damage (Botez-Marquard, 2001) originally suggested a cognitive role for the cerebellum. Since then, many studies have confirmed that the cerebellum contributes to cognitive and other non-motor functions. These neurofunctional deficits are one

of the key contributors to motor deficits and cellular stress associated with hypoglycemia in diabetes which is suggested to cause more damage at molecular level than hyperglycemia. Dysfunction of cerebellar cholinergic receptors are reported in hypoglycemia and hyperglycemia (Antony *et al*., 2010).

Brain stem

Brain stem reticular formation has been considered to play an important role in generating behavioural states as well as in the modulation of pain sensation (Pare & Steriade 1993). These reticular functions originate from interacting neuronal groups in the brain stem, including cholinergic, adrenergic and serotoninergic neurons (Steriade, 1996). Hypoglycemia result primarily from a lowered glucose level in the brain and it adversely affects the central and autonomic nervous systems (Charles *et al.*, 2005). When glucose level was lowered to 2.8 mmoles/L, the brain function was impaired in nondiabetic rats.

Symptoms of hypoglycemia result from the actions of hormones and neurotransmitters in the process of restoring blood glucose levels. Severe hypoglycemia triggers a cascade of events in vulnerable neurons that culminate in cell death even after glucose normalization (Suh *et al.* 2003; 2005). Brainstem is an important part of the brain in monitoring the glucose status and the regulation of feeding (Guillod *et al.*, 2003). Also, it serves as one of the key centres of the central nervous system for regulating body homeostasis. Present study on brainstem muscarinic receptors showed that the total muscarinic receptors increased during hypoglycemic condition compared to diabetic and control. Muscarinic M1 receptors are decreased and muscarinic M3 receptors are increased during hypoglycemia. RT-PCR analysis also revealed a down regulation of the muscarinic M1 receptor mRNA level during glucose deprivation in hypoglycemia. This is in accordance with our receptor binding studies. Also, confocal studies using specific antibodies of muscarinic M1 and M3 in brain stem confirmed the Real time PCR analysis and binding assays. The adverse effects of mild

hypoglycemia on brain function are not limited to higher centres but also involve the brainstem. Previous studies suggest that moderate hypoglycemia impairs brainstem function in normal humans and rats (McNay *et al.,* 2001). Recent studies suggest that moderate hypoglycemia impairs brainstem function in normal humans and rats. It is reported that mild hypoglycemia causes a functional impairment in the inferior colliculus (IC) region of the brainstem in both nondiabetic and diabetic rats (Jacob *et al.,* 1995). The brain stem provides the main motor and sensory innervation *via* the cranial nerves. Though some earlier studies reported that brainstem is less vulnerable to hypoglycemic brain damage (Auer, 2004), our results indicated that the cholinergic function mediated through muscarinic receptors in the brain stem is altered in hypoglycemic and hyperglycemic rats impairing ACh related functions of brain stem.

Corpus striatum

Corpus striatum regulates endocrine functions indirectly through the secretion of other hormones like thyroxine. In the rat striatum, Ach content, AchE and ChAT activity are among the highest in the brain (Calabresi *et al.,* 1998). Muscarinic modulation of ACh released by muscarinic autoreceptors are observed even in the presence of AChE, showing that this modulation plays a key role in the physiological function of cholinergic transmission (Dodt & Misgeld, 1986). Scatchard analysis of total muscarinic receptors revealed a decreased B_{max} in corpus striatum during hypoglycemia compared to diabetic and control rats. Within the striatum, the postsynaptic effects of Ach are mediated primarily by muscarinic receptors and the postsynaptic `excitatory' effects produced by muscarine seem to be generated by the activation of M1 receptors (Calabresi *et al.,* 2000).

Muscarinic M1 receptors were increased and muscarinic M3 receptors decreased during hypoglycemia. mRNA expression analysis also showed an up regulation of the muscarinic M1 receptor and down regulation of M3 receptor

during experimental condition. Various anatomical, electrophysiological and pathological observations provide evidence that ACh plays a major role in the control of striatal function and in the regulation of motor control (Jabbari *et al*., 1989). The results of confocal studies confirmed the alterations of muscarinic M1 and M3 receptor at protein level which mediated neuronal damage during condition of glucose deprivation induced by hypoglycemia. It is widely accepted that certain brain regions and specific neuronal cell types express differential sensitivity to hypoglycemic insults during glucose deprivation and striatal neurons are particularly prone to develop neuronal damage under energy deprivation (Haddad & Jiang, 1993). M1 receptors are responsible for the presynaptic inhibition of GABA release while M3 receptors exert an inhibitory control on the release of glutamate in the striatum (Sugita *et al.,* 1991). These studies shows the importance of maintaining synaptic neurotransmission mediated though muscarinic M1, M3 receptor subtypes which were found to be affected by recurrent hypoglycemia during diabetes.

CNS mAChRs regulate a large number of important central functions including cognitive, behavioural, sensory, motor and autonomic processes (Felder *et al*., 2000; Eglen, 2005). The striatum also expresses high sensitivity to hypoglycemic insults. During these pathological conditions, striatal synaptic transmission is altered depending on presynaptic inhibition of transmitter release and opposite membrane potential changes occur in neurones and in cholinergic interneurones (Calabresi *et al*., 2000) . Involvement of muscarinic M3 receptor in the presynaptic inhibition of synaptic transmission at excitatory synapses results in a decrease in neurotransmitter release and synaptic activity (Washburn & Moises, 1992). The high sensitivity of striatal spiny neurons to energy deprivation is expressed as a disruption of intrinsic membrane properties as well as an alteration of synaptic characteristics of the recorded cells (Xu, 1995). The present study on total muscarinic, muscarinic M1, M3 receptor expression levels in corpus striatum

shows impaired expression thereby function, during hypoglycemia which contributes to hypoglycemia associated functional deficit.

Hippocampus

The hippocampus is highly enriched in cholinergic terminals which originate from the medial septal pathways, and this system has a profound effect on hippocampal neuron functionality and reports demonstrated that the most abundant muscarinic receptor in the hippocampus is that of the musacrinic M1 subtype (Krnjevic, 1993; Levey, 1995). Excitatory modulations by muscarinic cholinergic receptors in hippocampal neurons have been well recognized. The high density of the muscarinic M1 receptor protein in this region of the CA1 subfield (Levey, 1995) supports the likely involvement of this subtype as a participant in the cholinergic modulation of protein synthesis and excitatory neuronal modulations. Diabetes mellitus and its most common treatment side effect, hypoglycemia, have multiple effects on the central nervous system. Prolonged or profound hypoglycemia leads to coma, seizures and potentially permanent brain damage (McCall, 2004).

Because of the numerous modulatory effects exerted through phosphoinositide- linked muscarinic receptors in the hippocampus, we evaluated how the muscarinic receptors respond to hypoglycemic stress. Significant alterations in binding parameters and gene expression in total muscarinic, muscarinic M1, M3 receptors were observed following hypoglycemia in diabetic and control rats. We observed a transient decreases in the mRNA levels of muscarinic receptor subtypes following recurrent episode of hypoglycemia. Immunohistochemistry study of muscarinic M1, M3 receptors in the hippocampus of control and experimental rats using confocal microscope confirmed a similar expression pattern which confirmed at protein level. Neurons react to the decrease in oxygen and glucose by initiating a series of adaptive responses to mitigate excitotoxicity. Both long term mechanisms involving alterations in gene

transcription and cell metabolism (Lipton, 1999), as well as immediate effects on electrophysiological properties (Krnjevic, 2008) serve to protect neurons, thereby allowing them to recover from ischemic insults of short duration. It has been demonstrated that neuronal activity in many systems is required to provide trophic factors essential for neuronal survival (Thoenen, 1995). Any sustained decrease in neuronal activity resulting from stress could therefore lead to a decreased supply of essential trophic factors, which would likely be detrimental to hippocampal neurons (Johnson & Deckwerth, 1993). Recent investigations have shown that the hypoglycemic condition, even in the absence of isoelectricity, induce discrete neuronal damage in vulnerable regions, such as hippocampus, when it is sustained for prolonged periods of time (Tkacs *et al.,* 2005; Ennis *et al.,* 2008; Haces *et al.,* 2010). Furthermore, increased lipoperoxidation has been observed in the hippocampus of hypoglycemic animals not subjected to a coma period (Patockova *et al.,* 2003; Haces *et al.,* 2010). The impairment in cognitive abilities is associated with insulin induced hypoglycemic neuronal damage (Roghani *et al.,* 2006). Present study suggests that cognitive and memory deficits associated with hypoglycemia is attributed to the altered cholinergic expression and thereby function mediated through muscarinic receptors in the hippocampus. Stress condition due to hypoglycemia result in deficits in several neurotransmitter systems, including cholinergic transmission (Ghajar *et al.,* 1985) which support the present findings that stress during glucose deprivations affects acetylcholine metabolism and cholinergic neurotransmission in the hippocampus.

MUSCARINIC RECEPTOR ALTERATIONS IN THE PANCREAS

Diabetes mellitus, characterized by uncontrolled hyperglycemia and maintenance of blood glucose within the physiological range is critical for the prevention of diabetes-related complications. Insulin secretion from pancreatic islet β-cells is a tightly regulated process, under the close control of blood glucose concentrations and several hormones and neurotransmitters. However, tight

glycemic control is also associated with an increased incidence of therapy-induced hypoglycemic events (Cryer, 1994). The pancreatic islets are richly innervated by parasympathetic, sympathetic and sensory nerves. Neurotransmitters are stored within the terminals of these nerves, both acetylcholine and noradrenalin and several neuropeptides. ACh has been shown to be present in normal and diabetic pancreas (Luiten *et al.,* 1986). Expression of muscarinic receptors in rat islets was established by reverse transcriptase-polymerase chain reaction and quantified by RNase protection. Both methods indicated that muscarinic M1 and M3 receptors were expressed approximately equally in the various cellular preparations (Lismaa *et al*., 2000) which signify the importance of muscarinic receptors in maintaining glucose homeostasis. During hypoglycemia, low circulating levels of glucose are detected by central and/or peripheral glucoreceptors. This information is relayed *via* central autonomic circuits that quickly correct this potentially dangerous condition causing the release of glucogenic hormones – glucagon from the α-cells of the pancreas and adrenaline from the adrenal medulla and inhibition of insulin secretion (Yamaguchi, 1992; Havel & Taborsky, 1994). Neuroglucopenia stimulates the secretion of counter-regulatory hormones, among which exists glucagon secretion by the pancreatic β-cells through activation of autonomic neurons (Flechtner *et al.,* 2006). Cholinergic input to the islets plays a particularly important role in the regulation of circulating insulin levels (Walter *et al*., 2004).

The ability of α cell to respond appropriately to hypoglycemia and to arginine during hyperglycemia is dependent on normal β-cells function (McCulloch *et al.,* 1989). Present study on pancreatic muscarinic receptor binding and gene expression along with immunocytochemistry revealed a significant decreased expression of muscarinic receptors. During hypoglycemic condition, total muscarinic, muscarinic M1 and M3 receptor binding parameters decreased when compared to diabetic and control. Gene expression studies also showed the down regulation of muscarinic M1, M3 receptors in hypoglycemic rats compared to diabetic rats. These muscarinic receptor alterations impair the functions

mediated by these receptors and contribute to defective counter regulatory response to hypoglycemia. In the present study, localization of muscarinic M1, M3 receptors using confocal laser scanning microscopy showed a decreased mean pixel value in pancreatic islets of hypoglycemic and diabetic rats. The presence of ACh in pancreas suggests a physiological role for acetylcholine in pancreatic function. ACh elicited a significant increase in glucagon secretion in the normal pancreas. Holst *et al* (1981) demonstrated that ACh stimulates glucagon secretion from the isolated canine and porcine pancreases. The response was completely resistant to hexamethonium and abolished by atropine. This indicates that the action of ACh on glucagon secretion is *via* muscarinic receptors. The inability of ACh to stimulate glucagons release from diabetic pancreas suggests that the mechanism of action of ACh in diabetes has been impaired. This is in accordance with our study on muscarinic receptors which showed decreased expression in diabetic rats.

Our results showed hypofunction of muscarinic receptors in the pancreas exposed to recurrent hypoglycemia. Havel, (1997) reported that muscarinic receptors blockade lowered the glucagon response to the insulin-induced hypoglycemia. Effects of cholinergic mechanisms on glucagon and epinephrine responses to insulin-induced hypoglycemia showed that glucagon response to insulin hypoglycemia in normal and short-term diabetic rats increased during cholinergic receptor activation. Patel (1984) demonstrates that the parasympathetic nervous system plays an important role in the glucagon release in response to insulin hypoglycemia in rats. The lack of glucagon response to insulin hypoglycemia observed in long-term diabetic rats could be due to deteriorated parasympathetic nervous system and also could be corrected with carbachol, drug that binds and activates the acetylcholine receptor (Patel, 1984).

Choline increased the acetylcholine content of the pancreas and enhanced acetylcholine release from minced pancreas, which suggests that choline stimulates insulin secretion indirectly by enhancing acetylcholine synthesis and

release (Ilcol *et al*., 2003). Muscarinic M3 receptor appears to be the predominant subtype expressed by pancreatic β-cells (Gilon & Henquin, 2001). Earlier study demonstrated that muscarinic stimulation of pancreatic insulin and glucagon release is mediated by the muscarinic M3 receptor subtype (Duttaroy *et al*., 2004). Acute hypoglycemia is associated with stimulation of the pancreatic α-cells and a concurrent, prolonged suppression of insulin secretion by the β- cells. The islets receive a rich autonomic innervation and may therefore be subject to control by adrenergic and cholinergic mechanisms (Roger, 1981). Acetylcholine mediates insulin release through vagal stimulation. Insulin secretion is stimulated by parasympathetic nerves and inhibited by sympathetic nerves (Ahren, 2000). Insulin signaling is important for the regulation of glucagon secretion in both normoglycemic and hypoglycemic states *in vivo* (Kawamori *et al.,* 2009). Glucagon, secreted from the pancreatic α cells, counters the actions of insulin and corrects hypoglycemia by enhancing hepatic glucose output and gluconeogenesis (Exton *et al.*, 1966; Maruyama *et al.*, 1984). Inappropriate glucagon secretion is often observed in patients with diabetes and a defective glucagon response to hypoglycemia in hyperinsulinemic states frequently exacerbates hypoglycemic attacks and limits intensive therapy (Amiel *et al.,* 1998). Reduction of the glucose counter regulatory hormonal responses during prolonged hypoglycemia affecting the glucagon response and reduced counter regulation to hypoglycemia could predispose to low plasma glucose concentrations (Guldstrand *et al.,* 2003).

Present study showed the effect of insulin induced hypoglycemia on pancreatic muscarinic receptors which plays an important role in counter regulatory mechanisms in response to hypoglycemia. Recurrent hypoglycemia caused cholinergic hypofunction in pancreas which contributes to defective counter regulatory response which in turn affects the insulin secretion and sensitivity of pancreatic cells.

α7 NICOTINIC ACETYLCHOLINE RECEPTOR GENE EXPRESSION IN BRAIN REGIONS OF EXPERIMENTAL RATS

In the brain, nicotinic receptors include several subtypes with differing properties and functions. The abundant presence of α7 nAChR's in the hippocampus, neocortex and basal ganglia (Clarke *et al.,* 1985), in conjunction with the memory-enhancing activity of selective α7 nicotinic agonists such as DMXB (Bitner *et al.,* 2007), suggests a significant role for α7 nAChR's in learning and memory.

Present study on analysis of α7 nAChR expression levels in different brain regions of hypoglycemic and diabetic rats showed that glucose deprivation significantly affects its expression thereby contributing to functional deficits. Our results showed an increased expression of α7 nAChR in cerebral cortex, cerebellum, brain stem, corpus striatum and decreased expression in hippocampus of hypoglycemic rats compared to diabetic and control rats. A previous study demonstrated a critical involvement of α7 nAChRs in hippocampal gamma band oscillations (Song *et al.,* 2005). Confocal studies using specific antibody for α7 nAChR confirmed the mRNA expression in cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus in experimental rats which showed significant alterations in hypoglycemic condition. Similar to the hippocampus, functional α7 nAChRs are present on GABAergic interneurons in the human cortex (Alkondon *et al.,* 2000) and the majority of parvalbumin-positive cells in the human cortex express α7 nAChRs (Krenz *et al.,* 2001) inviting the possibility that α7 nAChRs can directly modulate cortical GABAergic neurotransmission and gamma band oscillations, deficits of which underlie the cognitive deficits seen in schizophrenic patients. α7 nAChR activation is involved in performance on a variety of cognitive tests related to working, short-term, and longterm memory function in animal models (Boess *et al.,* 2007; Pichat *et al.,* 2007; Hashimoto *et al.,* 2008). Because the nAChR admits Ca^{2+} into the neuron (Vijayaraghavan *et*

al., 1992), it has the ability to affect neuronal migration (Komuro & Rakic, 1996) as well as other developmental functions such as apoptosis (Sastry & Rao, 2000).

In the present study, the up regulation of α7 nicotinic receptor is associated with altered and hypofunction of the receptor induced by glucose deprivation during hypoglycemia and diabetes which also leads to cognitive and memory deficits. Nicotinic receptor activation has been reported to improve performance in tests measuring several forms of learning and memory and its effects appear to be most robust for working memory (Levin 1992; Levin & Simon 1998). Present findings showed that the behavioural anomalities observed in glucose deprived /hypoglycemic rats are attributed to impaired function of α7 nicotinic receptors. These receptors mediate fast synaptic transmission (Alkondon *et al.,* 1998; Frazier *et al.,* 1998; Chang & Berg, 1999) and modulation of synaptic transmission mediated by transmitters such as acetylcholine, dopamine, glutamate, gamma aminobutyric acid (GABA) (MacDermott *et al.,* 1999) norepinephrine and and serotonin which is altered by recurrent hypoglycemic attacks in diabetic and non-diabetic rats.

CENTRAL GABA RECEPTOR ALTERATIONS IN EXPERIMENTAL RATS

Symptoms associated with hypoglycemic are related to the release of catecholamines, however, at times, transient neurological deficits, presumably resulting from a low intracerebral glucose concentration, accompany the typical clinical picture. These transient neurological deficits may be generalized such as confusion, motor restlessness, hypotonia and generalized seizures (Lahat, 1995). Epileptic episodes induced by hypoglycemia are the cause of neurological deficits (Wayne *et al.,* 1990). GABA, the major inhibitory neurotransmitter in the CNS (Sivilotti & Nistri, 1991) plays an important role in seizure generation. Excitatory and inhibitory neurotransmission in the CNS is mediated mainly by glutamate and GABA, respectively. A dysfunction of any of these neurotransmitter systems is

implicated in the generation of epilepsy, since an imbalance between excitation and inhibition produced by a decrease in GABAergic and/or an increase in glutamatergic transmission has been associated with the generation of this pathological condition, both in animal models and in humans (Bradford, 1995). There is an extensive literature showing that seizures can be provoked by blocking GABA synthesis with 3-mercaptoproprionic acid (MPA) *in vivo* (Mares *et al.*, 1993). These studies were demonstrating the involvement of GABA in the prevention of the over stimulation of neuronal networks.

Glutamate plays an important role in seizure initiation and spread exerting action not only on ionotropic, but also on metabotropic receptors (Chapman, 1999). Previous reports from our laboratory showed increased glutamate toxicity in cerebral cortex and cerebellum of hypoglycemic and diabetic rats (Joseph *et al.*, 2007, 2008). Inhibitory inter-neurons that make use of GABA as their neurotransmitter are found throughout the brain, but in any region they comprise a wide range of morphological and functional types that participate in different circuits with principal neurons. Through the mechanism of recurrent inhibitory feedback, GABAergic interneurons in the neurons terminate local sustained burst firing and, through inhibitory surround, limit the lateral spread of seizure activity. Precise GABAergic synaptic signaling is critical to the accurate transmission of information within neural circuits and even slight disruptions can produce hypersynchronous activity (Chagnac-Amitai & Connors, 1989). Moreover, changes in ambient GABA can alter tonic inhibition and thus the overall synaptic tone of a brain region (Farrant & Nusser, 2005).

The mechanisms of GABA synthesis and degradation are well understood. Glutamate is decarboxylated to GABA *via* glutamic acid decarboxylase (GAD). GABA that is released into the synaptic cleft, is transported in to both astrocytes and interneurons through specific transporters. Transported GABA can be repackaged for subsequent release in interneuronal terminals while astrocytic GABA is usually metabolized *via* GABA-transaminase (GABA-T). These

metabolic cycles are reviewed (Martin & Tobin, 2000; Bak *et al*., 2006). However, there is an increasing recognition that regulating neurotransmitter metabolism provides another avenue for neuromodulation. In terms of the GABAergic system, the anticonvulsant vigabatrin enhances the GABA content of neurons and glia by blocking its degradation, thereby increasing vesicular concentrations (French, 1993) while the expression of the synthetic enzyme, GAD, is enhanced following a seizure (Esclapez & Houser, 1999). Moreover, both experimental and modeling studies have shown that modulating the intracellular content determines the degree of vesicular GABA release (Wu *et al*., 2001; Engel *et al*., 2001; Axmacher *et al*., 2004).

The present study on GABA receptor subtypes; $GABA_{A\alpha1}$, $GABA_B$ and GAD in brain regions showed significant alterations in expression pattern which throws light on the fact that GABAergic neurotransmission is impaired during hypoglycemia and this contributes to hypoglycemic seizure and the subsequent neurological deficits.

Cerebral cortex

GABA neurons constitute 20–30% of all neurons in the cerebral cortex and perform critical roles in modulating cortical functional output (Cherubini & Conti, 2001; Krimer & Goldman-Rakic, 2001). GABA, the principal inhibitory neurotransmitter in the cerebral cortex, maintains the inhibitory tones that counter balances neuronal excitation. When this balance is perturbed, seizures ensue (Gregory & Mathews, 2007). GABA is formed within GABAergic axon terminals and released into the synapse, where it acts at one of two types of receptor $GABA_A$ and $GABA_B$ (Labrakakis *et al*., 1997). $GABA_A$ receptor binding influences the early portion of the GABA mediated inhibitory postsynaptic potential, whereas $GABA_B$ binding influences the late portion. $GABA_A$ receptor activation in neurons induced a complex physiological response, namely the activation of a Cl^- conductance in concert with a blockade of the resting K^+

outward conductance resulting in hyperpolarisation. Both responses were mediated by the activation of $GABA_A$ receptors, since they were both mimicked by the $GABA_A$ receptor agonist muscimol and antagonized by picrotoxin and bicuculline (Labrakakis *et al*., 1997).

Our study on GAD, the enzyme that catalyzes the decarboxylation of glutamate to GABA, GABA receptor binding and expression of GABA receptor subtypes $GABA_{A\alpha1}$ and $GABA_B$ showed significant alterations in the cerebral cortex region in hypoglycemic rats compared to diabetic. GAD is the rate limiting enzyme of GABA synthesis and it is used as a marker for GABAergic activity (Sophie *et al.,* 1990). GAD expression showed significant down regulation in hypoglycemic group. GABA receptor binding studies revealed decreased B_{max} which showed decreased receptor number eventhough the affinity of the receptor did not show significant change as shown by no change in K_d. It is reported that in both epileptic and histologically damaged cortex, there are significant decreases in GAD and GABA binding (Lloyd *et al*., 1981). Real time PCR analysis also showed similar results of GABA receptor binding in the cerebral cortex. Histological evidence as shown by specific $GABA_{A\alpha1}$ receptors showed decreased mean pixel value, which is in accordance with the gene expression study. In addition to augmentation of endogenous protective mechanisms following different pathophysiological conditions, alterations in the kinetics and pharmacology of $GABA_A$ receptors is associated with the development of spontaneous seizure activity (Treiman, 2001; Nishimura *et al.,* 2005).

Studies employing magnetic resonance spectroscopy (MRS) suggest that unipolar depression is associated with reductions in cortical GABA levels (Sanacora *et al*., 1999; 2000). Antidepressant and mood-stabilizing treatments also appear to raise cortical GABA levels and to ameliorate GABA deficits in patients with mood disorders (Krystal *et al*., 2002). Anxiety disorders have long been associated with disturbances of GABA function because of the ability of the benzodiazepine anxiolytics to facilitate brain GABA neurotransmission (Honig, *et*

al., 1988). Low blood glucose levels are associated with negative mood states, primarily self-reported ‘‘nervousness” (Boyle & Zrebiec, 2007). Moreover, patients with a history of severe hypoglycemia showed significantly increased levels of anxiety (Wredling *et al.*, 1990) or ‘‘negative mood” (Gonder-Frederick *et al.*, 2008) relative to other patients with IDDM.

Inhibitory neurotransmission play an important role in the generation and maintenance of certain seizure types in the corticothalamo - cortical circuits (Engel, 1996). GABA-mediated neuronal synchronization initiates seizures in some forebrain areas, mainly through the activation of $GABA_A$ receptors (Avoli *et al.*, 2005). Mechanisms like selective neuronal vulnerability, selective sensitivity of neurotransmitters and the loss of autoregulation of cerebral blood flow are suggested for neurological deficits followed by hypoglycemia (Lancet, 1985). The consequence of hypoglycemic shock in diabetics is that the administration of glucose did not result in immediate reversal of the neurological deficits (Wayne *et al.*, 1990).

It is evident from the present study that neurological dysfunction follows the hypofunction of GABA receptors and thereby impaired GABAergic transmission in the cortical regions. Decreased GABA neurotransmission in the present model of hypoglycemia is also suggested to lead to anxiety disorders associated with glucose deprivation.

Cerebellum

GABA is a predominant neurotransmitter, which is synaptically released, mediates fast inhibitory synaptic transmission and regulates excitatory activity of neurons (Martin & Tobin, 2000; Olsen & Avoli, 1997). The Aα1 subunit is the most dominant a subunit in all types of mature cerebellar neurons, and appears when and where GABAergic synapses are formed (Merlo *et al.*, 2007; Takayama & Inoue, 2003). Cerebellum is a region of the brain that plays an important role in the integration of sensory perception, memory consolidation, coordination and

motor control. In order to coordinate motor control, there are many neural pathways linking the cerebellum with the cerebral motor cortex and the spinocerebellar tract (Roberta & Peter, 2003).

Abundance of GABA receptors in the cerebellar cortex confirms the importance of GABAergic inhibitory action in signal processing and homeostasis. Results of our study on cerebellar GAD and GABA receptors showed decreased B_{max} and decreased expression of GAD in hypoglycemic rats compared to diabetic and control which put forward decreased GABAergic functional regulation in the cerebellum. There is currently enough anatomical, physiological and theoretical evidence to support the hypothesis that cerebellum is the region of the brain for learning, basal ganglia for reinforcement learning and cerebral cortex for unsupervised learning (Doya, 1999). $GABA_{A\alpha1}$ and $GABA_B$ receptor gene expression showed a significant downregulation in hypoglycemic rats. $GABA_{A\alpha1}$ subunit is considered to be an essential subunit for inhibitory synaptic transmission in the matured cerebellar cortex (Takayama & Inoue, 2004) and the decreased GABA receptor subunit contributes to blockade of inhibitory synaptic transmission which contributes to seizure initiation during recurrent hypoglycemia. $GABA_B$ receptors play a major role in inhibitory neurotransmission in the mammalian brain (Bowery, 2006) and are intimately involved in synaptic plasticity (Davies *et al.,* 1991), nociception (Gordon *et al.,* 1995) and some neuronal disease states. The changes in $GABA_A$ receptor subunit contribute to the changes in inhibitory function that underlie occurrence of recurrent seizures (Jean-Marc, 2008). Presynaptically, $GABA_B$ receptors inhibit the release of GABA, *via* autoreceptors, and excitatory neurotransmitters (Perkington & Sihra, 1998). Postsynaptic $GABA_B$ receptor activation elicits increased outward potassium current, causing hyperpolarisation. This gives rise to the slow late component of the inhibitory postsynaptic potential (IPSP) important in signal processing (Misgeld *et al.,* 1995). Postsynaptic GABA receptors also

modulate the second messenger responses to other neurotransmitters such as noradrenaline and 5-hydroxytryptamine (Karbon *et al.*,, 1984).

The cellular basis of motor learning has been mostly attributed to long term depression (LTD) at excitatory parallel fiber (PF)-Purkinje cell (PC) synapses. LTD is induced when PFs are activated in conjunction with a climbing fiber (CF), the other excitatory input to PCs. Recently, by using whole-cell patch-clamp recording from PCs in cerebellar slices, a new form of synaptic plasticity was discovered. Stimulation of excitatory CFs induced a long-lasting rebound potentiation (RP) of $GABA_A$ receptor mediated inhibitory postsynaptic currents (IPSCs) (Bibiana *et al*., 2008; Peer *et al*., 2009). Purkinje cells express a high density of $GABA_B$ receptor, a Gi/o-protein-coupled receptor for the inhibitory neurotransmitter GABA (Kuner *et al.,* 1999). As the entire output of the cerebellar cortex is carried by the firing of Purkinje cells, any mechanism able to modulate the firing pattern of Purkinje cells will influence cerebellar function. Purkinje cells fire spontaneously, even in the absence of glutamate input and the pattern of firing is very strongly influenced by high conductance, high frequency GABAergic inputs (Hausser & Clark, 1997). Considering the importance of GABA transmission in influencing Purkinje cell firing (Kanjhan *et al.,* 1999), decreased GABA receptor expression in cerebellum is suggested to affect GABAergic transmission. Immunohistochemical studies using specific antibodies of $GABA_{A\alpha1}$ have proved the decreased expression status of this receptor subtype in the cerebellum. In the present study, behavioural study using grid walk test showed impaired motor performance in hypoglycemic rats. Reduction in spontaneous motor activity follows recurrent hypoglycemia is reported by Duncan et al (2010). Loss of coordination of motor movement, inability to judge distance and timing, incapacity to perform rapid alternating movements, and hypotonia have been reported during cerebellar damage (Blumer *et al.,* 2004) and hypoglycemic induced cerebellar dysfunction . These studies indicate that during hypoglycemic stress, the subunits of GABA receptors assemble in different combinations with

varying inhibitory potential. Our results showed that changes in the GABAergic activity, motor learning and memory deficit are induced by the occurrence of hyperexcitability and seizures during hypoglycemia during intensive insulin therapy. The receptor analysis and gene expression studies implicated a role for GABA receptors in the modulation of neuronal network excitability and cerebellar motor learning.

Brain stem

GABA is one of the most important, identified neurotransmitters and generally have an inhibitory action (Cherubini *et al.,* 1998) and because GABA is unable to act without its receptors. GABA, inhibitory neurotransmitter in the Inferior colliculus neurons in the brainstem is known to be critical to audiogenic seizures (Faingold, 1999). IC in lower brainstem plays major roles in the processing of afferent and efferent information in the auditory system (Oliver & Huerta, 1992). GABA increases the production of α-brain waves (a state often achieved by meditation, characterized by being relaxed with greater mental focus and mental alertness) and reduces β- waves (associated with nervousness, scattered thoughts, and hyperactivity). GABA regulates proper function and neurotransmission.

Biosynthetic enzyme, GAD showed significant downregulation in hypoglycemic rats compared to diabetic and control. Expression $GABA_{A\alpha1}$ receptor showed upregulation and $GABA_B$ significantly down regulated in hypoglycemic rats. The increase in $GABA_A$ receptor density may represent a compensatory to reduced inhibitory input from local GABAergic interneurons. The reduction in GABA release would presumably evoke an up-regulation of GABA receptors on the postsynaptic neuron (Luo *et al.,* 2007). GABA receptor binding decreased in brain stem of hypoglycemic rats. Progressive decrease of neuronal malfunction results from decreased utilization of energy during hypoglycemic deprivation of glucose (Bendtson, 1993). An appropriate balance

between excitation and inhibition is essential for functional sensory networks (Wehr & Zador, 2003). For example, the relative strength and timing of excitatory and inhibitory inputs shapes the response to sensory stimuli, which is critical for the organization and tuning of receptive fields (Marino *et al.,* 2001; Higley & Contreras, 2006). Our study showed that differential expression of $GABA_A$ receptor subunits in the brainstem of hypoglycemic rats plays an important role in the excitation/inhibition balance. Depressed GABA mediated hyperpolarizations also contribute to the vulnerability of these neurons during an episode of energy failure.

Corpus striatum

Striatal neurons receive myriad of synaptic inputs originating from different sources. Massive afferents from all areas of the cortex and the thalamus represent the most important source of excitatory amino acids, whereas the nigrostriatal pathway and intrinsic circuits provide the striatum with dopamine, acetylcholine, GABA, nitric oxide and adenosine (Calabres *et al.,* 2000). The high sensitivity of striatal neurones to energy deprivation is expressed as a disruption of intrinsic membrane properties as well as an alteration of synaptic characteristics of cells (Xu, 1995).

In the present study, striatum was chosen because it plays an important role in the motor coordination and has abundant cortical and subcortical connections which are strongly activated in motor seizures (Kusske, 1979). The striatum play an important role in the spreading of seizure from cortical areas to deeper brain structures. Cortical neurons generate synchronous electrical activity under a variety of physiological and pathological conditions (Bragin *et al*., 1999; Grenier *et al*., 2003). All classes of striatal neurons receive prominent inhibitory GABAergic inputs. These inhibitory interactions are likely to be essential for striatal processing. GABA receptors are found at pre or postsynaptic locations in almost all neuronal elements in the striatum (Fujiyama, *et al*., 2000; Waldvogel *et*

al., 2004). GAD expression, a marker of GABAergic activity showed decreased expression in corpus striatum. GABA receptors decreased in hypoglycemic rats. Decreased GABA-mediated inhibition in the corpus striatum is attributed to changes in biosynthetic capacity of the GAD present (Raza *et al.*, 1994). $GABA_{A\alpha1}$ and $GABA_B$ receptor subtype expression showed down regulation in hypoglycemic rats compared to diabetic and control rats. Results were confirmed by immunohistochemical study. All classes of striatal neurons receive prominent inhibitory GABAergic inputs. These inhibitory interactions are likely to be essential for striatal processing (Waldvogel *et al*., 2004). Hypoglycemia induced decrease in GABAergic function thus impairs striatal inhibitory function. It is suggested that GABAergic inhibition in the striatum is one of the mechanisms that normally prevent excessive movements (Nakamura *et al*., 1990; Yoshida *et al*., 1991). Reduced inhibition in the striatum contributes to movement disorders such as some forms of dystonia (Kreil & Richter, 2005) or myoclonus (Darbin *et al*., 2000).

GABAergic inputs to striatal interneurons also originate from several sources and GABA receptor blockade at these sites, counteracting an ambient GABAergic tone that contributed to the changes in neuronal spiking. Cholinergic interneurons receive GABAergic input predominately from Medium Spiny Neurons collaterals (Martone *et al*., 1992). GABAergic inhibition in the striatum also act to inhibit seizure generation (Dematteis *et al*., 2003) or propagation (Sasaki *et al*., 2000; Benedek *et al*., 2004; Biraben *et al*., 2004; Bouilleret *et al*., 2005;). Short-term and long-term regulation of corticostriatal synaptic efficacy is critical in some pathophysiological conditions and an altered corticostriatal synaptic activity has been implicated in neurodegenerative diseases (Calabresi *et al.*, 1998). Modulations of striatal GABA levels therefore represent a pharmacological target of interest in the treatment of movement disorders and seizures. Our results showed decreased GABA receptors in the striatum of the hypoglycemic rats. This decreased GABA receptors facilitate the rapid spread of

hypoglycemic seizure to different part of the brain which leads to seizure generation. Evidence has accumulated that GABA is involved in the regulation of cholinergic (Ferkany & Enna, 1980; Scatton & Bartholini, 1982) neurons.

Hippocampus

Coulter (2001) reported that repeated seizures caused an attenuation of GABA mediated inhibition of the granule cells and in the pyramidal cells of the hippocampus. This change cannot be explained by a selective loss of GABAergic inhibitory interneuron, since the GABA immunoreactive neurons were shown to be more resistant to seizure-induced injury than other hippocampal neurons (Sloviter *et al*., 1987).

Analysis of GAD expression was used as a marker of over all GABAergic activity and was found to be lower in hypoglycemic rats. GAD plays a very important role in maintaining excitatory inhibitory balance of the central nervous system (Quan *et al*., 2003). GABA receptor binding significantly decreased along with decreased expression of its subtypes; $GABA_{A\alpha1}$ and $GABA_{B..}$ The decreased expression was confirmed by results from immunohistochemistry studies.

Dysfunction of GABAergic neurotransmitter systems is implicated in the generation of seizure, since an imbalance between excitation and inhibition produced by a decrease in GABAergic and/or an increase in glutamatergic transmission has been associated with the generation of this pathological condition, both in animal models and in humans (Bradford, 1995). Failure of GABAergic inhibition or enhancement of excitatory neurotransmission contributed to seizure initiation, especially at or near the site of seizure initiation (Balters & Francesco, 2002). Thus it is clear that the decreased GABA receptors along with decreased GAD gene expression in the hippocampus have an important role in hypoglycemic seizure and thereby neurological deficit. Research into seizures has gravitated to mechanisms associated with synaptic transmission because of its critical role in maintaining the balance between excitation and

inhibition. As more research have identified the molecular mechanisms of synaptic transmission, the defects in almost every step can lead to seizures (Babb & Brown, 1987; Sutula *et al.,* 1989). Glutamatergic and GABAergic transmission, as the major excitatory and inhibitory transmitters of the nervous system respectively, have a direct relationship to seizures.

The depression of synaptic transmission is one of the earliest events observed during glucose withdrawal and it has been observed in all brain regions in which it has been studied (Martin *et al.,* 1994) and the mechanism which mediates this include reduction in the release of neurotransmitters from the synaptic terminals. GABA receptor hypofunction during hypoglycemia in the present study is in accordance with these reports.

GABA RECEPTOR ALTERATIONS IN PANCREAS OF EXPERIMENTAL RATS

Neurotransmitters have been shown to be present in the nerves innervating the pancreas (Adeghate & Donath, 1990; 1991). This indicates that neurotransmitters, therefore, have a functional role in the regulation of pancreatic function. The presence of high concentrations of GABA and its metabolic enzymes in the pancreas has been known for nearly two decades (Taniguchi *et al.,* 1982; Sorenson *et al.,* 1991). The recent discovery of an association between GAD and insulin-dependent diabetes mellitus (IDDM) has generated increased interest in the physiologic role of GABA in the pancreas and the regulation of its metabolic enzymes. GABA acts on $GABA_A$ and $GABA_B$, receptors. $GABA_A$ receptors are ligand-gated chloride channels modulated by a variety of drugs. $GABA_B$ receptors are essentially presynaptic, usually coupled to potassium or calcium channels and they function *via* a GTP binding protein. The presence of GABA and functional GABA-A and GABA-B receptors in pancreatic endocrine cells and their ability to modulate secretion of insulin and glucagons (Brice *et al.,* 2002).

Insulin secretion from pancreatic islet β-cells is a tightly regulated process, under the close control of blood glucose concentrations and several hormones and neurotransmitters. Defects in glucose-triggered insulin secretion are ultimately responsible for the development of type II diabetes, a condition in which the total β-cell mass is essentially unaltered, but β-cells become progressively "glucose blind" and unable to meet the enhanced demand for insulin resulting for peripheral insulin resistance. In addition to its occurrence in central nervous system, GABA and GAD have been demonstrated in pancreatic β- cells (Garry *et al*,, 1986). Rorsman *et al* (1989) reported that GABA in pancreas is comparable to that in the central nervous system, demonstrating the importance of GABAergic neurotransmission in the pancreas. GABA plays an important role in the regulation of insulin biosynthesis and functions as an alternative energy source for the β-cell through GABA shunt.

GABA and its related enzymes have been demonstrated in pancreatic β-cells of normal rats. GABA induced significant increase in insulin secretion from pancreas (Adeghate & Ponery, 2002). Our study on pancreatic GABA receptor expression showed a significant decrease in hypoglycemic rats compared to diabetic and control rats. GAD expression decreased in hypoglycemic rats, suggests that hypoglycemia causes decreased GABA synthesis in the pancreas. This is in accordance with previous study which reported a significant decrease in GABA in diabetic pancreatic cells (Adeghate & Ponery, 2002) in diabetic pancreas. Hypoglycemia induced reduction in GABA receptors is suggested to progressively cause deficit in insulin secretory activity of pancreas thereby exacerbating the GABA dysfunction induced by diabetes. Thus the results throw light on maintaining euglycemia during diabetic condition and the avoidance of hypoglycemic episode during intensive insulin therapy. Gomez *et al.,* (1999) reported increased plasma insulin mediated by GABA agonists.

GLUT3 EXPRESSION IN BRAIN OF EXPERIMENTAL RATS

The brain requires a constant glucose supply which does not vary with the blood glucose concentration. This is brought about primarily by glucose metabolism itself being the rate-limiting step. Transport does have influence and is thought to be rate limiting at very low glucose concentrations (Robinson & Rapaport, 1986). Glucose transport into the brain is critical for the maintenance of brain metabolism. Although under basal conditions the rate of glucose transport is not the rate-limiting step for glycolysis in the central nervous system, hypoglycemia or hyperglycemia is known to change the glucose transport system in the brain (Devivo *et al*., 1991), suggesting that there is glucose-regulatable mechanisms associated with the transport of glucose. The expression, regulation and activity of glucose transporters play an essential role in neuronal homeostasis, because glucose represents the primary energy source for the brain (Pardridge, 1983). GLUT-3 mRNA is widely expressed in the brain, including the pyramidal neurons of the hippocampus and the granule neurons of the dentate gyrus (Nagamatsu *et al*., 1993) and immunohistochemical analysis has demonstrated that GLUT-3 protein expression also exhibits a widespread distribution in the brain (Zeller *et al*., 1995). Under normal physiological conditions, cerebral glucose metabolism is limited by the rate of glucose phosphorylation, but during hypoglycemia glucose transport can become rate limiting. Since glucose transport is facilitated by transport proteins for glucose, the global or local expression of such transport proteins are altered during hypoglycemia.

Severe hypoglycemia is a factor that can damage neurons. The gradient for glucose from capillaries to neurons has been reported by Gjedde, (1983). During hypoglycemia, the glucose concentrations in the brain tissue are decreased which could result in critical levels of glucose concentrations around neurons. Our study on GLUT3 expression in brain regions showed an upregulation of GLUT3 in cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus in hypoglycemic and diabetic hyperglycemic rats. The results showed that both

hyper and hypoglycemia causes altered neuronal glucose transport. These increases in GLUT3 expression accompanied by decreases in glucose utilization in the brain (Duelli *et al.,* 1999) which is detrimental to neuronal function. The levels of expression of GLUT3 transporters are regulated in concert with metabolic demand and regional rates of glucose utilization (Vannucci *et al.,* 1998). Adaptive responses to acute hypoglycemia vary according to both the degree and frequency of prior hypoglycemia and the presence of structural brain changes induced by chronic hyperglycemia (Kumagai *et al.,* 1995). It is reported that alterations in the glucose transport systems increase brain vulnerability to acute decrements in blood glucose levels (Pardridge *et al.,* 1990). Suda *et al* (1990) reported a 14% reduction in glucose utilization of the brain as a whole in acute moderate hypoglycemia. Previous reports showed an up regulation of GLUT1 transporters at the blood–brain barrier in experimental chronic hypoglycemia (Kumagai *et al.,* 1995). Our results showed that GLUT3 neuronal transporter was up regulated following episodes of hypoglycemia in diabetic and nondiabetic rats which in turn decreased the glucose metabolism in the brain regions, indicating that glucose directly regulate expression of GLUT3 mRNA.

INSULIN RECEPTOR ALTERATIONS IN BRAIN AND PANCREAS

Several studies have found high levels of insulin receptors in the CNS at specific locations. The highest concentrations of insulin receptors in the brain are in olfactory bulb, cerebral cortex, hippocampus, cerebellum and hypothalamus (Unger *et al.,* 1989; 1991). The insulin receptor is proposed to participate in a variety of functional activities of the CNS, including feeding, energy metabolism, reproduction and cognition (Bruning *et al.,* 2000; Schulingkamp *et al.,* 2000; Gerozissis, 2003; Schechter *et al.,* 2005;). The cognitive enhancing properties of insulin is mediated through insulin receptors expressed in the hippocampus (Kar *et al.,* 1993a), a region that is recognized as an important integration center for learning and memory (McEwen & Sapolsky, 1995). In streptozotocin-treated rats,

impairments in cognitive function and hippocampal synaptic plasticity was reversed by insulin replacement (Magarinos *et al.,* 2001) supporting a role for insulin in cognitive function. Insulin receptors are present in particularly high concentrations in neurons, and in lower levels in glia. The messenger RNA of insulin receptors is abundantly localized in neuronal somata, and receptor protein is found in both cell bodies and synapses (Zhao & Alkon, 2001). The major molecular structure and most of the properties of brain insulin receptors are identical to peripheral insulin receptors (Wozniak *et al.,* 1993).

Our study on insulin receptor expression in brain regions showed down regulation in cerebral cortex and up regulated expression in cerebellum, brain stem, corpus striatum and hippocampus of hypoglycemic and diabetic rats when compared to control. This indicates impairment in insulin metabolism in the brain during hypoglycemia. Insulin-associated modulation of neuroendocrine counter regulation, hypoglycemia perception and cerebral function occur in insulin-dependent diabetes mellitus, which indicates an intrinsic effect of insulin on the human brain (Lingenfelser *et al.,* 1996). Our results are consistent with the hypothesis that acute increases in insulin cross the blood-brain barrier and augment counter regulation *via* acute interactions with CNS insulin receptors as shown by increased insulin receptors in brain regions. Insulin injection can reduce blood glucose and lead to hypoglycemia which is associated with impaired memory (Kopf & Baratti, 1996). Given the importance of insulin signalling in neurons; altered expression of insulin receptors in brain regions in the present study during hypoglycemic condition is suggestive of cognitive impairments. In this study, intensive insulin treatment during diabetes treatment, proved to be detrimental to insulin signaling. Altered insulin receptor expression in the brain regions of hypoglycemic and diabetic rats elicits cognitive deficits.

Under conditions of hypoglycemia, when the autonomic nervous system is activated, elevated insulin levels have been shown to inhibit (Diamond *et al.,* 1991) the sympathoadrenal response. Thus impaired expression of insulin

receptors in glucose deprived condition contributes to neuronal dysfunction. An alteration in insulin signalling ability will have a major impact on cellular energy balance by affecting rate of uptake of glucose and other metabolic substrates and also directly by affecting the activity of enzymes involved in carbohydrate, lipid and protein metabolism (Dimitriadis, 2000). Many or all of the enzymes involved in the mitochondrial tricarboxylic acid cycle, the final common catabolic sequence, appear to be modulated by insulin independently of insulin-stimulated glucose transport. Expression of the genes for many enzymes involved in metabolism also appears to be regulated by insulin (O'Brien & Granner, 1996). Thus, an alteration of insulin signalling in brain regions have a profound effect on cellular energetic and is a contributing factor in the energetic deficit associated with the development of diabetes associated neurodegenerative diseases. Generalized impairment in the activation of the sympathoadrenal system in response to stress is mediated by CNS insulin receptors (Fischer *et al*, 2005). While the brain has classically been regarded as insulin insensitive, there has been clinical evidence to implicate that insulin act in the central nervous system (CNS) to influence sympathetic nervous activity (Paramore *et al.,* 1998). Therefore, insulin receptor activation and subsequent stimulation of insulin receptor second messenger cascades, including the translocation of insulinsensitive GLUTs, participate in the cognitive enhancing properties of insulin (Park, 2001; Watson & Craft, 2003).

Pancreas

Diabetes mellitus is characterized by uncontrolled hyperglycemia and maintenance of blood glucose within the physiological range is critical for the prevention of diabetes-related complications. However, tight glycemic control is also associated with an increased incidence of therapy-induced hypoglycemic events (DCCT, 1997). Glucagon, secreted from the pancreatic α-cells, counters the actions of insulin and corrects hypoglycemia by enhancing hepatic glucose output

and gluconeogenesis (Unger & Orci, 1977). Inappropriate glucagon secretion is often observed in patients with diabetes, and a defective glucagon response to hypoglycemia in hyperinsulinemic states frequently exacerbates hypoglycemic attacks and limits intensive insulin therapy.

Insulin receptor and post-receptor signaling mechanisms are required for pancreatic β- cell function (Kulkarni, 2002). Current study on insulin receptor expression in pancreas showed down regulation in diabetic rats. Decrease in insulin receptor mRNA expression is characteristic of the diabetic state. In hypoglycemic rats, the insulin receptor expression further down regulated which showed that insulin levels below and above homeostatic condition affects the functional regulation of neuronal cells. Strategies have shown that β-cell deletion of the insulin receptor reduces first-phase insulin release and β-cell insulin content and causes a progressive deterioration in glucose tolerance (Kulkarni *et al*., 2002). Previous studies showed impaired insulin synthesis and marked β-cell failure during deletion of the insulin receptor gene (Ueki *et al*., 2006). Recurrent hypoglycemia causes insulin insensitivity which adversely affects further insulin signalling in diabetic rats. This contributes to impairment in insulin receptor synthesis required for insulin signalling in pancreas. The study on the receptor shows the importance of maintaining euglycemia during diabetes treatment and the need of avoiding hypoglycemia. Insulin is known to inhibit its own release and reduce glucagon secretion.

Glucose stimulation of β cells in culture has been shown to result in the activation of the IR as does the application of exogenous insulin, suggesting that insulin secreted from β cells binds to its receptor eliciting a physiological response (O'Neill *et al.*, 2007). Insulin signaling has been shown to be associated with enhanced proliferation and reduced apoptosis in insulin target tissues (Datta *et al.*, 1999). Insulin signaling in the pancreas regulates mitochondrial function and has implications for β cell dysfunction in diabetes (Liu & Chang., 2009). Diabetes in itself cause β cell dysfunction affecting insulin receptor expression and

exacerbated by recurrent hypoglycemia. Our result is in accordance with early reports by Biessels *et al.,* (2004) which shows alterations in circulating insulin levels and insulin signaling pathways due to diabetes and its treatment; hypoglycemia, directly affecting the brain.

The profound benefits of intensive therapy, such as a decreased occurrence of neuropathy and microvascular complications reported by the Diabetes Control and Complications Trial (1993) demonstrate a clear need to improve long-term glycemic control in insulin-dependent diabetes mellitus. Unfortunately, this form of therapy results in more frequent episodes of severe hypoglycemia, which then lead to altered counter regulatory glycemic thresholds and hypoglycemia unawareness, provoking still more hypoglycemia. In addition, as the duration of IDDM increases, defects in the counter regulatory pathways themselves emerge, making hypoglycemia even more likely. In particular, the release of glucagon, which normally functions as a primary defense against hypoglycemia (Gerich *et al.,* 1974) is lost in diabetic patients (Santiago *et al.,* 1980). This plus an impairment in epinephrine release that develops later (Dagogo *et al.,* 1993) act in concert to limit the diabetic patient's ability to prevent serious hypoglycemic events that may adversely affect brain function (Ziegler *et al.,* 1992).

SOD EXPRESSION IN BRAIN AND PANCREAS

Oxidative stress has been suggested as a mechanism contributing to neuronal death. Early production of reactive species (RS) during the hypoglycemic episode has been reported. Recent investigations have suggested that oxidative stress is associated with hypoglycemic neuronal damage (Suh *et al.,* 2008; Haces *et al.,* 2008, 2010). The deleterious actions of diabetes and its complication; hypoglycemia, increase oxidative stress in the brain, leading to increases in neuronal vulnerability. The presence of oxidative stress during hypoglycemia has been recently suggested (Patocková *et al.,* 2003; Singh *et al.,*

2004; Suh *et al*., 2007), although its temporality and regional distribution in brain have not been explored in detail. Metabolism of glucose during stress such as hyperglycemia, lead to excess free-radical generation and oxidative stress. Oxidative stress has been suggested as a mechanism contributing to neuronal death induced by hypoglycemia, and an early production of reactive species (RS) during the hypoglycemic episode has been observed.

Our results showed a decreased expression of SOD in hypoglycemic and diabetic rats compared to control in cerebral cortex, cerebellum, corpus striatum and hippocampus and an increased expression in brain stem and pancreas. Regional difference in expression of antioxidant enzymes showed how the specific brain regions respond to changes in glucose homeostasis. The decreased SOD activity suggests that the accumulation of superoxide anion radical is responsible for increased lipid peroxidation. The inactivity of the antioxidant enzymes, SOD in the diabetes-induced groups was attributed to peroxidative damage to the tissues caused by administering STZ (Kwag, 2001). Chang *et al* (2007) reported that hypoglycemia down-modulate the activity of oxygen free radical scavengers and potentiate the excitotocity of brain cell. In line with this, our results showed that cerebral cortex, cerebellum, corpus striatum and hippocampus are more vulnerable to oxidative stress during impaired glucose metabolism during hypoglycemia and diabetes induced hyperglycemia. During prolonged periods of stress, exhaustion of neuronal defense mechanisms, such as anti-oxidant enzymes, reported to increase neuronal vulnerability to the point where neuronal adaptation shifts from neuronal plasticity towards neuronal damage (Reagan *et al*., 2000).

Severe hypoglycemia has been shown in adult rats and those with experimentally-induced diabetes to produce increases in markers of oxidative stress (Singh *et al.,* 2004). Recent investigations have shown that the hypoglycemic condition, even in the absence of isoelectricity, induce discrete neuronal damage in vulnerable regions, such as the cerebral cortex, when it is sustained for prolonged periods of time (Tkacs *et al.,* 2005; Ennis *et al.,* 2008;

Haces *et al.,* 2010). Furthermore, increased lipoperoxidation has been observed in the hippocampus, cerebral cortex and the striatum of hypoglycemic animals not subjected to a coma period (Patockova *et al.,* 2003). These observations together with the present results support the hypothesis that the hypoglycemic condition is sufficient to stimulate RS production.

Pancreas

SOD is implicated in the pathophysiology of various disease states including diabetes mellitus. Oxygen free radicals exert their cytotoxic effect by peroxidation of membrane phospholipids leading to change in permeability and loss of membrane integrity (Meerson *et al*., 1982). Pancreatic β-cell death underlies the pathogenesis of Type I (insulin-dependent) diabetes mellitus and liver is an important organ which offers an adequate site for various metabolic functions. Oxygen free radicals have been implicated in β cell destruction as well as in liver injury (Roza *et al.*, 1995; Poli *et al*., 1989; Paty *et al*., 1990). Our results showed a decreased expression of SOD in the pancreas of hypoglycemic and diabetic rats when compared to control which suggest the oxidative stress in pancreas during hypoglycemia and hyperglycemia. Increases in oxidative stress following hyper/hypoglycemia results from modulation of anti-oxidant pathways.

BAX PROTEIN IN BRAIN AND PANCREAS

Bax, a member of the Bcl-2 family proteins, are distinct regulators of early stages of the apoptotic process by forming oligomers onto the mitochondrial outer membrane and creating a channel for the release of cytochrome *C* and other apoptotic substances (Eskes *et al.,* 2000; Antonsson *et al.,* 2001). Study also suggested that Bax can bind to the voltage-dependent anion channel (VDAC) and promote the release of cytochrome *C* from VDAC, despite the fact the opening and closing of VDAC is Bax independent (Vander Heiden *et al.,* 1997). Bax

translocation onto the mitochondrial membrane therefore becomes one of the important indicators for the onset of mitochondria-mediated apoptosis.

In the present study, we examined whether gene expression of pro-apoptotic Bax is altered or affected by hypoglycemia and hyperglycemia. Results showed that diabetes induced up regulation in bax protein expression in cerebral cortex, cerebellum, brainstem, corpus striatum, hippocampus and pancreas. Hypoglycemia further up regulated the increased mRNA expression due to diabetes. During diabetic condition, the lack of insulin activity results in failure of transfer of glucose from the plasma into the cells and the situation so called "starvation in the midst of plenty" causes glucose deprivation. Whereas hypoglycemia, starve the cell since there is deprivation of glucose. Up regulation of Bax mRNA and protein has been reported for CA1 neurons after global brain ischemia in rat (Chen *et al.,* 1996; Campagne *et al.,* 1998) and is associated with DNA damage (Miyashita & Reed, 1995; Levine *et al.,* 1997). Thus, in both hypo and hyperglycemia, the cells are starved which is suggested to up regulate the pro - apoptotic protein, Bax expression.

PHOSPHOLIPASE C EXPRESSION IN BRAIN AND PANCREAS OF EXPERIMENTAL RATS

Phospholipase C mediates transduction of neurotransmitter signals across membranes *via* hydrolysis of phosphatidylinositol-4,5-bisphosphate, leading to generation of second messengers inositol- 1,4,5-trisphosphate and diacylglycerol. In the CNS, neurotransmitter receptor coupling to phospholipase C (PLC) has been extensively documented in [^{3}H] inositol-labeled tissue slices and synaptosomes obtained from animal brains (Fisher & Agranoff, 1987; Stephens & Logan, 1989; Chandler & Crews, 1990). Phospolipase C performs a catalytic mechanism, generating inositol triphosphate (IP3) and diacylglycerol (DAG).

We have therefore conducted studies on gene expression analysis of second messenger enzyme; phospholipase C in brain regions and pancreas of

hypoglycemic and diabetic rats to know whether the observed cholinergic and GABAergic neurotransmitter changes at receptor level induced by hypoglycemia and hyperglycemia is modulated at second messenger level. Results showed a decreased expression of phospholipase C in the cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus of hypoglycemic rats compared to diabetic- hyperglycemic and control rats.

Muscarinic M1, M3, M5 receptors typically couple *via* α subunits of the Gq/11 family to activate phospholipase C, stimulating phosphoinositide (PI) hydrolysis (Caulfield & Birdsall, 1998). In particular, reconstitution experiments with purified muscarinic M1 receptors, G protein subunits, and PLC suggested that the β1 subtype of PLC serves as the primary effector for the muscarinic M1 receptor (Jose *et al.,* 1995). We considered that the down regulation of the Phospholipase C in rat brain regions during hypoglycemia contribute to the impaired signal transduction of G-protein coupled neurotransmitter receptors. Altered phospholipase C expression fails to modulate the activity of downstream proteins important for cellular signaling. Defective expression of phospholipase C results in low levels of IP3 causing the impaired release of Ca^{2+} and bring down the level of intracellular calcium and thus failed to execute the normal neuronal function in brain regions. Previous studies reported that phospholipase C mediated signaling, initiated by growth factor receptor types, are involved in long-term memory formation, a process that requires gene expression (Paul *et al*., 2008).

Reports also showed that under abnormal conditions, excessive phospholipase activation, along with a decreased ability to resynthesize membrane phospholipids, lead to the generation of free radicals, excitotoxicity, mitochondrial dysfunction and apoptosis/necrosis.

Pancreas

The physiologic regulation of insulin secretion from the pancreatic β cell is dependent upon the coordinated interaction of metabolic and neurohumoral

signals (Zawalich & Rasmussen, 1990). While the preeminent nutrient fuel that regulates secretion is glucose, it is also clear that the vagally derived signal acetylcholine plays an important role (Shiota *et al.,* 2002). The underlying explanation for impaired insulin secretion in diabetes resides, in the inability of glucose to activate information flow in the phospholipase C/protein kinase C (PLC/PKC) signal transduction system to the same quantitative extent in mouse islets as it does in rat and, presumably, human islets as well. Our study showed a decreased expression of phospholipase C expression in the pancreatic islets of hypoglycemia and diabetic rats which shows that hypoglycemia and the subsequent glucose deprivation adversely affects the pancreatic second messenger enzyme regulation. The impact of acetylcholine on the β cell is initiated by the interaction of the neurotransmitter with the muscarinic cholinergic receptor, the activation of phospholipase C and the subsequent generation of inositol phosphates and diacylglycerol (Berridge, 1987; Gilon &. Henquin, 2001). The decreased phospholipase C expression is associated with impaired cholinergic and GABAergic eceptor signaling during hypoglycemia and hyperglycemia.

Cholinergic stimulation of the β cell activates the insulin secretory process, a response that is mediated by PLC (Kelley *et al*., 1995). The nature of the receptor type that couples the activation of PLC to release has yet to be precisely determined and both the muscarinic M1 and M3 receptor types appear to be expressed in the β cell (Lismaa *et al*., 2000). Our study on PLC expression shows that glucose deprivation is detrimental to innate insulin secreting function and other functions associated with pancreas. Thus, hypoglycemia exacerbates the dysfunction caused by diabetes.

CREB PROTEIN EXPRESSION IN BRAIN OF EXPERIMENTAL RATS

The cAMP responsive element binding protein (CREB) is a nuclear protein that modulates the transcription of genes with cAMP responsive elements in their promoters. CREB is a basally expressed, post-translationally activated

transcription factor that has been implicated in the trans-activation of a number of genes in response to cAMP and calcium signals. This transcription factor is a component of intracellular signaling events that regulate a wide range of biological functions, from spermatogenesis to circadian rhythms and memory. Interruption of CREB phosphorylation pathway interferes with important cognitive performance and behavioural aspects associated with CNS.

Current study showed a significant down regulation of CREB in cerebral cortex, cerebellum, brainstem, hippocampus and up regulated expression in corpus striatum of diabetic rats when compared to control. Recurrent hypoglycemia caused further decrease in the CREB expression in all the brain regions studied which showed a significant decreased CREB transcription in hypoglycemic and diabetic – hyperglycemic condition. Interruption of CREB phosphorylation pathway interferes with important cognitive performance and behavioural aspects associated with CNS. Alterations in CREB-mediated transcription have been implicated in multiple cognitive and psychiatric disorders including depression, anxiety, addiction, and cognitive decline and reports show decreased CREB activity in the brains of depressed patients (Mayr & Montminy, 2001). Schwechter *et al.,* (2003) reported susceptibility to seizures positively correlated with blood glucose concentrations as the increased glucose concentration was associated with proconvulsant effects. As previously reported, forebrain susceptibility to seizures is increased during moderate *in vivo* hypoglycemia and the hippocampus is involved during hypoglycemic seizures during glucose deprivation (Kirchner *et al.,* 2006). CREB phosphorylation is decreased by seizures in experimental animals. Therefore our studies on CREB mRNA expression in hypoglycemic and hyperglycemic rats suggest the correlation of decreased expression of CREB to seizures induced by variation in glucose metabolism.

To conclude, our results on hypoglycemic and hyperglycemic changes at molecular level showed that both conditions adversely affect the neuronal cells. The alterations in cholinergic, GABAergic receptors and subsequent changes of

second messenger enzymes and transcription factor resulted in neurobehavioural deficits associated with hypoglycemia and hyperglycemia. Present study confirmed the deleterious effect of therapeutic hypoglycemia during diabetes causing enhanced behavioral deficits and neurochemical changes in brain. This ascertains the importance of maintaining normoglycemia or near euglycemia but never hypoglycemia during diabetes treatment.

Summary

1. Insulin induced hypoglycemia and streptozotocin induced diabetes in Wistar rats were used as models to study the alterations in cholinergic, GABAergic receptors, GLUT3, insulin receptors, SOD, Bax protein, second messenger enzyme - phospholipase C and transcription factor - CREB in the brain and pancreas during hypoglycemia and diabetes.

2. The body weight was analyzed to study the changes in body weight in hypoglycemic and diabetic rats compared to control. Diabetes caused a reduction in the body weight while hypoglycemic rats showed increased body weight compared to diabetic rats.

3. Blood glucose level in the serum was measured to analyze the circulating glucose level changes due to hypoglycemia and diabetes in rats compared to control. Diabetic rats showed increased blood glucose level. The D + IIH and C + IIH rats showed significant reduction in blood glucose level. The blood glucose analysis also revealed that the D + IIH rats became hypoglycemic at the 3^{rd} hour and the C+IIH rats became hypoglycemic at the 1^{st} hour.

4. The circulating insulin level was analysed to study the changes in insulin concentration in hypoglycemic and diabetic rats compared to control. Diabetic rats showed a significant decrease in insulin level. The D + IIH and C + IIH rats showed significant increase in the insulin concentration.

5. Behavioural studies: Y maze and grid walk test were conducted to assess the exploratory, memory deficit, motor function and learning in control and experimental rats. The experiment demonstrated the impairment in the motor

function, learning and memory in the diabetic, D + IIH and C + IIH rats compared to control. Behavioural deficit was more prominent in hypoglycemic rats.

6. AChE expression level has been used as a marker for cholinergic activity. AChE expression was analysed in the brain regions and pancreas. During diabetes, the expression was increased in the cerebral cortex, cerebellum, brainstem, hippocampus and pancreas while hypoglycemia caused further increase in expression. In corpus striatum, AChE expression decreased significantly in hypoglycemic and diabetic rats.

7. ChAT expression level has been used as a marker for acetylcholine synthesis. ChAT showed decreased expression in the cerebral cortex, cerebellum, corpus striatum, hippocampus, pancreas while in brainstem it was increased in diabetic rats. D + IIH and C + IIH group showed significant decreased expression in all the brain regions and the pancreas.

8. Total muscarinic receptor was analysed in the brain regions and pancreas of control and experimental rats. The receptor binding was decreased in cerebral cortex, corpus striatum and hippocampus while cerebellum and brainstem showed increased expression in diabetic, D + IIH and C + IIH rats. The Scatchard analysis and gene expression studies of muscarinic M1 receptor revealed a down regulation in cerebral cortex, brainstem and hippocampus whereas in cerebellum and corpus striatum it was up regulated. Muscarinic M3 receptor binding and expression in cerebral cortex, cerebellum, brain stem and hippocampus were increased and in corpus striatum there was a decrease in diabetic, D + IIH and C + IIH rats compared to control. In pancreas, total muscarinic, muscarinic M1 and muscarinic M3 receptors were down regulated in hypoglycemia and diabetic condition. Immunohistochemical studies using

specific antibodies confirmed the Scatchard analysis and Real Time PCR analysis of muscarinic receptor expression at protein level in control and experimental rats.

9. α7 nicotinic acetylcholine receptor gene expression was studied in brain regions of experimental rats. In hypoglycemic and diabetic condition, the receptor was increased in cerebral cortex, cerebellum, brain stem, corpus striatum and decreased in hippocampus when compared to control. Immunohistochemical studies using specific antibody confirmed the gene expression of α7 nicotinic acetylcholine receptor expression at protein level in control and experimental rats.

10. Total GABA receptor binding and GAD expression was analysed in the brain regions and pancreas of control and experimental rats. The receptor binding decreased in cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus of hypoglycemic and diabetic rats. The gene expression studies of $GABA_{A\alpha1}$ and $GABA_B$ receptor showed down regulation in cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus. GAD mRNA expression significantly decreased in all the brain regions studied. In pancreas, GABA receptor binding along with $GABA_{A\alpha1}$, $GABA_B$ and GAD significantly decreased during hypoglycemia and diabetes. Immunohistochemical studies using specific antibody confirmed the binding analysis and Real Time PCR analysis of $GABA_{A\alpha1}$ receptor expression in control and experimental rats.

11. Decreased GLUT3 in brain regions- cerebral cortex, cerebellum, brainstem, corpus striatum and hippocampus of diabetic rats were decreased further in hypoglycemic group compared to diabetic and control.

12. Insulin receptor mRNA level was studied in the brain regions and pancreas of experimental rats. A decreased expression of insulin receptor was observed in cerebral cortex whereas in cerebellum, brain stem, corpus striatum and hippocampus, there was an increased expression in hypoglycemic and diabetic rats. Pancreas of both hypoglycemic and diabetic rats showed decreased insulin receptor expression.

13. Antioxidant enzyme, SOD expression was studied in experimental rats. Results showed that in diabetic rats, its mRNA level was down regulated in cerebral cortex, cerebellum and hippocampus whereas in brain stem and corpus striatum, it was up regulated when compared to control. In D + IIH and C + IIH rats, SOD expression decreased in cerebral cortex, cerebellum, corpus striatum and hippocampus whereas brain stem showed increased expression. Pancreatic expression of SOD mRNA in hypoglycemic rats decreased significantly compared to diabetic and control. Oxidative stress seen in diabetic brain and pancreas were found to exacerbate by hypoglycemia.

14. Pro-apoptotic protein- Bax mRNA expression significantly up regulated in hypoglycemic brain regions - cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus and pancreas compared to diabetic and control.

15. Second messenger enzyme - phospholipase C showed a decreased expression in hypoglycemic and diabetic brain regions - cerebral cortex, cerebellum, brain stem, corpus striatum and hippocampus. In pancreas, there was a significant decrease in mRNA expression of phospholipase C in diabetic, D + IIH and C + IIH rats compared to control.

16. Transcription factor, CREB expression in the brain regions - cerebral cortex, cerebellum, brain stem and hippocampus showed decreased expression in hypoglycemic and diabetic rats. In corpus striatum, there was an increased CREB expression in diabetic rats compared to control whereas D + IIH and C + IIH rats showed decreased expression compared to diabetic.

It is evident from our results that cholinergic, GABAergic receptor functional balance plays a major role in hypoglycemia and diabetes associated disorders. Gene expression studies along with immunohistochemistry showed a prominent functional disturbance in brain regions and pancreas of hypoglycemic and diabetic rats. These findings have important implications for understanding the molecular mechanisms underlying memory and cognitive impairment at second messenger and transcription level during recurrent hypoglycemia. Our studies showed hypoglycemic and hyperglycemic effect on brain functions by acetylcholine and GABA mediated through their receptor subtypes, second messenger enzymes and transcription factors. It is suggested that the corrective measures for the brain functional damage caused during diabetes and its treatment, through cholinergic and GABAergic receptors have therapeutic role in the management of diabetes and hypoglycemia - induced by anti-hyperglycemic treatment in diabetes.

Conclusion

Hypoglycemia is the major problem to blood glucose homeostasis in treatment of diabetes. The CNS neurotransmitters play an important role in the regulation of glucose homeostasis. These neurotransmitters mediate rapid intracellular communications not only within the central nervous system but also in the peripheral tissues. They exert their function through receptors present in both neuronal and non-neuronal cell surface that trigger second messenger signaling pathways. Our findings demonstrated a prominent functional disturbance in cholinergic and GABAergic system in the hypoglycemic and hyperglycemic brain which contributes to neuronal damage and reduced behavioural, cognitive and motor function. Disorders in the transport and metabolism of glucose through GLUT3 in hypo and hyperglycemia, contributes to neuronal dysfunction and triggers apoptotic cascade. Bax, apoptotic protein expression, increased in hypoglycemic rat brain regions and pancreas which demonstrated the central and peripheral nervous system damage induced by recurrent hypoglycemia during diabetes treatment. Altered CREB expression shows derangement of CREB related intracellular signal transduction which closely parallels neuronal dysfunction. Cholinergic and GABA receptors alteration affected normal neurotransmission through second messenger enzyme – Phospholipase C and transcription factor – CREB protein. It was also apparent from the present results that biochemical alterations produced by hyperglycemia was exacerbated by recurrent hypoglycemia and adversely affected neurological functions of CNS and PNS. Higher brain functions cannot adapt to recurrent hypoglycemia which shows that hypoglycemic condition has more functional damage at the molecular level than hyperglycemia. It is suggested that the corrective measures for the brain functional damage caused during diabetes and anti-diabetic treatment, through cholinergic and GABAergic receptors, have therapeutic role in the management of hypoglycemia and hyperglycemia.

References

Abdelmalik PA, Shannon P, Yiu A, Liang P, Adamchik Y, Weisspapir M, Samoilova M, Burnham WM, Carlen PL. (2007). Hypoglycemic seizures during transient hypoglycemia exacerbate hippocampal dysfunction. Neurobiol Dis, 26: 646–660.

Abdul–Ghani AS, Ghneim H, El–Lati S, Saca'an A. (1989). Changes in the activity of glutamate related enzymes in cerebral cortex, during insulin–induced seizures. Int. J. Neurosci, 44: 67–74.

Abraham A, Paulose CS. (1999). Age–related alterations in noradrenergic function in the brain stem of streptozotocin–diabetic rats. J Biochem Mol Bio Biophys, 3: 171–176.

Adams CE, Freedman R. (1997). Brain Res. Nicotinic antagonist alpha–bungarotoxin binding to rat hippocampal neurons containing nitric oxide synthase. 776: 111–116.

Adeghate E, Donáth T. (1990). Intramural serotonin immunoreactive cells in normal and transplanted pancreas. Biogenic Amines, 7: 385–390.

Adeghate E, Donath T. (1991). Dopamine–beta–hydroxylasepositive nerves in normal and transplanted tissue in the anterior eye–chamber of rats. Journal of Chemical Neuroanatomy, 4: 223–227.

Adeghate E, Ponery AS, Pallot DJ, Singh J. (2001). Distribution of vasoactive intestinal polypeptide, neuropeptide–Y and substance P and their effects on

insulin secretion from the in vitro pancreas of normal and diabetic rats. Peptides, 22: 99–107.

Adeghate E, Ponery AS. (2002). GABA in the endocrine pancreas: cellular localization and function in normal and diabetic rats. Tissue & Cell, 34: 1–6.

Adler CM, Goldberg TE, Malhotra AK, Pickar D, Breier A. (1998). Effects of ketamine on thought disorder, working memory, and semantic memory in healthy volunteers. Biol Psychiatry, 43: 811–816.

Ahren B, Karlsson S, Lindskog S. (1990). Cholinergic regulation of the endocrine pancreas. Prog Brain Res, 84: 209–218.

Ahren B, Sauerberg P, Thomsen C. (1999). Increased insulin secretion and normalization of glucose tolerance by cholinergic agonism in high fat–fed mice. Am J Physiol, 277: 93–102.

Ahren B, Taborsky GJ, Porte D. (1986). Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. Diabetologia, 29: 827–836.

Ahren. (2000). Autonomic regulation of islet hormone secretion—implications for health and disease. Diabetologia, 43: 393–410.

Akinci MK, Schofield PR. (1999). Widespread expression of GABA (A) receptor subunits in peripheral tissues. Neurosci Res, 35: 145–153.

Akria T, Hirohisa I, Yasushi I, Eiko KI, Yujui M. (1994). Hypothalamic cholinergic and noradrenergic neurons in hyperglycaemia induced by 2–deoxyglucose. Brain Research, 665: 13–17.

Akwa Y, Ladurelle N, Covey F, Baulieu E. (2001). The synthetic enantiomer of pregnenolone sulfate is very active on memory in rats and mice, even more so than its physiological neurosteroid counterpart: distinct mechanisms? Proc Natl Acad Sci U S A, 98: 14033–14037.

Akyol A, Kiylioglu N, Bolukbasi O, Guney E, Yurekli Y. (2003). Repeated hypoglycemia and cognitive decline: A case report. Neuro Endocrinol Lett, 24: 54–56.

Albanus L. (1970). Central and peripheral effects of anticholinergic compounds. Acta Pharmacol Toxicol, 28: 305–326.

Alkondon M, Pereira EFR, Eisenberg HM, Albuquerque EX. (1999). Choline and selective antagonists identify two subtypes of nicotinic acetylcholine receptors that modulate GABA release from CA1 interneurons in rat hippocampal slices. J. Neurosci, 19: 2693–2705.

Alkondon M, Pereira EF, Albuquerque EX. (1998). alpha–bungarotoxin– and methyllycaconitine–sensitive nicotinic receptors mediate fast synaptic transmission in interneurons of rat hippocampal slices. Brain Res, 810: 257–263.

Alkondon M, Pereira EF, Eisenberg HM, Albuquerque EX. (2000). Nicotinic receptor activation in human cerebral cortical interneurons: a mechanism for inhibition and disinhibition of neuronal networks. J Neurosci, 20: 66–75.

Alvarez EO, Beauquis J, Revsin Y, Banzan A, Roig P, De Nicola AF, Saravia F. (2009). Cognitive dysfunction and hippocampal changes in experimental type 1 diabetes. Behav Brain Res, 198: 224–230.

Amiel S. (1998). Cognitive function testing in studies of acute hypoglycaemia: rights and wrongs? Diabetologia, 41: 713–719.

Anderson CM, Swanson RA. (2000). Astrocyte glutamate transport: review of properties, regulation, and physiological functions. Glia, 32: 1–14.

Antonsson B, Montessuit S, Sanchez B, Martinou JC. (2001). Bax is present as a high molecular weight oligomer/complex in the mitochondrial membrane of apoptotic cells. J Biol Chem, 276: 11615–11623.

Antony Sherin, Peeyush Kumar T, Mathew J, Anju TR, Paulose CS. (2010). Hypoglycemia induced changes in cholinergic receptor expression in the cerebellum of diabetic rats. J Biomed Sci, 5: 17–21.

Appleyard ME, Taylor SC and. Little HJ. (1990). Acetylcholinesterase activity in regions of mouse brain following acute and chronic treatment with a benzodiazepine inverse agonist. Br J Pharmacol, 101: 599–604.

Aragno M, Parola S, Tamagno E, Brignardello E, Manti R, Danni O, Boccuzzi G. (2000). Oxidative derangement in rat synaptosomes induced by hyperglycemia: restorative effect of dehydroepiandrosterone treatment. Biochem Pharmacol, 60: 389–395.

Aral YZ, Gucuyener K, Atalay Y, Hasanoglu A, Turkyilmaz C, Sayal A, Biberoglu G. (1998). Role of excitatory aminoacids in neonatal hypoglycemia. Acta Paediatr Jpn, 40: 303–306.

Arison RN, Ciaccio EI, Glitzer MS, Cassaro JA, Pruss MP. (1967). Light and electron microscopy of lesions in rats rendered diabetic with streptozotocin. Diabetes, 16: 51–56.

Asada H, Kawamura Y, Maruyama K, Kume H, Ding R, Ji FY, Kanbara N, Kuzume H, Sanbo M, Yagi T, Obata K. (1996). Mice lacking the 65 kDa isoform of glutamic acid decarboxylase (GAD65) maintain normal levels of GAD67 and GABA in their brains but are susceptible to seizures. Biochem Biophys Res Commun, 229: 891–895.

Ashkenazi A, Winslow JW, Peralta EG. (1987). An M2 muscarinic receptor subtype couple to both adenylyl cyclase and phosphoinositide turnover. Science, 238: 672–675.

Auer RN, Hugh J, Cosgrove E, Curry B. (1989). Neuropathologic findings in three cases of profound hypoglycemia. Clin Neuropathol, 8: 63–68.

Auer RN, Kalimo H, Olsson Y, Siesjö BK. (1985). The temporal evolution of hypoglycemic brain damage. II. Light– and electron–microscopic findings in the hippocampal gyrus and subiculum of the rat. Acta Neuropathol, 67: 25–36.

Auer RN, Siesjo BK. (1988). Biological differences between ischemia, hypoglycaemia, and epilepsy. Ann. Neural, 24: 699–707.

Auer RN, Siesjo BK. (1993) Hypoglycaemia: brain neurochemistry and neuropathology. Baillieres Clin Endocrinol Metab, 7: 611–625.

Auer RN. (2004). Hypoglycaemic brain damage. Forensic Science International, 146: 105–110.

Auerbach JM, Segal M. (1996). Muscarinic receptors mediating depression and long–term potentiation in rat hippocampus. J. Physiol, 492: 479–493.

Avoli M, Louvel J, Pumain R, Köhling R. (2005). Cellular and molecular mechanisms of epilepsy in the human brain. Prog Neurobiol, 97: 166–200.

Axmacher N, Stemmler M, Engel D, Draguhn A, Ritz R. (2004). Transmitter metabolism as a mechanism of synaptic plasticity: a modeling study. J Neurophysiol, 91: 25–39.

Baba H, Kohno T, Okamoto M, Goldstein PA, Shimoji K, Yoshimura M. (1998). Muscarinic facilitation of GABA release in substantia gelatinosa of the rat spinal dorsal horn. The Journal of Physiology, 508: 83–93.

Babb TL, Brown WJ. (1987). Pathological findings in epilepsy. In: Engel JJr (ed) Surgical treatment of the epilepsies. Raven Press, New York, P: 511–540.

Babb TL, Pretorius JK, Kupfer WR, Crandall PH. (1989). Glutamate decarboxylase–immunoreactive neurons are preserved in human epileptic hippocampus. J Neurosci, 9: 2562–2574.

Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter–Olesen H, De Camilli P. (1990). Identification of the 64K autoantigen in insulin–dependent diabetes as the GABA–synthesizing enzyme glutamic acid decarboxylase. Nature, 347: 151–156.

Bak LK, Schousboe A, Waagepetersen HS. (2006). The glutamate/GABA–glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. J Neurochem, 98: 641–653.

Balters E, Francesco M. (2002). New research on epilepsy and behaviour. In Keith. J Holaway (ed). Nova Publishers, New York, P: 76–89.

Bankier A, Turner M, Hopkins IJ. (1983). Pyridoxine–dependent seizures a wider clinical spectrum. Arch Dis Child, 58: 415–418.

Barnard EA, Skolnick P, Olsen RW, Moher H, Sieghart W, Biggio G, Braestrup C, Bateson A, Langer SZ. (1998). International union of pharmacology XV. Subtypes of γ–Aminobutyric Acid A receptors: classification on the basis of subunit structure and receptor function. Pharmacol Rev, 50: 291–314

Barnard EA. (2001). The molecular architecture of GABAA receptors Pharmacology of GABA and glycine neurotransmission. In: Möhler H (eds). Handbook of Experimental Pharmacology. Berlin, Springer, 150: 94–100.

Bartus RT, Dean RL, Beer B, Lippa AS. (1982). The cholinergic hypothesis of geriatric memory dysfunction. Science, 217: 408–414.

Bartus RT. (2000). On Neurodegenerative Diseases, Models, and Treatment Strategies: Lessons Learned and Lessons Forgotten a Generation Following the Cholinergic Hypothesis. Experimental Neurology, 163: 495–529.

Baumgold J, Fishman PH. (1988). Muscarinic receptor mediated increase in cAMP levels in SK–N–SH human neuroblastoma cells. Biochem Biophys Res Comm, 154: 1137–1143.

Becker D, Ryan C. (2000). Hypoglycemia: a complication of diabetes therapy in children. Trends Endocrinol Metab, 11: 198–202.

Ben–Ami H, Nagachandran P, Mendelson A, Edoute Y. (1999). Drug–induced hypoglycemic coma in 102 diabetic patients. Arch Intern Med, 159: 281–284.

Benedek K, Juhasz C, Muzik O, Chugani DC, Chugani HT. (2004). Metabolic changes of subcortical structures in intractable focal epilepsy. Epilepsia, 45: 1100–1105.

Benveniste H, Drejer J, Schousboe A, Diemer NH. (1984). Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem, 43: 1369–1374.

Berridge MJ. (1987). Inositol trisphosphate and diacylglycerol: two interacting second messengers. Rev Biochem, 56: 159–193.

Berstein G, Blank JL, Smrck A. (1992). Reconstitution of agonist–stimulated phosphatidylinositol 4,5–bisphosphate hydrolysis using purified m1 muscarinic receptor, Gq/11, and phospholipase C–beta 1. J Biol Chem, 267: 8081–8088.

Berthoud HR, Powley TL. (1991). Morphology and distribution of efferent vagal innervation of rat pancreas as revealed with anterograde transport of Dil. Rain Res, 553: 336–341.

Berthoud, HR, Fox EA, Powley T L. (1990). Localization of vagal preganglionics that stimulate insulin and glucagon secretion. Am J Physiol, 258: 160–168.

Bettler B, Kaupmann K, Bowery N. (1998). GABAB receptors: drugs meet clones. Curr Opin Neurobiol, 8: 345–350.

Bettler B, Kaupmann K, Mosbacher J, Gassmann M. (2004). Molecular structure and physiological functions of GABA (B) receptors. Physiol Rev, 84: 835–867.

Bhardwaj SK, Sandhu SK, Sharma P, Kaur G. (1999). Impact of diabetes on CNS: role of signal transduction cascade. Brain Res. Bul, 49: 155–162.

Bhattacharya SK, Saraswathi M. (1991). Effect of intracerebroventricularly administered insulin on brain monoamines and acetylcholine in euglycemic and alloxan– induced hyperglycemic rats. Indian J. Exp. Biol, 29: 1095–1100.

Bibiana S, Benedetto S, Piergiorgio S. (2008). Learning–related long–term potentiation of inhibitory synapses in the cerebellar cortex. Proc Natl Acad Sci U S A, 105: 769–774.

Biessels GJ, Bravenboer B, Gispen WH. (2004). Glucose, insulin and the brain: modulation of cognition and synaptic plasticity in health and disease: a preface. Eur J Pharmacol, 490: 1–4.

Biessels GJ, Kappelle AC, Bravenboer B, Erkelens DW, Gispen WH. (1994). Cerebral function in diabetes mellitus. Diabetologia, 37: 643–650.

Billups D, Billups B, Challiss RA, Nahorski SR. (2006). Modulation of gq–protein–coupled inositol trisphosphate and Ca2+ signalling by the membrane potential. J Neurosci, 27: 9983–9995.

Bilotta F, Caramia R, Cernak I, Paoloni FP, Doronzio A, Cuzzone V, Santoro A, Rosa G. (2008). Intensive Insulin Therapy after Severe Traumatic Brain Injury: A Randomized Clinical Trial. Neurocrit Care, 9: 159–166.

Biraben A, Semah F, Ribeiro MJ, Douaud G, Remy P, Depaulis A. (2004). PET evidence for a role of the basal ganglia in patients with ring chromosome 20 epilepsy. Neurology, 63: 73–77.

Bitar M, Koulu M, Rapoport SI, Linnoila M. (1986). Diabetes induced alterations in brain monoamine metabolism in rats. Journal of Pharmacology and Experimental Therapeutics, 236: 432–437.

Bitner RS, Bunnelle WH, Anderson DJ, Briggs CA, Buccafusco J, Curzon P, Decker MW, Frost JM, Gronlien JH, Gubbins E, Li J, Malysz J, Markosyan S, Marsh K, Meyer MD, Nikkel AL, Radek RJ, Robb HM, Timmermann D, Sullivan JP, Gopalakrishnan M. (2007). Broad–spectrum efficacy across cognitive domains by alpha7 nicotinic acetylcholine receptor agonism correlates with activation of ERK1/2 and CREB phosphorylation pathways. J Neurosci, 27: 10578–10587.

Bizzarri C, Girolamo MD, D'Orazio MC. (1990). Evidence that a guanine neucleotide binding protein linked to a muscarinic receptor inhibits directly phospholipase C. Proc Natl Acad Sci, 87: 4889–4893.

Blumer D, Montouris G, Davies K. (2004). The interictal dysphoric disorder: recognition, pathogenesis, and treatment of themajor psychiatric disorder of epilepsy. Epilepsy Behav, 5: 826–840.

Blusztajn JK, Berse B. (2000). The cholinergic neuronal phenotype in Alzheimer's disease. Met. Brain Dis, 15: 45–64.

Bock P. (1986). Fine structure of the neuro–insular complex type II in the cat. Arch Histol Jpn, 49: 189–197.

Boess FG, De Vry J, Erb C, Flessner T, Hendrix M, Luithle J, Methfessel C, Riedl B, Schnizler K, van der Staay FJ, van Kampen M, Wiese WB, Koenig G. (2007). The novel alpha7 nicotinic acetylcholine receptor agonist N– [(3R)–1–azabicyclo[2.2.2]oct–3–yl]–7–[2–(methoxy)phenyl]–1–benzofuran–2–arboxamide improves working and recognition memory in rodents. J. Pharmacol. Exp. Ther, 321: 716–725.

Bond RW, Wyborski RJ, Gottlieb DI. (1990). Developmentally regulated expression of an exon containing a stop codon in the gene for glutamic acid decarboxylase. Proc Natl Acad Sci U S A, 87: 8771–8775 Szabo G, Katarova Z, Greenspan R. (1994). Distinct protein forms are produced from alternatively spliced bicistronic glutamic acid decarboxylase mRNAs during development. Mol Cell Biol, 14: 7535–7545.

Bonner–Weir S, Deery D, Leahy JL, Weir GC. (1989). Compensatory growth of pancreatic beta–cells in adult rats after short– term glucose infusion. Diabetes, 38: 49–53.

Bormann J. (2000). The 'ABC' of GABA receptors. Trends Pharmacol Sci, 21: 16–19.

Bosch D, Schmid S. (2008). Cholinergic mechanism underlying prepulse inhibition of the startle response in rats. Neuroscience, 155: 326–35.

Botez–Marquard TC, Bard J, Leveille MI, Botez. (2001). A severe frontal–parietal lobe syndrome following cerebellar damage. Eur J of Neurol, 8: 347–353.

Bouilleret V, Semah F, Biraben A, Taussig D, Chassoux F, Syrota A, Ribeiro MJ. (2005). Involvement of the basal ganglia in refractory epilepsy: an 18F–fluoro–L–DOPA PET study using 2 methods of analysis. J Nucl Med, 46: 540–547.

Bowery NG. (2006). GABAB receptor: a site of therapeutic benefit. Curr Opin Pharmacol, 6: 37–43.

Boyle PJ, Nagy RJ, O'Connor AM, Kempers SF, Yeo RA, Qualls C. (1994). Adaptation in brain glucose uptake following recurrent hypoglycemia. Proc Natl Acad Sci U S A, 91: 9352–9356.

Boyle PJ, Zrebiec J. (2007). Physiological and behavioral aspects of glycemic control and hypoglycemia in diabetes. South Med J, 100: 175–182.

Bradford HF. (1995). Glutamate, GABA and epilepsy. Prog. Neurobiol, 47: 477–511.

Bragin A, Engel J, Wilson CL, Fried, I, Buzsaki G. (1999). High–frequency oscillations in human brain. Hippocampus, 9: 137–142.

Brands AM, Biessels GJ, Kappelle LJ, de Haan EH, de Valk HW, Algra A, Kessels RPC. (2007). Cognitive functioning and brain MR1 in patients with type 1 and type 2 diabetes mellitus: a comparative study. Dement Geriatr Cogn Disord, 23: 343– 350.

Brann MR, Buckley NJ, Bonner TI. (1988). The striatum and cerebral cortex express different muscarinic receptor mRNAs. FEBS, 230: 90–94.

Braun M, Wendt A, Birnir B, Broman J, Eliasson L, Galvanovskis J, Gromada J, Mulder, H, Rorsman P. (2004). Regulated exocytosis of GABA–containing synaptic–like microvesicles in pancreatic beta–cells. J. Gen Physiol, 123: 191–204.

Brezenoff HE, Xiao YF. (1986). Acetylcholine in the hypothalamic nucleus is involved in the elevated blood pressure in the spontaneously hypertensive rats. Life Sci, 45: 1163–1170.

Brice NL, Varadi A, Ashcroft SJ, Molnar E. (2002). Metabotropic glutamate and GABA(B) receptors contribute to the modulation of glucose–stimulated insulin secretion in pancreatic beta cells. Diabetologia, 45: 242–252.

Brown DA, Forward A, Marsh S. (1980). Antagonist discrimination between ganglionic and ileal muscarinic receptors. Br J Pharmacol, 71: 362–364.

Brunicardi FC, Shavelle DM, Andersen DK. (1995). Neural regulation of the endocrine pancreas. Int J Pancreatol, 18: 177–195.

Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Müller–Wieland D, Kahn CR. (2000). Role of brain insulin receptor in control of body weight and reproduction. Science, 289: 2122–2125.

Bu DF, Erlander MG, Hitz BC, Tillakaratne NJ, Kaufman DL, Wagner–McPherson CB, Evans GA, Tobin AJ. (1992). Two human glutamate decarboxylases, 65–kDa GAD and 67–kDa GAD, are each encoded by a single gene. Proc Natl Acad Sci U S A, 89: 2115–2119.

Buccafusco JJ. (1996). The central cholinergic neurons in the regulation of blood pressure and in experimental hypertension. Pharm Rev, 48: 179–211.

Butterworth R F. (1999). Metabolic Encephalopathies, Basic neurochemistry. Published by Lippincott Williams and Wilkins, 5: 38.

Burcelin R, Eddouks M, Maury J, Kande J, Assan R, Girard J. (1995). Excessive glucose production, rather than insulin resistance, accounts for hyperglycaemia in recent–onset streptozotocin–diabetic rats. Diabetologia, 38: 283–290.

Byrne JH, Zwartjes R, Homayouni R, Critz SD, Eskin A. (1993). Roles of second messenger pathways in neuronal plasticity and in Aplysia. In Advances in Second Messenger and Phosphoprotein Research, ed. S Shenolikar, AC Nairn. New York: Raven, 47–108.

Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G. (1998). Endogenous Ach enhances striatal NMDA–responses via M1–like muscarinic receptors and PKC activation. Eur. J. Neurosci, 10: 2887–95.

Calabresi P, Centonze D, Gubellini P, Marfia GA, Pisani A, Sancesario G, Bernardi G. (2000). Synaptic transmission in the striatum: from plasticity to neurodegeneration. Progress in Neurobiology, 61: 231–265.

Campagne van Lookeren M, Gill R. (1998). Increased expression of cyclin G1 and p21 in neurons following transient forebrain ischemia: Comparison with early DNA damage. J. Neurosci. Res, 53: 279–296.

Cao WH, Morrison SF. (2001). Differential chemoreceptor reflex responses of adrenal preganglionic neurons. Am J Physiol Regul Integr Comp Physiol, 281: 1825–1832.

Carroll MF, Burge MR, Schade DS. (2003). Severe hypoglycemia in adults. Rev Endocr Metab Disord, 4: 149–157.

Caulfield MP, Birdsall NJM. (1998). International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol Rev, 50: 279–290.

Caulfield MP. (1993). Muscarinic receptors: Characterisation, coupling and function. Pharmacol Ther, 58: 319–379.

Cebers G, Cebere A, and Liljequist S. (1998). Metabolic inhibition potentiates AMPA–induced Ca2+ fluxes and neurotoxicity in rat cerebellar granule cells. Brain Res, 779: 194–204.

Chagnac–Amitai Y, Connors BW. (1989). Horizontal spread of synchronized activity in neocortex and its control by GABA–mediated inhibition. J Neurophysiol, 61: 747–758.

Chandler LJ, Crews FT. (1990). Calcium–versus G protein–mediated phosphoinositide hydrolysis in rat cerebral cortical synaptoneurosomes. Journal of Neurochemistry, 55: 1022–1030.

Chang KT, Berg DK. (1999). Nicotinic acetylcholine receptors containing alpha7 subunits are required for reliable synaptic transmission in situ. J Neurosci, 19: 3701–3710.

Chang YH, Hsieh MC, Hsin SC, Shin SJ, Lin KD. (2007). Insulinoma–associated transient hypothalamus–pituitary–adrenal axis impairment and amelioration by steroid therapy and surgical intervention: a case report. Kaohsiung J Med Sci, 23: 526–530.

Chapman CA, Lacaille JC. (1999). Cholinergic induction of theta–frequency oscillations in hippocampal inhibitory interneurons and pacing of pyramidal cell firing. Neurosci, 19: 8637–8645.

Charles RA, Goh SY. (2005). Occult hypoglycemia in a diabetic patient on peritoneal dialysis. Eur J Emerg Med, 12: 322–333.

Chebib M, Johnston GA. (1999). The 'ABC' of GABA receptors: a brief review. Clin Exp Pharmacol Physiol, 26: 937–940.

Chebib M, Johnston GAR. (2000). GABA–activated ligand gated ion channels: medicinal chemistry and molecular biology. J Med Chem, 43: 1427–1447.

Chen J, Zhu RL, Nakayama M, Kawaguchi K, Jin K, Stetler RA, Simon RP, Graham SH. (1996). Expression of the apoptosis. effector gene, Bax, is up–regulated in vulnerable hippocampal CA1 neurons following global ischemia. J. Neurochem, 67: 64–71.

Chen XH, Itoh M, Sun W, Miki T, Takeuchi Y. (1996). Localization of sympathetic and parasympathetic neurons innervating pancreas and spleen in the cat. J Auton Nerv Syst, 59: 12–16.

Chen S, Kobayashi M, Honda Y, Kakuta S, Sato F, Kishi K. (2007). Preferential neuron loss in rat piriform cortex following pilocarpine induced status epilepticus. Epilepsy Res, 74: 1–18.

Cherubini E, Conti F. (2001). Generating diversity at GABAergic synapses. Trends Neurosci, 24: 155–162.

Cherubini E, Martina M, Sciancalepore M, Strata F. (1998). GABA excites immature CA3 pyramidal cells through bicuculline–sensitive and insensitive chloride–dependent receptors. Perspect. Dev. Neurobiol, 5: 289–304.

Choeiri C, Staines W, Messier C. (2002). Immunohistochemical localization and quantification of glucose transporters in the mouse brain. Neuroscience, 111: 19–34.

Choi DW. (1988). Glutamate neurotoxicity and diseases of the nervous system. Neuron, 1: 623–634.

Chu PC, Lim MT, Shian LR, Liu SY. (1986). Alterations in physiologic functions and in monoamine content in streptozotocin – diabetic rats. Diabetes, 35: 481–485.

Clarke PB, Schwartz RD, Paul SM, Pert CB and Pert A. (1985). J Neurosci, 5: 1307–1315.

Claude Messier, MiehOle Gagnon. (1996). Glucose regulation and cognitive functions: relation to Alzheimer's disease and diabetes Behavioural Brain Research, 75:1–11.

Connors B, Long M. (2004). Electrical synapses in the mammalian brain. Annu Rev Neurosci, 27: 393–418.

Coulter DA. (2001). Epilepsy–associated plasticity in gamma–aminobutyric acid receptor expression, function, and inhibitory synaptic properties. Int. Rev. Neurobiol, 45: 237–252.

Courchesne E. (1995). New Evidence of Cerebellar and Brainstem Hypoplasia in Autistic Infants, Children and Adolescents: The MR Imaging Study by Hashimoto and Colleagues. J. Autism Dev Disord, 25: 19–22.

Couve A, Thomas P, Calver AR, Hirst WD, Pangalos MN, Walsh FS, Smart TG, Moss SJ. (2002). Cyclic AMP–dependent protein kinase phosphorylation facilitates GABA (B) receptor–effector coupling. Nat Neurosci, 5: 415–424.

Coyle JT, Price DL, DeLong MR. (1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. Science, 219: 1184–1190.

Crossman AR, Mitchell IJ, Sambrook MA, Jackson A. (1988). Chorea and myoclonus in the monkey induced by gamma–aminobutyric acid antagonism in the lentiform complex. The site of drug action and a hypothesis for the neural mechanisms of chorea. Brain, 111: 1211–1233.

Cryer PE, Davis SN, Shamoon H. (2003). Hypoglycemia in diabetes. Diabetes Care, 26: 1902–1912.

Cryer PE. (1994). Banting Lecture. Hypoglycemia: the limiting factor in the management of IDDM. Diabetes, 43: 1378–1389.

Cryer PE, Polonsky KS. (1998). Glucose homeostasis and hypoglycemia. In: Williams textbook of endocrinology, 9th edition (Wilson JD, Foster DW, eds), Philadelphia: WB Saunders Co. p 939–971.

Cryer PE. (2002a). Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes. Diabetologia, 45: 937–948.

Cryer PE. (2002b). The pathophysiology of hypoglycaemia in diabetes. Diabetes Nutr Metab, 15: 330–333.

Cryer PE. (2003). Hypoglycaemia in diabetes. Diabetes Care, 26: 1902–1912.

Cryer PE. (2004). Diverse causes of hypoglycemia associated autonomic failure in diabetes. N Engl J Med, 350: 2272–2279.

Cryer PE. (2006). Mechanisms of sympathoadrenal failure and hypoglycemia in diabetes. J Clin Inv, 116: 1470–1473.

Cukierman T, Gerstein HC, Williamson JD. (2005). Cognitive decline and dementia in diabetes–systematic overview of prospective observational studies. J Diabetologia, 48: 12–19.

Czech, M.P, Corvera, S. (1999). Signaling mechanisms that regulate glucose transport. J Biol Chem. 274: 1865–1868.

Dagogo–Jack SE, Craft S, Cryer PE. (1993). Hypoglycaemia–associated autonomic failure in insulin–dependent diabetes mellitus. Recent antecedent hypoglycaemia reduces autonomic response to, symptoms of, and defense against subsequent hypoglycaemia. J Clin Invest, 91: 819–828.

Dalby NO, Mody I. (2001). The process of epileptogenesis: a pathophysiological approach. Curr Opin Neurol, 14: 187–192.

Darbin O, Risso JJ, Rostain JC. (2000). High pressure enhanced NMDA activity in the striatum and the globus pallidus: relationships with myoclonia and locomotor and motor activity in rat. Brain Res, 852: 62–67.

Datta SR, Brunet A, Greenberg ME. (1999). Cellular survival: a play in three Akts. Genes Dev, 13: 2905–2927.

Davies CH, Starkey SJ, Pozza MF, Collingridge GL. (1991). GABAB autoreceptors regulate the induction of LTP. Nature, 349: 609–611.

Davis EA, Keating B, Byrne GC, Russell M, Jones TW. (1998). Impact of improved glycaemic control on rates of hypoglycaemia in insulin dependent diabetes mellitus. Arch Dis Child, 78: 111–115.

Davis MR, Shamoon H. (1991). Counterregulatory adaptation to recurrent hypoglycaemia in normal humans. J Clin Endocrinol Metab, 73: 995–1001.

Deary IJ. (1993). Effects of hypoglycemia on cognitive function. In: Frier BM, Fisher BM, editors. Hypoglycemia and diabetes: clinical and physiological aspects. London: Edward Arnold, p: 80–92.

De Filippi G, Baldwinson T, Sher E. (2001). Evidence for nicotinic acetylcholine receptor activation in rat cerebellar slices. Pharmacol Biochem Behav, 70: 447–455.

de Fernandes LA, Saad ML, Velloso LA. (1999). Insulin induces tyrosine phosphorylation of the insulin receptor and SHC, and SHC/GRB2 association in cerebellum but not in forebrain cortex of rats. Brain Res, 826: 74–82.

DeKosky ST, Ikonomovic MD, Styren SD, Beckett L, Wisniewski S, Bennet DA, Cochran EJ, Kordower JH, Mufson EJ. (2002). Upregulation of cholinetransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. Ann. Neurol, 51: 145–155.

Delgado Esteban M, Almeida A, Bolanos JP. (2000). Glucose prevents glutathione oxidation and mitochondrial damage after glutamate receptor stimulation in rat cortical primary neurons. J Neurochem, 75: 1618–1624.

Dematteis M, Kahane P, Vercueil L, Depaulis A. (2003). MRI evidence for the involvement of basal ganglia in epileptic seizures: an hypothesis. Epileptic Disord, 5: 161–164.

Devivo DC, Trifiletti RR, Jacobson RI, Rosen GM, Behmand RA, Harik SI. (1991). Defective glucose transport across the blood–brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. N EngI J Med, 325: 703–709.

Dey J, Misra A, Desai NG, Mahapatra AK, Padma MV. (1997). Cognitive function in younger. type II diabetes. Diabetes Care, 20: 32–35.

Diabetes Control and Complications Trial. (1993). The effect of intensive treatment of diabetes on the development and progression of long–term complications in insulin–dependent diabetes mellitus. N Engl J Med, 329: 977–986.

Diamond MP, Hallarman L, Starick–Zych K, Jones TW, Connolly–Howard M, Tamborlane WV, Sherwin RS. (1991). Suppression of counterregulatory hormone response to hypoglycemia by insulin per se. J Clin Endocrinol Metab, 72: 1388–1390.

Dimitriadis GD, Raptis SA, Newsholme EA. (2000). Integration of some biochemical and physiologic effects of insulin that may play a role in the control of blood glucose concentration. In: LeRoith D, Taylor SI, Olefsky JM, editors. Diabetes

mellitus: A fundamental and clinical text, Philadelphia: Lippincott, Williams & Wilkins, p: 161–176.

Dodt HU, Misgeld U. (1986). Muscarinic slow excitation and muscarinic inhibition of synaptic transmission in the rat neostriatum. J Physiol, 380: 593–608.

Dolivo M. (1974). Metabolism of mammalian sympathetic ganglia. Fed Proc, 33: 1043–1048.

Doya K. (1999). What are the computations of the cerebellum, the basal ganglia and the cerebral cortex? Neural Network, 12: 961–974.

Duelli R, Staudt R, Duembgen L, Kuschinsky W. (1999). Increase in glucose transporter densities of Glut3 and decrease of glucose utilization in rat brain after one week of hypoglycemia. Brain Research, 831: 254–262.

Duncan CE, Webster MJ, Rothmond DA, Bahn S, Elashoff M, Shannon Weickert C. (2010). Prefrontal GABA(A) receptor alpha–subunit expression in normal postnatal human development and schizophrenia. J Psychiatr Res (in press).

Dunne MJ, Cosgrove KE, Shepherd RM, Aynsley G, Lindley KJ. (2004). Hyperinsulinism in infancy: From basic science to clinical disease. Physiol Rev, 84: 239–275.

Dupuy ST, Houser CR. (1996). Prominent expression of two forms of glutamate decarboxylase in the embryonic and early postnatal rat hippocampal formation. J Neurosci, 16: 6919–6932.

Dutar P, Nicoll RA. (1989). Pharmacological characterization of muscarinic responses in rat hippocampal pyramidal cells. EXS, 57: 68–76.

Duttaroy A, Zimliki CL, Gautam D, Cui Y, Mears D. (2004). Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in m3 muscarinic acetylcholine receptor–deficient mice. Diabetes, 53: 1714–1720.

Eglen RM. (2005). Muscarinic receptor subtype pharmacology and physiology. Prog Med Chem, 43: 105–136.

Engel D, Pahner I, Schulze K, Frahm C, Jarry H, Ahnert–Hilger G, Draguhn A. (2001). Plasticity of rat central inhibitory synapses through GABA metabolism. J Physiol, 535: 473–482.

Engel J. (1996). Surgery for seizures. N Engl J Med, 334: 647–652.

Ennis K, Tran PV, Seaquist ER, Rao R. (2008). Postnatal age influences hypoglycemia–induced neuronal injury in the rat brain. Brain Res, 1224: 119–126.

Enz R. (2001). GABAC receptors: a molecular view. Biol Chem, 382: 1111–1122.

Eppolito AK, Smith RF. (2006). Long–term behavioral and developmental consequences of pre– and postnatal nicotine. Pharmacol. Biochem. Behav, 85: 835–841.

Erecinska M, Silver A. (1994). Ions and energy in mammalian brain. Prog. Neurobiol, 43, 37–71.

Erecinska M, Nelson D, Yudkoff M, Silver IA. (1994). Energetics of the nerve terminal in relation to central nervous system function. Biochem Soc Trans, 22: 959–965.

Eriksson P, Ankarberg E, Fredriksson A. (2000). Exposure to nicotine during a defined period in neonatal life induces permanent changes in brain nicotinic receptors and behaviour of adult mice. Brain Res, 853: 41–48.

Erlander MG, Tillakaratne NJ, Feldblum S, Patel N, Tobin AJ. (1991). Two genes encode distinct glutamate decarboxylases. Neuron, 7: 91–100.

Ertl AC, Davis SN. (2004). Evidence for a vicious cycle of exercise and hypoglycaemia in type 1 diabetes mellitus. Diabetes Metab Res, 20: 124–130.

Esclapez M, Houser CR. (1999). Up–regulation of GAD65 and GAD67 in remaining hippocampal GABA neurons in a model of temporal lobe epilepsy. J Comp Neurol, 412: 488–505.

Eskes R, Desagher S, Antonsson B, Martinou JC. (2000). Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. Mol. Cell Biol, 20: 929–935.

Eswar Shankar, P. N., Anu Joseph, C. S. Paulose. (2007). Decreased [3H] YM–09151–2 binding to dopamine D2 receptors in the hypothalamus, brainstem and pancreatic islets of streptozotocin–induced diabetic rats. European Journal of Pharmacology, 557: 99–105.

Exton JH, Jefferson LS, Butcher RW, Park CR. (1966). Gluconeogenesis in the perfused liver. The effects of fasting, alloxan diabetes, glucagon, epinephrine, adenosine monophosphate and insulin. Am. J. Med, 40: 709–715.

Faingold CL. (1999). Neurons in the deep layers of superior colliculus play a criticalrole in the neuronal network for audiogenic seizures: mechanisms for production of wild running behavior. Brain Res, 815: 250–258.

Farrant M, Nusser Z. (2005). Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci, 6: 215–229.

Farris TW, Oh JD, Butcher LL, Woolf NJ. (1995). Trophic–factor modulation of cortical acetylcholinesterase reappearance following transection of the medial cholinergic pathway in adult rat. Exp Neurol, 131:180–192.

Feigenspan A, Bormann J. (1994). Modulation of GABAC receptors in rat retinal bipolar cells by protein kinase C. The Journal of Physiology, 481: 325–330.

Felder CC, Bymaster FP, Ward J, DeLapp N. (2000). Therapeutic opportunities for muscarinic receptors in the central nervous system. J Med Chem, 43: 4333–4353.

Feldman, EL, Stevens MJ, Greene, DA. (1997). Pathogenesis of diabetic neuropathy. Clin Neurosci, 4: 365–370.

Ferkany JW, Enna SJ. (1980). Interaction between GABA agonists and the cholinergic muscarinic system in rat corpus striatum. Life Sci, 27: 143–149.

Fischer CP, Perstrup LB, Berntsen A, Eskildsen P, Pedersen BK. (2005). Elevated plasma interleukin–18 is a marker of insulin–resistance in type 2 diabetic and non–diabetic humans. Clin Immunol, 117: 152–160.

Fisher SK, Agranoff BW. (1987). Receptor activation and inositol lipid hydolysis in neural tissues. Journal of Neurochemistry, 48: 999–1017.

Fladeby C, Skar R, Serck–Hanssen G. (2003). Distinct regulation of glucose transport and GLUT1/GLUT3 transporters by g lucose deprivation and IGF–I in chromaffin cells. Biochim Biophys Acta, 1593: 201–208.

Flanagan DE, Evans ML, Monsod TP, Rife F. (2003). The influence of insulin on circulating ghrelin. Am J Physiol Endocrinol Metab, 284: 313–316.

Flechtner I, de Lonlay P, Polak M. (2006). Diabetes and hypoglycaemia in young children and mutations in the Kir6.2 subunit of the potassium channel Therapeutic consequences. Diabetes Metab, 32: 569–580.

Frazier CJ, Buhler AV, Weiner JL, Dunwiddie TV. (1998). Synaptic potentials mediated via alpha–bungarotoxin–sensitive nicotinic acetylcholine receptors in rat hippocampal interneurons, J Neurosci, 8228–8235.

French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, Spencer DD. (1993). Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. Ann Neurol, 34: 774–780.

Frier BM. (2008). How hypoglycaemia can affect the life of a person with diabetes. Diabetes Metab Res Rev, 24: 87–92.

Frier B. (2000). Hypoglycaemia—clinical consequences and morbidity. International Journal of Clinical Practice, 51–55.

Fujita T, Kobayashi S. (1979). Proposal of a neurosecretory system in the pancreas: an electron microscope study in the dog. Arch Histol Jpn, 42: 277–295.

Fujiyama F, Fritschy JM, Stephenson FA, Bolam JP. (2000). Synaptic localization of GABA(A) receptor subunits in the striatum of the rat. J Comp Neurol, 416: 158–172.

Fukami K. (2002). Structure, Regulation, and Function of Phospholipase C Isozymes. J Biochem, 131: 293–299.

Fuster D, Moe OW, Hilgemann DW. (2004). Lipid and mechanosensitivities of sodium/hydrogen exchangers analyzed by electrical methods. Proc Natl Acad Sci USA, 101: 10482–10487.

Garris DR. (1995). Developmental and regional changes in brain norepinephrine levels in diabetic C57BL/KsJ mice: effects of estradiol and progesterone. Brain Res. Dev. Brain. Res, 89: 314–319.

Garris. (1990). Age diabetes associated alterations in regional brain norepinephrine concentrations and adrenergic populations in C57BL/KsL mice. Developmental Brain Research, 51: 161–166.

Garry DJ, Sorenson RL, Elde RP, Maley BE, Madsen A. (1986). Immunohistochemical colocalization of GABA and insulin in beta–cells of rat islet. Diabetes, 35: 1090–1095.

Gaskins HR, Baldeón ME, Selassie L, Beverly JL. (1995). Glucose modulates gamma–aminobutyric acid release from the pancreatic beta TC6 cell line. J. Biol. Chem, 270: 30286–30289.

Gazit V, Ben–Abraham R, Pick CG, Ben–Shlomo I, Katz Y. (2003). Long–term neurobehavioral and histological damage in brain of mice induced by L–cysteine. Pharmacology biochemistry and behavior, 75: 795–799.

Gee CE, Benquet P, Demont–guignard S, Wendling F, Gerber U. (2010). Energy deprivation transiently enhances rhythmic inhibitory events in the CA3 hippocampal network in vitro. Neuroscience, doi: 10.1016/j.neuroscience.2010.04.021.

George E, Harris N, Bedford C, Macdonald IA, Hardisty CA, Heller SR. (1995). Prolonged but partial impairment of the hypoglycaemic physiological response following short–term hypoglycaemia in normal subjects. Diabetologia, 38: 1183–1190.

Gerber DJ, Sotnikova TD, Gainetdinov RR, Huang SY, Caron MG, Tonegawa S. (2001). Hyperactivity, elevated dopaminergic transmission, and response to amphetamine in M1 muscarinic acetylcholine receptor– deficient mice. Proc Natl Acad Sci USA, 98: 15312–15317.

Gerber, Hare TA. (1979). Gamma–aminobutyric acid in peripheral tissue, with emphasis on the endocrine pancreas: presence in two species and reduction by streptozotocin. Diabetes, 28: 1073–1076.

Gerich JE, Schneider V, Dippe SE, Langlois M, Noacco C, Karam JH, Forsham PH. (1974). Characterization of the glucagon response to hypoglycemia in man. J Clin Endocrinol. Metab, 38: 77–82.

Gerozissis K. (2003). Brain insulin: regulation, mechanisms of action and functions. Cell. Mol. Neurobiol, 23: 1–25.

Ghajar JB, Gibson GE, Duffy TE. (1985). Regional acetylcholine metabolism in brain during acute hypoglycemia and recovery. J Neurochem, 44: 94–98.

Gibson GE, Blass JP. (1976). Impaired synthesis of acetylcholine in brain accompanying hypoglycaemia and mild hypoxia. J. Neurochem, 27: 37–42.

Gilon P, Henquin JC. (2001). Mechanisms and physiological significance of the cholinergic control of pancreatic beta cell function. Endocr Rev, 22: 565–604.

Gilon P, Mallefet J, De Vriendt C, Pauwels S, Geffard M, Campistron G, Remacle C. (1990). Immunocytochemical and autoradiographic studies of the endocrine cells interacting with GABA in the rat stomach. Histochemistry, 93: 645–654.

Gilon P, Tappaz M, Remacle C. (1991). Localization of GAD–like immunoreactivity in the pancreas and stomach of the rat and mouse. Histochemistry, 96: 355–365.

Gioiosa L, Raggi C, Ricceri L, Jasmin JF, Frank PG, Capozza F, Lisanti MP, Alleva E, Sargiacomo M, Laviola G. (2008). Altered emotionality, spatial memory and cholinergic function in caveolin–1 knock–out mice. Behavioural Brain Research, 188: 255–262.

Girard J. (1994). Insulin resistance: role in type 2 diabetes. Diabete Metab, 20: 330–336.

Gireesh G, Reas SK, Jobin M, Paulose CS. (2008a). Decreased muscarinic M1 receptor gene expression in the cerebral cortex of streptozotocin–induced diabetic rats and Aegle marmelose leaf extract's therapeutic function. J Ethnopharmacol, 116: 296–304.

Gireesh G, Kaimal SB, Kumar TP, Paulose CS. (2008b). Decreased muscarinic M1 receptor gene expression in the hypothalamus, brainstem, and pancreatic islets of streptozotocin–induced diabetic rats. J Neurosci Res, 86: 947–953.

Girod R, Role LW. (2001). Long–lasting enhancement of glutamatergic synaptic transmission by acetylcholine contrasts with response adaptation after exposure to low–level nicotine. J Neurosci, 21: 5182–5190.

Gjedde. (1983). Modulation of substrate transport to the brain. Acta Neurol. Scand, 67: 3–25.

Glowinski J, Iversen LL. (1966). Regional studies of catecholamines in the rat brain: The disposition of [3H] norepinephrine, [3H] dopamine and [3H] dopa in various regions of the brain. J Neurochem, 13: 655–669.

Godfrey DA, Matschinsky FM. (1975). Enzymes of the cholinergic system in islets of Langerhans. J Histochem Cytochem, 23: 645–651.

Gold AE, Deary IJ, Frier BM. (1993). Recurrent severe hypoglycaemia and cognitive function in type 1 diabetes. Diabet Med, 10: 503–508.

Golding DW, Pow DV. (1990). Neurosecretion by synaptic terminals and glandular discharge in the endocrine pancreas: application of tannic acid to the teleost Xiphophorus helleri. Neuroendocrinology, 51: 369–375.

Gomez J. (1999). Medicinal chemistry section. IDrugs, 2: 1115–1116.

Gonder–Frederick L, Zrebiec J, Bauchowitz A, Lee J, Cox D, Ritterband L, Kovatchev B, Clarke W. (2008). Detection of hypoglycemia by children with type 1 diabetes 6 to 11 years of age and their parents: a field study. Pediatrics, 121: 489–495.

Gordon NC, Gear RW, Heller PH, Miaskowski SPC, Levine JD. (1995). Enhancement of morphine analgesia by the GABAB agonist baclofen. Neuroscience, 69: 345–349.

Green A, Ellis KA, Ellis J, Bartholomeusz CF, Ilic S, Croft RJ, Phan KL, Nathan PJ. (2005). Muscarinic and nicotinic receptor modulation of object and spatial n–back working memory in humans. Pharmacol Biochem Be, 8: 575–584.

Greenbaum CJ, Havel PJ, Taborsky GJ Jr, Klaff LJ. (1991). Intra–islet insulin permits glucose to directly suppress pancreatic A cell function. J Clin Invest, 88: 767–773.

Gregory C, Mathews MD. (2007). The Dual Roles of GABA in Seizures and Epilepsy Generate More Excitement. Epilepsy Curr, 7: 28–30.

Grenier F, Timofeev I, Steriade M. (2003). Neocortical very fast oscillations (ripples, 80–200 Hz) during seizures, intracellular correlates. J Neurophysiol, 89: 841–852.

Guillod J, Schmid–Saugeon P, Décaillet F, Panizzon R, Kunt M, Thiran JP. (2003). An open Internet platform to distributed image processing applied to dermoscopy. Stud Health Technol Inform, 95: 107–112.

Guldstrand Marie, Bo Ahren, Regina Wredling, Lars Backman, Per Eric Lins, and Ulf Adamson. (2003). Alteration of the Counterregulatory Responses to Insulin–Induced Hypoglycemia and of Cognitive Function After Massive Weight Reduction in Severely Obese Subjects. Metabolism, 52: 900–907.

Gurcharan K, Sukwinder LS. (1994). Effect of alloxan–induced diabetes on Na+/K+–ATPase activity from descrete areas of rat brain. Biochem. Mol. Biol. Int, 34: 781–788.

Gurwitz D, Haring R, Heldman E. (1994). Discrete activation of transduction pathways associated with acetylcholine M1 receptor by several muscarinic ligands. Eur J Pharmacol, 267: 21–31.

Gusovsky F, Leuden JE, Kohn EC. (1993). Muscarinic receptor mediated –tyrosine phophorylation of phospholipase C–g. J Biol Chem, 268: 7768–7772.

Haces ML, Hernández–Fonseca K, Medina–Campos ON, Montiel T, Pedraza–Chaverri J, Massieu L. (2008). Antioxidant capacity contributes to protection of ketone bodies against oxidative damage induced during hypoglycemic conditions. Exp Neurol, 211: 85–96.

Haces ML, Montiel T, Massieu L. Selective vulnerability of brain regions to oxidative stress in a non–coma model of insulin–induced hypoglycemia. (2010). Neuroscience, 165: 28–38.

Haddad GG, Jiang C. (1993). Mechanisms of anoxia–induced depolarization in brainstem neurons: in vitro current and voltage clamp studies in the adult rat. Brain Res, 625: 261–268.

Hales TG, Tyndale RF. (1994). Few cell lines with GABAA mRNAs have functional receptors. J Neurosci, 14: 5429–5436.

Halliwell B. (2006). Oxidative stress and neurodegeneration: where are we now?. J Neurochem, 97: 1634–1658.

Hamaguchi T, Fukushima H, Uehara M, Wada S, Shirotani T, Kishikawa H, Ichinose K, Yamaguchi K, Shichiri M. (1991). Abnormal glucagons response to arginine and its normalization in obese hyperinsulinaemic patients with glucose intolerance: importance of insulin action on pancreatic alpha cells. Diabetologia, 34: 801–806.

Hamilton SE, Loose MD, Qi M, Levey AI, Hille B, McKnight GS. (1997). Disruption of the m1 receptor gene ablates muscarinic receptor– dependent M current regulation and seizure activity in mice. Proc Natl Acad Sci USA, 94: 13311–13316.

Han Y, Qin J, Bu DF, Chang XZ, Yang ZX. (2006). Successive alterations of hippocampal gamma–aminobutyric acid B receptor subunits in a rat model of febrile seizure. Life Sci, 78: 2944–2952.

Hannonen R, Tupola S, Ahonen T, Riikonen R. (2003). Neurocognitive functioning in children with type–1 diabetes with and without episodes of severe hypoglycaemia. Dev. Med. Child Neurol, 45: 262–268.

Hansen AJ. (1985). Effect of anoxia on ion distribution in the brain. Physiol Rev, 65: 101–148.

Hara A, Niwa M, Kumada M, Kitaori N, Yamamoto T, Kozawa O, Mori H. (2004). Fragmented DNA transport in dendrites of retinal neurons during apoptotic cell death. Brain Res, 1007: 183–187.

Harty RF, Boharski MG, Bochna GS, Carr TA, Eagan PE, Rings M, Lassiter DC, Pour MP, Schafer DF, Markin RS. (1991). gamma–Aminobutyric acid localization and function as modulator of cholinergic neurotransmission in rat antral mucosal/submucosal fragments. Gastroenterology, 101: 1178–1186.

Hashimoto K, Ishima T, Fujita Y, Matsuo M, Kobashi T, Takahagi M, Tsukada H, Iyo M. (2008). Phencyclidine–induced cognitive deficits in mice are improved by subsequent subchronic administration of the novel selective alpha7 nicotinic receptor agonist SSR180711. Biol. Psychiatry, 63: 92–97.

Hassall C, Stanford C, Burnstock, Buckley NnJ. (1993). Co–expression of four muscarinic receptor genes by the intrinsic neurons of the rat and guinea–pig heart. Neuroscience, 56: 1041–1048.

Hausser M, Clark BA. (1997). Tonic synaptic inhibition modulates neuronal output pattern and spatiotemporal synaptic integration. Neuron, 19: 665–678.

Havel PJ, Parry SJ, Stern JS, Akpan JO, Gingerich RL, Taborsky GJ Jr, Curry DL. (1994). Redundant parasympathetic and sympathoadrenal mediation of increased glucagon secretion during insulin–induced hypoglycemia in conscious rats. Metabolism, 43: 860–866.

Havel PnJ, Dunning BnE, Verchere CnB, Baskin DnG, O'Dorisio T, Taborsky Jr GnJ. (1997). Evidence that vasoactive intestinal polypeptide is a parasympathetic neurotransmitter in the endocrine pancreas in dogs. Regul Pept, 71: 163–170.

Havrankova J, Roth J, Brownstein M. (1978). Insulin receptors are widely distributed in the central nervous system of the rat. Nature, 272: 827–829.

Hawdon JM. (1999). Hypoglycaemia and the neonatal brain. Eur J Pediatr, 158: 9–12.

Heffner TG, Hartman JA, Seiden LS. (1980). A rapid method for the regional dissection of the rat brain. Pharmacol. Biochem. Behav, 13: 453–456.

Heidenreich KA, Gilmore PR, Garvey WT. (1989). Glucose transport in primary cultured neurons. J. Neurosci Res, 22: 397–407.

Heller SR, Cryer PE. (1991). Reduced neuroendocrine and symptomatic responses to subsequent hypoglycaemia after 1 episode of hypoglycaemia in nondiabetic humans. Diabetes, 40: 223–226.

Henquin JC, Nenquin M. (1988). The muscarinic receptor subtype in mouse pancreatic B–cells. FEBS Lett, 15: 89–92.

Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman B, Montminy M. (2001). CREB regulates hepatic gluconeogenesis through the coactivator PGC–1. Nature, 413: 179–183.

Higley MJ, Contreras D. (2006). Balanced excitation and inhibition determine spike timing during frequency adaptation. J Neurosci, 26: 448–457.

Hohenegger M, Rudas B. (1971). Kidney function in experimental diabetic ketosis. Diabetologia, 7: 334–338.

Holst JJ, Grønholt R, Schaffalitzky de Muckadell OB, Fahrenkrug J. (1981). Nervous control of pancreatic endocrine secretion in pigs. I. Insulin and glucagon responses to electrical stimulation of the vagus nerves. Acta Physiol Scand, 111: 1–7.

Honig A, Bartlett JR, Bouras N, Bridges PK. (1988). Amino acid levels in depression: a preliminary investigation. Journal of Psychiatric Research, 22: 159–164.

Hosey MM. (1992). Diversity of structure, signaling and regulation within the family of muscarinic cholinergic receptors. FASEB Journal, 6: 845–852.

Hossein FS, Timothy DF, Teri JR, Paul DT. (2008). Expression of GABAB Receptors Is Altered in Brains of Subjects with Autism. The Cerebellum, 8: 64–69.

Houser CR, Esclapez M. (1994). Localization of mRNAs encoding two forms of glutamic acid decarboxylase in the rat hippocampal formation. Hippocampus, 4: 530–545.

Howell SL, Taylor KW. (1968). Pottassium ions and the secretions of insulin by islets of langerhans incubated in vitro. Biochem. J, 108: 17–24.

Hoyer S. (1998). Is sporadic Alzheimer disease the brain type of non insulin dependent diabetes mellitus? A challenging hypothesis. J Neural Transm, 105: 415–422.

Hsu KS, Huang CC, Gean PW. (1995). Muscarinic depression of excitatory synaptic transmission mediated by the presynaptic M3 receptors in the rat striatum. Neurosci. Lett, 197: 141–144.

Hulme EC, Birdsall NJM, Buckley NJ. (1990). Muscarinic receptor subtypes. Annu Rev Pharmacol Toxicol, 30: 633–673.

Hvidberg A, Fanelli CG, Hershey T, Terkamp C, Craft S, Cryer PE. (1996). Impact of recent antecedent hypoglycaemia on hypoglycaemic cognitive dysfunction in nondiabetic humans. Diabetes, 46: 1031–1036.

Iismaa TP, Kerr EA, Wilson JR, Carpenter L, Sims N, Biden TJ. (2000). Quantitative and functional characterization of muscarinic receptor subtypes in insulin–secreting cell lines and rat pancreatic islets. Diabetes, 49: 392–398.

Ilcol YO, Gurun MS, Taga Y, Ulus IH. (2003). Choline increases serum insulin in rat when injected intraperitoneally and augments basal and stimulated aceylcholine release from the rat minced pancreas in vitro. Eur J Biochem, 270: 991–999.

Inglis WL, Olmstead MC, Robbins TW. (2001). Selective deficits in attentional performance on the 5–choice serial reaction time task following pedunculopontine tegmental nucleus lesions. Behav Brain Res, 123: 117–131.

Ishida K, Mizuno A, Sano T, Shi K, Shima K. (1993). Plasma glucagon responses to insulin–induced hypoglycemia and arginine in spontaneous non–insulin–dependent diabetes mellitus (NIDDM) rats, Otsuka Long–Evans Tokushima Fatty (OLETF) strain. Acta Endocrinol, 129: 585–593.

Ishihara H, Maechler P, Gjinovci A, Herrera PL, Wollheim CB. (2003). Islet beta–cell secretion determines glucagon release from neighbouring alpha–cells. Nat. Cell Biol, 5: 330–335.

Jabbari B, Scherokman B, Gunderson CH, Rosenberg ML, Miller. (1989). Treatment of movement disorders with trihexyphenidyl. J Mov Disord, 4: 202–212.

Jackson AL, Rashed H, Cardoso S, Wong F, Werkman R, Thompson J, Abell TL. (2000). Assessment of gastric electrical activity and autonomic function among diabetic and nondiabetic patients with symptoms of gastroesophageal reflux. Dig Dis Sci, 45: 1727–1730.

Jackson J, Paulose CS. (1999). Enhancement of [m–methoxy [3 H] MDL 100907 binding to 5–HT 2A receptors in the cerebral cortex and brain stem of streptozotocin induced diabetic rats. Mol. Cell. Biochem, 199: 81–85.

Jacob RJ, Weber AB, Dziura J, Morgen J, Sherwin RS. (1995). Brainstem dysfunction is provoked by a less pronounced hypoglycaemic stimulus in diabetic BB rats. Diabetes, 44: 900–905.

Jane EM . (1999). Neonatal Hypoglycaemia. Pediatrics in Review, 20: 6–15.

Jean–Marc F. (2008). Epilepsy, E/I Balance and GABAA Receptor Plasticity. Front Mol Neur, Doi: 10.3389/neuro.02.005.

Jobin M, Peeyush T, Reas S, Paulose CS. (2010). Behavioural Deficit and Decreased GABA Receptor in the Cerebellum of Epileptic Rats: Effect of Bacopa monnieri and Bacoside–A. Epilepsy and Behavior, doi:10.1016/j.yebeh.2010.01.012.

Johnson E, Deckwerth T. (1993). Molecular mechanisms of developmental neuronal death, Armu. Rev. Neurosci, 16: 31–46.

Johnston MV. (2005). Excitotoxicity in perinatal brain injury. Brain Pathol, 15: 234–240.

Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, Yao WJ, Johnson M, Gunwaldsen C, Huang LY, Tang C, Shen Q, Salon JA, Morse K, Laz T, Smith KE, Nagarathnam D, Noble SA, Branchek T A, Gerald C. (1998). GABA B receptors function as a heteromeric assembly of the subunits GABABR1 and GABABR2. Nature, 396: 674–679.

Jones TW, Davis EA. (2003). Hypoglycemia in children with type 1 diabetes: current issues and controversies. Pediatr Diabetes, 4: 143–150.

Jose PA, Yu PY, Yamaguchi I, Eisner GM, Mouradian MM, Felder CC, Felder RA. (1995). Dopamine D1 receptor regulation of phospholipase C. Hypertens Res, 18: 39–42.

Joseph A, Remya R, Paulose CS. (2007). Enhanced [3H] Glutamate Binding in the Cerebellum of Insulin Induced Hypoglycaemic and Streptozotocin Induced Diabetic Rats. Cellular and Molecular Neurobiology, 27: 1085–1095.

Joseph A, Sherin B, Paulose CS. (2008). Increased glutamate receptor gene expression in the cerebral cortex of insulin induced hypoglycaemic and streptozotocin induced diabetic rats. Neuroscience, 156: 298–304.

Joseph A, Peeyush KT, Nandhu MS, Paulose CS. (2010). Enhanced NMDAR1, NMDA2B and mGlu5 receptors gene expression in the cerebellum of insulin

induced hypoglycaemic and streptozotocin induced diabetic rats. European Journal of Pharmacology, 630: 61–68.

Julius D, Livelli T, Jessell TM, Axel R. (1989). Ectopic expression of the serotonin 1c receptor and the triggering of malignant transformation. Science, 244: 1057–1062.

Junod A, Lambert AE, Staufferacher W, Renold AE. (1969). Diabetogenic action of Streptozotocin: Relationship of dose to metabolic response. J Clin Invest, 48: 2129–2139.

Kadekaro M, Ito M, Gross PM. (1988). Local cerebral glucose utilization is increased in acutely adrenalectomized rats. Neuroendocrinology, 47: 329–334.

Kaimal Balarama S, Gireesh G, Paulose CS. (2007). Decreased GABAA receptor function in the brain stem during pancreatic regeneration in rats. Neurochem Res, 32: 1813–1822.

Kale AY, Paranjape SA, Briski KP. (2006). Type II glucocorticoid receptor involvement in habituated activation of lateral hypothalamic area orexin–A–immunopositive neurons during recurring insulin–induced hypoglycemia. Neurosci Res, 56: 309–313.

Kalimo H, Olsson Y. (1980). Effects of severe hypoglycemia on the human brain. Neuropathological case reports. Acta Neurol Scand, 62: 345–356.

Kaneda M, Mochizuki M, Aoki K, Kaneko A. (1997). Modulation of GABA (C) response by Ca2+ and other divalent cations in horizontal cells of the catfish retina. Journal of General Physiology, 110: 741–747.

Kanjhan R, Housley GD, Burton LD, Christie DL, Kippenberger A, Thorne PR, Luo L, Ryan AF. (1999). Distribution of the P2X2 receptor subunit of the ATP–gated ion channels in the rat central nervous system. J Comp. Neurol, 407: 11–32.

Kar S, Chabot JG, Quirion R. (1993). Quantitative autoradiographic localization of [125I] insulin–like growth factor I, [125I] insulin–like growth factor II, and [125I] insulin receptor binding sites in developing and adult rat brain. J Comp Neurol, 333: 375–397.

Karbon EW, Duman RS, Enna SJ. (1984). GABAB receptors and norepinenphrine–stimulated c–AMP production in rat brain cortex. Brain Res, 290: 327–332.

Karlsson S, Ahren B. (1998). Insulin and glucagon secretion by ganglionic nicotinic activation in adrenalectomized mice. Eur J Pharmacol, 342: 291–295.

Karp MM. (1989). Hypoglycemia in diabetes among children and adolescents. Indian J Pediatr, 56: 93–98.

Kash SF, Johnson RS, Tecott LH, Noebels JL, Mayfield RD, Hanahan D, Baekkeskov S. (1997). Epilepsy in mice deficient in the 65–kDa isoform of glutamic acid decarboxylase. Proc Natl Acad Sci U S A, 94: 14060–14065.

Kashihara K, Varga EV, Waite SL, Roeske WR, Yamamura HI. (1992). Cloning of the rat M3, M4 and M5 muscarinic acetylcholine receptor genes by the polymerase chain reaction PCR and the pharmacological characterization of the expressed genes. Life Sci, 51: 955–971.

Katz RJ, Chudler R. (1980). Y–Maze behavior after an analog of ACTH 4–9, evidence for an attentional alteration. Psychopharmacology, 71: 95–96.

Kaufman DL, Houser CR, Tobin AJ. (1991). Two forms of the gamma–aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. J Neurochem, 56: 720–723.

Kaufman FR. (1998). Diabetes in children and adolescents. Areas of controversy. Med Clin North Am, 82: 721–738.

Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W, Bettler B. (1997). Expression cloning of GABAB receptors uncovers similarity to metabotropic glutamate receptors. Nature, 386: 239–246.

Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, Karschin A, Bettler B. (1998). GABA B–receptor subtypes assemble into functional heteromeric complexes. Nature, 396: 683–687.

Kaupmann K, Cryan JF, Wellendorph P, Mombereau C, Sansig G, Klebs K, Schmutz M, Froestl W, van der Putten H, Mosbacher J, Brauner–Osborne H, Waldmeier P, Bettler B. (2003). Specific gamma–hydroxybutyrate–binding sites but loss of pharmacological effects of gamma–hydroxybutyrate in GABA(B)(1)–deficient mice. Eur J Neurosci, 18: 2722–2730.

Kawamori D, Kurpad AJ, Hu J, Liew CW, Shih JL, Ford EL, Herrera PL, Polonsky KS, McGuinness OP, Kulkarni RN. (2009). Insulin Signaling in alpha Cell Modulates Glucagon Secretion In Vivo. Cell Metabolism, 9: 350–361.

Kayadjanian N, Menétrey A, Besson MJ. (1997). Activation of muscarinic receptors stimulates GABA release in the rat globus pallidus. Synapse, 26: 131–139.

Kelley GG, Zawalich KC, Zawalich WS. (1995). Am J Physiol, 269: 575–582.

Kenakin TP, Boselli C. (1990). Promiscuous or heterogenous muscarinic receptors in rat atria? Schild analysis with competitive antagonists. Eur J Pharmacol, 191: 39–48.

Kinami S, Miwa K, Sato T, Miyazaki I. (1997). Section of the vagal celiac branch in man reduces glucagon–stimulated insulin release. J Auton Nerv Syst, 64: 44–48.

Kirchgessner AL, Liu MT. (1998). Immunohistochemical localization of nicotinic acetylcholine receptors in the guinea pig bowel and pancreas. J Comp Neurol, 390: 497–514.

Kirchner A, Velískova J, Velisek L. (2006). Differential effects of low glucose concentrations on seizures and epileptiform activity in vivo and in vitro. Eur J Neurosci, 23: 1512–1522.

Kitaichi K, Hori T, Srivastava LK, Quirion R. (1999). Antisense oligodeoxynucleotides against the muscarinic m2, but not m4, receptor supports its role as autoreceptors in the rat hippocampus. Brain Res Mol, 67: 98–106.

Kittler JT, Moss SJ. (2003). Modulation of GABAA receptor activity by phosphorylation and receptor trafficking: implications for the efficacy of synaptic inhibition. Curr Opin Neurobiol, 13: 341–347.

Kobayashi S, Fujita T. (1969). Fine structure of mammalian and avian pancreatic islets with special reference to D cells and nervous elements. Z Zellforsch Mikrosk Anat, 100: 340–363.

Komuro H, Rakic P. (1996). Intracellular Ca fluctuations modulate the rate of neuronal migration. Neuron, 17: 275–285.

Kopf SR, Baratti CM. (1996). Effects of posttraining administration of glucose on retention of a habituation response in mice: participation of a central cholinergic mechanism. Neurobiol Learn Mem, 65: 253–260.

Krantis A, Tufts K, Nichols K, Morris GP. (1994). [3H] GABA uptake and GABA localization in mucosal endocrine cells of the rat stomach and colon. J Auton Nerv Syst, 47: 225–232.

Kreil A, Richter A. (2005). Antidystonic efficacy of gamma–aminobutyric acid uptake inhibitors in the dtsz mutant. Eur J Pharmacol, 521: 95–98.

Krenz I, Kalkan D, Wevers A, de Vos RA, Steur EN, Lindstrom J, Pilz K, Nowacki S, Schütz U, Moser N, Witter B, Schröder H. (2001). Parvalbumin–containing interneurons of the human cerebral cortex express nicotinic acetylcholine receptor proteins. J Chem Neuroanat, 21: 239–246.

Krimer LS, Goldman–Rakic PS. (2001). Prefrontal microcircuits: membrane properties and excitatory input of local, medium, and wide arbor interneurons. J Neurosci, 21: 3788–3796.

Krinsley JS, Grover A. (2007). Severe hypoglycemia in critically ill patients: risk factors and outcomes. Crit Care Med, 35: 2262–2267.

Krishnakumar A, Nandhu MS, Paulose CS. (2009). Upregulation of 5–HT2C receptors in hippocampus of pilocarpine–induced epileptic rats: antagonism by Bacopa monnieri. Epilepsy Behav, 16: 225–230.

Krnjevic K. (1993). Central cholinergic mechanisms and function, Prog. Brain Res, 98: 285–92.

Krnjevic´ K. (2008). Electrophysiology of cerebral ischemia. Neuropharmacology, 55: 319–33.

Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, Epperson CN, Goddard A, Mason GF. (2002). Glutamate and GABA systems as targets for novel antidepressant and mood–stabilizing treatments. Molecular Psychiatry, 7: 71–80.

Kuhad A, Pilkhwal S, Sharma S, Tirkey N, Chopra K. (2007). Effect of curcumin on inflammation and oxidative stress in cisplatin–induced experimental nephrotoxicity. J Agric Food Chem, 55: 10150–10155.

Kuhl DE, Koeppe RA, Minoshima S, Snyder SE, Ficaro EP, Foster NL, Frey KA, Kilbourn MR. (1999). In vivo mapping of cerebral acetylcholinesterase activity in aging and Alzheimer's disease. Neurology, 52: 691–699.

Kulkarni RN. (2002). Receptors for insulin and insulin–like growth factor–1 and insulin receptor substrate–1 mediate pathways that regulate islet function. Biochem Soc Trans, 30: 317–322.

Kumagai AK. (1995). Upregulation of blood brain barrier Glut–1 glucose transporter protein and mRNA in experimental chronic hypoglycemia. Diabetes, 44: 1399–1404.

Kumagai AK. (1999). Glucose transport in brain and retina: implications in the management and complications of diabetes. Diabetes Metab Res Rev, 15: 261–273.

Kumar PJ, Clark M. (2002). Diabetes mellitus and other disorders of metabolism. In. Clinical Medicine, 1069–1121.

Kuner R, Kohr G, Grunewald S, Eisenhardt G, Bach A, Kornau HC. (1999). Role of heteromer formation in GABAB receptor function. Science, 283: 74–77.

Kurioka S, Toshiaki k, Makoto M. (1981). Effect of Sodim and Bicarbonate Ions on GABA Receptor binding in synaptic membrane of rat brain. Journal of Neurochemistry, 37: 418–421.

Kusama T, Sakurai M, Kizawa Y, Uhl GR, Murakami H. (1995). GABA rho 1 receptor: inhibition by protein kinase C activators. European Journal of Pharmacology, 291: 431–434.

Kusske JA. (1979).Corticocaudatothalamic interactions in experimental focal epilepsy in the cat. Exp Neurol, 65: 616–624.

Kwag OG, Kim SO, Choi JH, Rhee IK, Choi MS, Rhee SJ. (2001). Vitamin E improves microsomal phospholipase A2 activity and the arachidonic acid cascade in kidney of diabetic rats. J Nutr, 131: 1297–1301.

Labrakakis, C., Müller, T., Schmidt, K. and Kettenmann, H. (1997). GABA(A) receptor activation triggers a Cl– conductance increase and a K+ channel blockade in cerebellar granule cells. Neuroscience, 79: 177–189.

Lackovic Z, Salkovic M, Kuci Z, Relja M. (1990). Effect of long–lasting diabetes mellitus on rat and human brain monoamines. J Neurochem, 51: 143–147.

Lahat E, Barr J, Bistritzer T. (1995). Focal epileptic episodes associated with hypoglycemia in children with diabetes. Clin Neurol Neurosurg. 97: 314–316.

Lang C. (1995). Inhibition of central GABAA receptors enhances hepatic glucose production and peripheral glucose uptake. Brain Res Bull, 37: 611–616.

Langan SJ, Deary IJ, Hepburn DA, Frier BM. (1991). Cumulative cognitive impairment following recurrent severe hypoglycaemia in adult patients with insulin–treated diabetes mellitus. Diabetologia, 34: 337–344.

Laughlin SB. (2004). The implications of metabolic energy requirements for the representation of information in neurons. The cognitive neurosciences, 3: 187–196.

Lazareno S, Buckley NJ, Roberts FF. (1990). Characterisation of muscarinic M4 binding sites in rabbit lung, chicken heart and NG108–15 cells. Mol Pharmacol, 53: 573–589.

Lees GJ. (1991). Inhibition of sodium–potassium–ATPase: a potentially ubiquitous mechanism contributing to central nervous system neuropathology. Brain Res Rev, 16: 283–300.

Legg PG. (1967). The fine structure and innervation of the ß and ∂cells in the islet of Langerhans of the cat. Z Zellforsch Mikrosk Anat, 80: 307–321.

Levey A, Edmunds S, Koliatsos V, Wiley R, Heilman C. (1995). Expression of ml–m4 muscarinic acetylcholine receptor proteins in rat hippocampus and regulation by cholinergic innervation. J. Neurosci, 15: 4077–4092.

Levey AI. (1993). Immunological localization of M1–M5 muscarinic acetylcholine receptors in peripheral tissue and brain. Life Sci, 52: 441–448.

Levin ED, Bradley A, Addy N, Sigurani N. (2002). Hippocampal alpha 7 and alpha 4 beta 2 nicotinic receptors and working memory. Neuroscience, 109: 757–765.

Levin BE, Routh VH, Kang L, Sanders NM, Dunn_Meynell AA. (2004). Neuronal glucosensing: what do we know after 50 years? Diabetes, 53: 2521–2528.

Levin ED, Simon BB. (1998). Nicotinic acetylcholine involvement in cognitive function in animals. Psychopharmacology, 138: 217–230.

Levin ED. (1992). Nicotinic systems and cognitive function. Psychopharmacology, 108: 417–431.

Levine AJ. (1997). p53, the cellular gatekeeper for growth and division. Cell, 88: 323–331.

Lingenfelser T, Overkamp D, Renn W, Buettner U, Kimmerle K, Schmalfuss A, Jakober B. (1996). Insulin–associated modulation of neuroendocrine counterregulation, hypoglycemia perception, and cerebral function in insulin–dependent diabetes mellitus: evidence for an intrinsic effect of insulin on the central nervous system. Journal of Clinical Endocrinology & Metabolism, 81: 1197–1205.

Lipton P. (1999). Ischemic cell death in brain neurons. Physiol Rev, 79: 1431–568.

Lismaa TP, Kerr E, Wilson J, Carpenter L, Sims N, Bidden T. (2000). Quantitative and functional characterization of muscarinic rceptor subtypes in insulin–secreting cell lines and rat pancreatic islets. Diabetes, 49: 392–408.

Liu L, Brown JC 3rd, Webster WW, Morrisett RA, Monaghan DT. (1995). Insulin potentiates N–methyl–D–aspartate receptor activity in Xenopus oocytes and rat hippocampus. Neurosci Lett, 192: 5–8.

Liu WH, Chang LS. (2009). Reactive oxygen species and p38 mitogen–activated protein kinase induce apoptotic death of U937 cells in response to Naja nigricollis toxin–gamma. J Cell Mol Med, 8B: 1695–1705.

Lloyd KG, Munari C, Bossi L, Stoeffels C, Talairach J, Morselli PL. (1981). Biochemical evidence for the alterations of GABA– mediated synaptic transmission in pathological brain tissue (stereo EEG or morphological definition) from epileptic patients. In: Morselli PL (eds). Neuro– transmitters, Seizures, and Epilepsy. Raven press, New York, P: 325–338.

Love JA, Szebeni K. (1999). Morphology and histochemistry of the rabbit pancreatic innervation. Pancreas, 18: 53–64.

Low PA, Nickander KK, Tritschler HJ. (1997). The role of oxidative stress and antioxidant treatment in experimental diabetic neuropathy. Diabetes, 46: 38–42.

Lowry OH, Roserbrough NJ, Farr AL, Randall RJ. (1951). Protein measurements and folin phenol reagent. J Biol Chem, 193: 265–275.

Luiten PG, Ter Horst GJ, Buijs RM, Steffens AB. (1986). Autonomic innervation of the pancreas in diabetic and non–diabetic rats: A new view on intramural sympathetic structural organization. J Auton Nerv Syst, 15: 33–44.

Luo Z, McMullen NT, Costy–Bennett S, Fregosi RF. (2007). Prenatal nicotine exposure alters glycinergic and GABAergic control of respiratory frequency in the neonatal rat brainstem–spinal cord preparation. Respiratory Physiology & Neurobiology, 157: 226–234.

Ma MX, Chen YM, He J, Zeng T, Wang JH. (2007). Effects of morphine and its withdrawal on Y–maze spatial recognition memory in mice. Neuroscience, 147: 1059–1065.

MacDermott AB, Role LW, Siegelbaum SA. (1999). Presynaptic ionotropic receptors and the control of transmitter release. Annu Rev Neurosci, 22: 443–485.

Maechler P, Wollheim CB. (2000). Mitochondrial signals in glucose–stimulated insulin secretion in the beta cell. J Physiol, 529: 49–56.

Magarinos AM Jain K, Blount ED, Reagan L, Smith BH, McEwen BS. (2001). Peritoneal implantation of macroencapsulated porcine pancreatic islets in diabetic rats ameliorates severe hyperglycemia and prevents retraction and simplification of hippocampal dendrites. Brain Res, 902: 282–287.

Maher F, Simpson IA. (1994). Modulation of expression of glucose transporters GLUT3 and GLUT1 by potassium and N–methyl–D–aspartate in cultured cerebellar granule neurons. Mol Cell Neurosci, 5: 369–375.

Mares P, Kubova H, Zouhar A, Folbergrova J, Koryntova H, Stankova L. (1993). Motor and electrocorticographic epileptic activity induced by 3–mercaptopropionic acid in immature rats. Epilepsy Res, 16: 11–18.

Margaill I, Plotkine M, Lerouet D. (2005). Antioxidant strategies in the treatment of stroke. Free Radic Biol Med, 39: 429–443.

Marino MJ, Wittmann M, Bradley SR, Hubert GW, Smith Y, Conn PJ. (2001). Activation of group I metabotropic glutamate receptors produces a direct excitation and disinhibition of GABAergic projection neurons in the substantia nigra pars reticulata. J Neurosci, 21: 7001–7012.

Marshall JF, Friedman MI, Heffner TG. (1976). Reduced anorexic and locomotor–stimulant action of D–amphetamine in alloxan–diabetic rats. Brain Res, 111: 428–432.

Martin DL & Tobin AJ. (2000). Mechanisms controlling GABA synthesis and degredation in the brain. In: Martin DL & Olsen R (eds). GABA in the Nervous System. Williams & Wilkins, Philadelphia, P: 25–41.

Martin DL, Rimvall K. (1993). Regulation of gamma–aminobutyric acid synthesis in the brain. J Neurochem, 60: 395–407.

Martin RL, Lloyd HGE, Cowan AI. (1994). The early events of oxygen and glucose deprivation: setting the scene for neuronal death? Trends Neurosci, 17: 251–257.

Martone ME, Armstrong DM, Young SJ, Groves PM. (1992). Ultrastructural examination of enkephalin and substance P input to cholinergic neurons within the rat neostriatum. Brain Res, 594: 253–262.

Maruyama H, Hisatomi A, Orci L, Grodsky GM, Unger RH. (1984). Insulin within islets is a physiologic glucagon release inhibitor. J. Clin. Invest, 74: 2296–2299.

Matsui M, Motomura D, Karasawa H, Fujikawa T, Jiang J, Komiya Y, Takahashi S, Taketo MM. (2000). Multiple functional defects in peripheral autonomic organs in mice lacking muscarinic acetylcholine receptor gene for the M3 subtype. PNAS, 97: 9579–9584.

Matsumoto K, Ueda S, Hashimoto T, Kuriyama K. (1991). Ischemic neuronal injury in the rat hippocampus following transient forebrain ischemia: evaluation using in vivo microdialysis. Brain Res, 543: 236–242.

Mayr B, Montminy M. (2001). Transcriptional regulation by the phosphorylation–dependent factor CREB. Nat Rev Mol Cell Biol, 2: 599–609.

McAulay V, Deary IJ, Frier BM. (2001). Symptoms of hypoglycaemia in people with diabetes. Diabet Med, 18: 690–705.

McCall AL. (1992). The impact of diabetes on the CNS. Diabetes, 41: 557–570.

McCall AL. (2004). Cerebral glucose metabolism in diabetes mellitus. Eur J Pharmacol, 490: 147–158.

McCrimmon RJ, Sherwin RS. (1999). Effect of pancreatic transplantation on counterregulatory hormonal responses to hypoglycemia. Acta Diabetol, 36: 11–20.

McCulloch DK, Raghu PK, Koerker DJ, Palmer JP, Klaff LJ. (1989). Responses of the pancreatic A cell during hypoglycemia and hyperglycemia are dependent on the B cell. Metabolism, 38: 702–707.

McEwen BS, Sapolsky RM. (1995). Stress and cognitive function. Curr Opin Neurobiol, 5: 205–216.

McNay EC, Cotero VE. (2010). Impact of recurrent hypoglycemia on cognitive and brain function. Physiol Behav, doi:10.1016/j.physbeh.2010.01.004.

McNay EC, McCarty RC, Gold PE. (2001). Fluctuations in brain glucose concentration during behavioral testing: Dissociations between brain areas and between brain and blood. Neurobiology of Learning and Memory, 75: 325–327.

McNay EC, Williamson A, McCrimmon RJ, Sherwin RS. (2006). Cognitive and Neural Hippocampal Effects of Long–Term Moderate Recurrent Hypoglycemia. Diabetes, 55: 1088–1095.

Meerson FZ, Kagan VE, Kozlov YP, Belkina LM, Arkhipenko YV. (1982). The role of lipid peroxidation in pathogenesis of ischemic damage and antioxidant protection of the heart. Basic Res Cardiol, 77: 465–468.

Meisenhelder J, Suh PG, Rhee SG. (1989). Phopholipase C–g is a substrate for the PDGF and EGF receptor protein–tyrosine kinases in vivo and in vitro. Cell, 57: 1109–1122.

Merlo D, Mollinari C, Inaba Y, Cardinale A, Rinaldi AM, D'Antuono M, D'Arcangelo G, Tancredi V, Ragsdale D, Avoli M. (2007). Reduced GABAB receptor subunit

expression and paired–pulse depression in a genetic model of absence seizures. Neurobiol Dis, 25: 631–641.

Messier C, Gagnon M. (1996). Glucose regulation and cognitive functions: relation to Alzheimer's disease and diabetes Behavioural Brain Research, 75: 1–11.

Meyer T, Wellner– Kienitz MC, Biewald A, Bender K, Eickel A, Pott L. (2001). Depletion of phosphatidylinositol 4, 5–bisphosphate by activation of phospholipase C–coupled receptors causes slow inhibition but not desensitization of G protein–gated inward rectifier K+ current in atrial myocytes. J Biol Chem, 276: 5650–5658.

Miller GH. (1980). Devices to help smokers stop don't. Am Pharm, 20: 53.

Miller RE. (1981). Pancreatic neuroendocrinology peripheral neural mechanisms in the regulation of the islets of Langerhans. Endocr Rev, 2: 471–494.

Minna LK, Pasi IS, Juhani AKE, Juha ST, Aimo R, Timo HM, Heikki VH. (2005). Effects of Sustained Insulin–Induced Hypoglycaemia on Cardiovascular Autonomic Regulation in Type 1 Diabetes. Diabetes, 54: 744–750.

Misgeld U, Bijak M, Jarolinek W. (1995). A physiological role for GABAB receptors and the effects of baclofen in the mammalian central nervous system. Prog. Neurobiol, 46: 423–462.

Mita Y, Dobashi K, Suzuki K, Mori M, Nakazawa T. (1996). Induction of muscarinic receptor subtypes in monocytic/macrophagic cells differentiated from EoL–1 cells. Eur J Pharmacol, 297: 121–127.

Miyakawa T, Yamada M, Duttaroy A, Wess J. (2001). Hyperactivity and intact hippocampus–dependent learning in mice lacking the M1 muscarinic acetylcholine receptor. J Neurosci, 21: 5239–5250.

Miyashita T, Reed JC. (1995). Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell, 80: 293–299.

Moan A, Høieggen A, Nordby G, Os I, Eide I, Kjeldsen SE. (1995). Mental Stress Increases Glucose Uptake During Hyperinsulinemia:Associations With Sympathetic and Cardiovascular Responsiveness. Metabolism, 44: 1303–1307.

Mody I, Pearce RA. (2004). Diversity of inhibitory neurotransmission through GABA(A) receptors. Trends Neurosci, 27: 569–575.

Moley KH, Mueckler MM. (2000). Glucose transport and apoptosis. Apoptosis, 5: 99–105.

Monmaur P, Sharif A, M'Harzi M. (1997). Involvement of septal muscarinic receptors in cholinergically mediated changes in rat rearing activity. Pharmacol Biochem Behav, 58: 577–582.

Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg PH. (1992). Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. Science, 256: 1217–1221.

Mooradian AD. (1988). Diabetic complications of the central nervous system. Endocr. Rev, 9: 346–356.

Moos DD. (2007). Central anticholinergic syndrome: a case report. J Perianesth Nurs, 22: 309–321.

Moss SJ, Smart TG. (1996). Modulation of amino acid–gated ion channels by protein phosphorylation. International Review of Neurobiology, 39: 1–52.

Murugesan T. (2005). Evaluation of psychopharmacological effects of Linn. Extract. Phytomedicine, 8: 472–476.

Myojin T, Kitamura N, Hondo E, Baltazar ET, Pearson GT, Yamada J. (2000). Immunohistochemical localization of neuropeptides in bovine pancreas. Anat Histol Embryol, 29: 167–172.

Nagamatsu SH, Sawa K, Kamada Y, Nakamichi K, Yoshimoto, Hoshino T. (1993). Neuron–specific glucose transporter (NSGT): CNS distribution of GLUT3 rat glucose transporter (RGT3) in rat central neurons. FEBS Lett, 334: 289–295.

Naguib JM, Kulinskaya E, Lomax CL, Garralda ML. (2009). Neurocognitive performance in children with type 1 diabetes–a metaanalysis. J Pediatr Psychol, 34: 271–282.

Nakamura S, Muramatsu S, Yoshida M. (1990). Role of the basal ganglia in manifestation of rhythmical jaw movement in rats. Brain Res, 535: 335–338.

Nishimura T, Schwarzer C, Gasser E, Kato N, Vezzani A, Sperk G. (2005). Altered expression of GABAA and GABAB receptor subunit mRNAs in the hippocampus after kindling and electrically induced status epilepticus. Neuroscience, 134: 691–704.

Nusser Z, Sieghart W, Somogyi P. (1998). Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J Neurosci, 18: 1693–1703.

O'Leary KT, Leslie FM. (2003). Developmental regulation of nicotinic acetylcholine receptor–mediated [3H] norepinephrine release from rat cerebellum. J Neurochem, 84: 952–959.

O'Neill BT KJ, Wende AR, Theobald HA, Tuinei J, Buchanan J, Guo A, Zaha VG, Davis DK, Schell JC, Boudina S, Wayment B, Litwin SE, Shioi T, Izumo S, Birnbaum MJ, Abel ED. (2007). A conserved role for phosphatidylinositol 3–kinase but not Akt signaling in mitochondrial adaptations that accompany physiological cardiac hypertrophy. Cell Metab, 6: 294–306.

O'Brien RM, Granner DK. (1996). Regulation of gene expression by insulin. Physiol Rev, 76: 1109 –1161.

Ohtani N, Ohta M, Sugano T. (1997). Microdialysis study of modification of hypothalamic neurotransmitters in streptozotocin–diabetic rats. J. Neurochem, 69: 1622–1628.

Olianas MC, Adem A, Karlsson E, Onali P. (1996). Rat striatal muscarinic receptors coupled to inhibition of adenylyl cyclase activity: Potent block by the selective M4 ligand muscarinic toxin 3 (MT3). Br J Pharmacol, 118: 283–288.

Oliver DL, Huerta MF. (1992). Inferior and superior colliculus. In: The Mammalian Auditory Pathway (eds Webster, D.A., Popper, A.N. and Fay, R.R.), Springer Verlag, Berlin, New York. P: 168–221.

Olson AL, Pessin JE. (1996). Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. Annu. Rev. Nutr, 16: 235–256.

Olsen WR, Avoli M. (1997). GABA and epileptogenesis. Epilepsia, 38: 399–407.

Ouyang C, Guo L, Lu Q, Xu X, Wang H. (2007). Enhanced activity of GABA receptors inhibits glutamate release induced by focal cerebral ischemia in rat striatum. Neurosci Lett, 420: 174–178.

Owen C, Wanling Z, Yuyan D, Rory JM, Robert SS. (2006). Blockade of GABAA Receptors in the Ventromedial Hypothalamus Further Stimulates Glucagon and Sympathoadrenal but Not the Hypothalamo–Pituitary–Adrenal Response to Hypoglycaemia. Diabetes, 55: 1080–1087.

Padayatti PS, Paulose CS. (1999). Alpha2 adrenergic and high affinity serotonergic receptor changes in the brain stem of streptozotocin–induced diabetic rats. Life Sci, 65: 403–414.

Paik SG, Fleischer N, Shin SI. (1980). Insulin–dependent diabetes mellitus induced by subdiabetogenic doses of streptozotocin: obligatory role of cell –mediated autoimmune processes. Proc. Natl. Acad. Sci, 77: 6129–6133.

Palovcik RA, Phillips MI, Kappy MS, Raizada MK. (1984). Insulin inhibits pyramidal neurons in hippocampal slices. Brain Res, 309: 187–191.

Pang KC, Nocera R, Secor AJ, Yoder RM. (2001). GABAergic septohippocampal neurons are not necessary for spatial memory. Hippocampus, 11: 814–827.

Paramo B, Hernández–Fonseca K, Estrada–Sánchez AM, Jiménez N, Hernández–Cruz A, Massieu L. (2010). Pathways involved in the generation of reactive oxygen and nitrogen species during glucose deprivation and its role on the death of cultured hippocampal neurons. Neuroscience, 167: 1057–1069.

Paramore DS, Fanelli CG, Shah SD, Cryer PE. (1998). Forearm norepinephrine spillover during standing, hyperinsulinemia, and hypoglycemia. Am J Physiol, 275: E872–E881.

Pardridge WM, Triguero D, Farrell CR. (1990). Down–regulation of blood brain barrier glucose transporter in experimental diabetes. Diabetes, 39: 1040–1044.

Pardridge WM. (1983). Brain metabolism: a perspective from the blood–brain barrier. Physiol. Rev, 63: 1481–1535.

Pare D, Steriade M. (1993). The reticular thalamic nucleus projects to the contralateral dorsal thalamus in macaque monkey. Neurosci Lett, 154: 96–100.

Park CR, Seeley RJ, Craft S, Woods SC. (2000). Intracerebroventricular insulin enhances memory in a passive–avoidance task. Physiol Behav, 68: 509–514.

Park CR. (2001). Cognitive effects of insulin in the central nervous system. Neurosci. Biobehav. Rev, 25: 311–323.

Parvizi J, Damasio AR. (2003). Neuroanatomical correlates of brainstem coma. Brain, 126: 1524–1536.

Patel DG. (1984). Role of parasympathetic nervous system in glucagon response to insulin–induced hypoglycemia in normal and diabetic rats. Metabolism, 33: 1123–1127.

Patockova J, Marhol P, Tumova E, Krsiak M, Rokyta R, Stipek S, Crkovska J, Andel M. (2003). Oxidative stress in the brain tissue of laboratory mice with acute post insulin hypoglycemia. Physiol Res, 52: 131–135.

Patrick AW, Campbell IW. (1990). Fatal hypoglycaemia in insulin–treated diabetes mellitus: clinical features and neuropathological changes. Diabet Med, 7: 349–354.

Paty PB, Graeff RW, Mathes SJ, Hunt TK. (1990). Superoxide production by wound neutrophils. Evidence for increased activity of the NADPH oxidase. Arch Surg, 125: 65–69.

Paul C, Schöberl F, Weinmeister P, Micale V, Wotjak CT, Hofmann F, Kleppisch T. (2008). Signaling through cGMP–dependent protein kinase I in the amygdala is critical for auditory–cued fear memory and long–term potentiation. J Neurosci, 28: 14202–14212.

Paulose CS, Padayatti PS, Sudha B. (1999). Neurotransmitter– Hormonal regulation of diabetes and liver regeneration. Comparative Endocrinology and Reproduction, Narosa Publishing House, NewDelhi, India, P: 559–568.

Peer W, Martijn S, Massimiliano R, Laura V, Marco SP, Aleksandra B, Zhenyu G, Freek E, Hoebeek SD, William W, Mark F, Chris ID. (2009). Synaptic inhibition of Purkinje cells mediates consolidation of vestibulo–cerebellar motor learning. Nature Neuroscience, 12: 1042–1049.

Peeyush KT, Savitha B, Sherin A, Anju TR, Jes P, Paulose CS. (2010). Cholinergic, dopaminergic and insulin receptors gene expression in the cerebellum of streptozotocin–induced diabetic rats: functional regulation with Vitamin D3 supplementation. Pharmacol Biochem Behav, 95: 216–222.

Perkington MS, Sihra TS. (1998). Presynaptic GABAB receptor modulation of glutamate exocytosis in rat cerebrocortical nerve terminals: receptor decoupling by protein kinase C. J. Neurochem, 70: 1513–1522.

Pichat P, Bergis OE, Terranova JP, Urani A, Duarte C, Santucci V, Gueudet C, Voltz C, Steinberg R, Stemmelin J, Oury–Donat F, Avenet P, Griebel G, Scatton B. (2007). SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (II) efficacy in experimental models predictive of activity against cognitive symptoms of schizophrenia. Neuropsychopharmacol, 32: 17–34.

Pin J–P, Galvez T, Prézeau L. (2003). Evolution, structure and activation mechanism of family 3/C G–protein coupled receptors. Pharmacol Ther, 98: 325–354.

Pipeleers DG, Schuit FC, Van Schravendijk CF, Van WM. (1985). Interplay of nutrients and hormones in the regulation of glucagon release. Endocrinology, 117: 817–823.

Piroli GG, Grillo CA, Hoskin EK, Znamensky V, Katz EB, Milner TA, McEwen BS, Charron MJ, Reagan LP. (2002). Peripheral glucose administration stimulates the translocation of GLUT8 glucose transporter to the endoplasmic reticulum in the rat hippocampus. J Comp Neurol, 452: 103–114.

Polak RL, Meeuws MM. (1966). The influence of atropine on the release and uptake of acetylcholine by the isolated cerebral cortex of the rat. Biochem Pharmacol, 15: 989–992.

Polenzani L, Woodward RM, Miledi R. (1991). Expression of mammalian γ–aminobutyric acid receptors with distinct pharmacology in Xenopus oocytes. Neurobiology, 88: 4318–4322.

Poli G, Chiarpotto E, Biasi F. (1989). Role of aldehydes in the propagation of lipid peroxidation–induced liver toxicity. (Poli G, Cheeseman KH, Dianzani MU, Slates TF, eds). Oxford: Pergamon Press, Advances In Bioscience, 76: 73–81.

Pramming S, Thorsteinsson B, Bendtson I, Binder C. (1991). Symptomatic hypoglycaemia in 411 type 1 diabetic patients. Diabetic Med, 8: 217–222.

Prickaerts J, Fahrig T, Blokland A. (1999). Cognitive performance and biochemical markers in septum, hippocampus and striatum of rats after an i.c.v. injection of streptozotocin: a correlation analysis. Behav Brain Res, 102: 73–88.

Princivalle AP, Richards DA, Duncan JS, Spreafico R, Bowery NG. (2003). Modification of GABA(B1) and GABA(B2) receptor subunits in the somatosensory cerebral cortex and thalamus of rats with absence seizures (GAERS). Epilepsy Res, 55: 39–51.

Prosser HM, Gill CH, Hirst WD, Grau E, Robbins M, Calver A. (2001). Epileptogenesis and enhanced prepulse inhibition in GABA(B1)–deficient mice. Mol Cell Neurosci, 17: 1059–1070.

Quan L, Meili G, Chang BS, Lowenstein DH. (2003). Epilepsy. N Engl J Med, 349: 1257–1266.

Radke R, Stach W. (1986). Are the islets of Langerhans neuro–paraneuronal control centers of the exocrine pancreas? Arch Histol Jpn, 49: 411–420.

Ramakrishna R, Namasivayam A. (1995). Norepinephrine and Epinephrine levels in the brain of alloxan diabetic rats. Neuarosci Let, 186: 200–202.

Rang, Dale, Ritter, Moore. (2003). ISBN 0443071454, 5:th ed., Churchill Livingstone. Pharmacology, P: 138–147.

Raza Ahmad, Joseph C, Milbrandt, Stephen P, Arneric DM. (1994). Caspary.Age–related changes in brainstem auditory neurotransmitters: Measures of GABA and acetylcholine function. Hearing Research, 77: 221–230.

Reagan LP, Magariños AM, Yee DK, Swzeda LI, Van Bueren A, McCall AL, McEwen BS. (2000). Oxidative stress and HNE conjugation of GLUT3 are increased in the 1 hippocampus of diabetic rats subjected to stress. Brain Research, 862: 292 –300.

Reetz A, Solimena M, Matteoli M, Folli F, Takei K, De Camilli P. (1991). GABA and pancreatic beta–cells: colocalization of glutamic acid decarboxylase (GAD) and GABA with synaptic–like microvesicles suggests their role in GABA storage and secretion. J. EMBO, 10: 1275–1284.

Reever CM, Ferrari–DiLeo G, Flyn DD. (1997). The M5 (m5) receptor subtype: Fact or fiction? Life Sci, 60: 1105–1112.

Reno LA, Zago W, Markus RP. (2004). Release of [3H]–L–glutamate by stimulation of nicotinic acetylcholine receptors in rat cerebellar slices. Neuroscience, 124: 647–653.

Renuka TR, Paulose CS. (2004). Alterations in the brain stem muscarinic M1 and M3 receptor gene expression during pancreatic regeneration and insulin secretion in weanling rats. Life Science, 75: 2269–2280.

Renuka TR, Remya R, Paulose CS. (2006). Increased insulin secretion by muscarinic M1 and M3 receptor function from rat pancreatic islets in vitro. Neurochemical Research, 31: 313–320.

Rhee SG, Choi KD. (1992). Regulation of inositol phospholipid–specific phosphpolipase C isoenzymes. J Biol Chem, 2678: 12393–12396.

Ribak CE, Harris AB, Vaughn JE, Roberts E. (1979). GABAergic nerve terminals decrease at sites of focal epilepsy. Science, 205: 211–214.

Roberta MK, Peter LS. (2003). Cerebellar Loops with Motor Cortex and Prefrontal Cortex of a Nonhuman Primate. Journal of Neuroscience, 23: 8432–8444.

Roberts RO, Geda YE, Knopman DS, Christianson TJH, Pankratz VS, Boeve BF, Vella A, Rocca WA, Petersen RC. (2008). Duration and severity of diabetes are associated with mild cognitive impairment. Arch Neurol, 65: 1066–1073.

Robinson PJ, Rapoport SI. (1986). Glucose transport and metabolism in the brain. Am J Physiol, 250: 127–136.

Robinson R, Krishnakumar A, Paulose CS. (2009). Enhanced dopamine D1 and D2 receptor gene expression in the hippocampus of hypoglycaemic and diabetic rats. Cell Mol Neurobiol, 29: 365–372.

Rodrigo J, Fernández–Vizarra P, Castro–Blanco S, Bentura ML, Nieto M, Gómez–Isla T, Martínez–Murillo R, MartInez A, Serrano J, Fernández AP. (2004). Nitric oxide in the cerebral cortex of amyloid precursor protein (SW) Tg2576 transgenic mice. Neuroscience, 128: 73–89.

Roettger V, Lipton P. (1996). Mechanism of glutamate release from rat hippocampal slices during in vitro ischemia. Neuroscience, 75: 677–685.

Roger JM, Corrall BM, Frier. (1981). Acute hypoglycemia in man: Neural control of pancreatic islet cell function. Metabolism, 30: 160–164.

Roghani, Mehrdad, Mohammad Taghi Joghataie, Mohammad Reza Jalali and Tourandokht Baluchnejadmojarad. (2006). Time Course of Changes in Passive Avoidance and Y–maze Performance in Male Diabetic Rats. Iranian Biomedical Journal, 10: 99–104.

Rorsman P, Berggren PO, Bokvist K, Ericson H, Mohler H, Ostenson CG, Smith PA. (1989). Glucose–inhibition of glucagon secretion involves activation of GABAA–receptor chloride channels. Nature, 341: 233–236.

Rossi DJ, Hamann M, Attwell D. (2003). Multiple modes of GABAergic inhibition of rat cerebellar granule cells. J Physiol, 548: 97–110.

Rostron CL, Farquhar MJ, Latimer MP, Winn P. (2009). The pedunculopontine tegmental nucleus and the nucleus basalis magnocellularis: do both have a role in sustained attention? Neurol, 513: 532–541.

Rovet JF, Ehrlich RM. (1999). The effect of hypoglycaemic seizures on cognitive function in children with diabetes: a 7–year prospective study. J. Pediatr, 134: 503 –506.

Roza AM, Pieper GM, Johnson CP, Adams MB. (1995). Pancreatic antioxidant enzyme activity in normoglycemic diabetic prone BB rats. Pancreas, 10: 53–58.

Rudolph U, Möhler H. (2004). Analysis of GABAA receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. Annu Rev Pharmacol Toxicol, 44: 475–498.

Rutter GA. (2001). Nutrient–secretion coupling in the pancreatic islet β–cell: recent advances. Molecular Aspects of Medicine, 22: 247–284.

Ryan C, Vega A, Drash A. (1985). Cognitive deficits in adolescents who developed diabetes early in life. Pediatrics, 75: 921–927.

Rye DB, Saper CB, Lee HJ, Wainer BH. (1987). Pedunculopontine tegmental nucleus of the rat: cytoarchitecture, cytochemistry, and some extrapyramidal connections of the mesopontine tegmentum. J Comp Neurol, 259: 483–528.

Sakurai T, Yang B, Takata T, Yokono K. (2002). Synaptic adaptation to repeated hypoglycemia depends on the utilization of monocarboxylates in guinea pig hippocampal slices. Diabetes, 51: 430–438.

Salkovic M, Lackovic Z. (1992). Brain D1 dopamine receptor in alloxan–induced diabetes. Diabetes, 41: 1119–1121.

Sanacora G, Mason GF, Krystal JH. (2000). Impairment of GABAergic transmission in depression : new insights from neuroimaging studies. Critical Reviews in Neurobiology; 14: 23–45.

Sanacora G, Mason GF, Rothman DL, Behar KL, Hyder F, Petroff OA, Berman RM, Charney DS, Krystal JH. (1999). Reduced cortical gamma–aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. Archives of General Psychiatry, 56: 1043–1047.

Sanders RD, Brian D, Maze M. (2008). G–protein–coupled receptors. Handb Exp Pharmacol, 182: 93–117.

Sandrini M, Vitale G, Vergoni A, Othani A, Bertolini A. (1997). Streptozotocin–induced diabetes provokes changes in serotonin concentration and on 5HT1A and 5HT2A receptors in rat brain. Life Sci, 60: 1393–1397.

Sang WS, Aoyama K, Matsumori Y, Liu J, Swanson RA. (2005). Pyruvate administered after severe hypoglycemia reduces neuronal death and cognitive impairment. Diabetes, 54: 1452–1458.

Sankar R, Thamotharan S, Shin D, Moley KH, Devaskar SU. (2002). Insulin–responsive glucose transporters–GLUT8 and GLUT4 are expressed in the developing mammalian brain. Brain Res. Mol. Brain Res, 107: 157–165.

Santiago JV, Clarke WL, Shah SD, Cryer PE. (1980). Epinephrine, norepinephrine, glucagon and growth hormone release in association with physiological

decrements in the plasma glucose concehtration in normal and diabetic man. J Clin Endocrinol Metab, 51: 877–883.

Sarter M, Bruno JP, Givens B. (2003). Attentional functions of cortical cholinergic inputs: what does it mean for learning and memory? Neurobiol Learn Mem, 80: 245–256.

Sarter M, Bruno JP, Givens B. (2003). Attentional functions of cortical cholinergic inputs: what does it mean for learning and memory? Neurobiol Learn Mem, 80: 245–256.

Sasaki M., Matsuda H, Omura I, Sugai K, Hashimoto T. (2000). Transient seizure disappearance due to bilateral striatal necrosis in a patient with intractable epilepsy. Brain Dev, 22: 50–55.

Sastry PS, Rao KS. (2000). Apoptosis and the nervous system. J Neurochem, 74: 1–20.

Scatchard G. (1949). The attractions of proteins for small molecules and ions. Annels of New York Academy of Sciences, 51: 660–672.

Scatton B, Bartholini G. (1982). y–Aminobutyric acid (GABA) receptor stimulation. IV effect of progabide (SL 76002) and other GABAergic agents on acetylcholine turnover in rat brain areas. J Pharmacol Exp Ther, 220: 689–695.

Schechter R, Beju D, Miller KE. (2005). The effect of insulin deficiency on tau and neurofilament in the insulin knockout mouse. Biochem Biophys Res Commun, 334: 979–986.

Scheucher A. Alvarez AL, Torres N, Dabsys SM, Finkielman S, Nathmod VE, Pirola CJ. (1991). Cholinergic hyperactivity in the lateral septal area of spontaneoulsy hypertensive rats: depressor effect of hemicholinum –3 and pirenzepine. Neuropharmacology, 30: 391–397.

Schmahmann JD, David Caplan (2006) Cognition, emotion and the cerebellum Brain, 129: 290–292.

Schuler V, Luscher C, Blanchet C, Klix N, Sansig G, Klebs K, Schmutz M, Heid J, Gentry C, Urban L, Fox A, Spooren W, Jaton A, Vigouret J, Pozza M, Kelly PH, Mosbacher J, Froestl W, Kaslin E, Korn R, Bischoff S, Kaupmann K, van der Putten H, Bettler B. (2001). Epilepsy, hyperalgesia, impaired memory, and loss of pre– and postsynaptic GABAB responses in mice lacking GABAB1. Neuron, 31: 47–58.

Schulingkamp RJ, Pagano TC, Hung D, Raffa RB. (2000). Insulin receptors and insulin action in the brain: review and clinical implications. Neurosci Biobehav Rev, 24: 855–872.

Schwartz JC, Giros B, Martres M, Schwartz, Sokoloff P. (1998). The Dopamine Receptor Family: Molecular Biology and Pharmacology. Seminars in the Neurosciences, 4: 99–108.

Schwechter EM, Velísková J, Velísek L. (2003). Correlation between extracellular glucose and seizure susceptibility in adult rats. Ann Neurol, 53: 91–101.

Shah SJ, Bhandarkar SD, Satoskar RS. (1984). Drug interaction between chlorpropamide and non–steroidal anti–inflammatory drugs, ibuprofen and phenylbutazone. Int J Clin Pharmacol Ther Toxicol, 22: 470–472.

Shapovalova KB. (1998). Increasing the activity of the neostriatum cholinergic system alters an established type of motor behavior in animals. Neurosci Behav Physiol, 28: 244–251.

Sheetz MJ, King GL. (2002). Molecular understanding of hyperglycaemia's adverse effects for diabetic complications. JAMA, 228: 2579–2588.

Shekhar A, Hingtgen JN, DiMicco JA. (1990). GABA receptors in the posterior hypothalamus regulate experimental anxiety in rats. Brain Research, 512: 81–88.

Shen KZ, Johnson SW. (2000). Presynaptic dopamine D2 and muscarine M3 receptors inhibit excitatory and inhibitory transmission to rat subthalamic neurones in vitro. J Physiol, 2: 331–341.

Shi Y, Kanaani J, Menard–Rose V, Ma YH, Chang PY, Hanahan D, Tobin A, Grodsky G, Baekkeskov S. (2000). Increased expression of GAD65 and GABA in pancreatic beta–cells impairs first–phase insulin secretion. Am J Physiol Endocrinol Metab, 279: 684–694.

Shin BC, McKnight RA, Devaskar SU. (2004). Glucose transporter GLUT8 translocation in neurons is not insulin responsive. J. Neurosci. Res, 75: 835–844.

Shinotoh H, Namba H, Fukushi K, Nagatsuka S, Tanaka N, Aotsuka A, Ota T, Tanada S, Irie T. (2000). Progressive loss of cortical acetylcholinesterase activity in association with decline in Alzheimer’s disease: a positron emission tomography study. Ann. Neurol, 48: 194–200.

Shiota C, Larsson O, Shelton KD, Shiota M, Efanov AM, Hoy M, Lindner J, Kooptiwut S, Juntti–Berggren L, Gromada J, Berggren PO, Magnuson MA.

(2002). Sulfonylurea receptor type 1 knock–out mice have intact feeding–stimulated insulin secretion despite marked impairment in their response to glucose. J. Biol. Chem, 277: 37176–37183.

Shorr SS, Bloom FE. (1970). Fine structure of islet–cell innervation in the pancreas of normal and alloxan–treated rats. Z Zellforsch Mikrosk Anat, 103: 12–25.

Sicree R, Shaw J, Zimmet P. (2006). Diabetes and impaired glucose tolerance. in Diabetes Atlas, 3rd edition, Gan D, (Ed)., International Diabetes Federation: Brussels. P: 15–109.

Siddiqui MF, Levey AI. (1999). Cholinergic therapies in Alzheimer's disease. Drugs Future, 24: 417–444.

Silva AJ, Jeffrey HK, Paul WF, Satoshi K. (1998). Creb and Memory. Annu Rev Neurosci, 21: 127–148.

Singh P, Jain A, Kaur G. (2004). Impact of hypoglycemia and diabetes on CNS: correlation of mitochondrial oxidative stress with DNA damage. Mol Cell Biochem, 260: 153–159.

Sivilotti L, Nistri A. (1991). GABA receptor mechanisms in the central nervous system. Prog Neurobiol, 36: 35–92.

Sloviter RS, Nilaver G. (1987). Immunocytochemical localization of GABA–cholecystokinin–vasoactive intestinal polypeptide and somatostatin–like immunoreactivity in the area dentata and hippocampus of the rat. J Comp Neurol, 256: 42–60.

Sloviter RS. (1987). Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. Science, 235: 73–76.

Sokoloff. (1981). Relationships among local functional activity, energy metabolism, and blood flow in the central nervous system. Fed. Proc., 40: 2311–2316.

Song C, Murray TA, Kimura R, Wakui M, Ellsworth K, Javedan SP, Marxer–Miller S, Lukas RJ, Wu J. (2005). Role of alpha7–nicotinic acetylcholine receptors in tetanic stimulation–induced gamma oscillations in rat hippocampal slices. Neuropharmacology, 48: 869–880.

Sonkusare S, Srinivasan K, Kaul C, Ramarao P. (2005). Effect of donepezil and lercanidipine on memory impairment induced by intracerebroventricular streptozotocin in rats. Life Sci, 77: 1–14.

Sophie F, Robert FA, Allan J. (1990). Tobin Long term increase of glutamate decarboxylase mRNA in a rat model of temporal lobe epilepsy. Neuron, 5: 361–371.

Sorenson R, Garry D, Brelje T. (1991). Structural and functional conciderations of GABA in the islets of Langerhans: β–cells and nerves. Diabetes, 40: 1365–1374.

Stagner JI, Samols E. (1986). Modulation of insulin secretion by pancreatic ganglionic nicotinic receptors. Diabetes, 35: 849–854.

Stephens LR, Logan SD. (1989). Formation of [3H]inositol metabolites in rat hippocampal formation slices prelabelled with [3H]inositol and stimulated with carbachol. Journal of Neurochemistry, 52: 713–721.

Steriade M. (1996). Arousal: revisiting the reticular activating system. Science, 272: 225–226.

Stillman MJ, Shukitt–Hale B, Galli RL, Levy A, Lieberman HR. (1996). Effects of M2 antagonists on in vivo hippocampal acetylcholine levels. Brain Res Bull, 41: 221–226.

Straessle A, Loup F, Arabadzisz D, Ohning GV, Fritschy JM. (2003). Rapid and long–term alterations of hippocampal GABAB receptors in a mouse model of temporal lobe epilepsy. Eur J Neurosci, 18: 2213–2226.

Strubbe JH, Steffens AB. (1993). Neural control of insulin secretion. Horm. Metab. Res., 25: 507–12.

Suda S, Shinohara M, Miyaoka M, Lucignani G, Kennedy C, Sokoloff L. (1990). The lumped constant of the deoxyglucose method in hypoglycemia: effects of moderate hypoglycemia on local cerebral glucose utilization in the rat. J Cereb Blood Flow Metab, 10: 499–509.

Sugita S, Uchimura N, Jiang ZG, North RA. (1991). Distinct muscarinic receptors inhibit release of g–aminobutyric acid and excitatory amino acids in mammalian brain. Proc Natn Acad Sci. USA, 88: 2608–2611.

Suh SW, Aoyama K, Chen Y, Garnier P, Matsumori Y, Gum E, Liu J, Swanson RA. (2003). Hypoglycemic neuronal death and cognitive impairment are prevented by poly(ADP–ribose) polymerase inhibitors administered after hypoglycemia. J Neurosci, 23: 10681–10690.

Suh BC, Hille B. (2005). Regulation of ion channels by phosphatidylinositol 4, 5–bisphosphate. Curr Opin Neurobiol, 15: 370–378.

Suh SW, Gum ET, Hamby AM, Chan PH, Swanson RA. (2007). Hypoglycemic neuronal death is triggered by glucose reperfusion and activation of neuronal NADPH oxidase. J Clin Invest, 117: 910–918.

Suh SW, Hamby AM, Gum ET, Shin BS, Won SJ, Sheline CT, Chan PH, Swanson RA. (2008). Sequential release of nitric oxide, zinc, and superoxide in hypoglycemic neuronal death. J Cereb Blood Flow Metab, 28: 1697–1706.

Sumiyoshi T, Ichikawa J, Meltzer HY. (1997). The effect of streptozotocin– induced diabetes on Dopamine D2, Serotonine 1A & serotonine 2A receptors in the rat brain. Neuropscypharmacol, 16: 183–190.

Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. (1989). Mossy fiber synaptic reorganization in the epileptic human temporal lobe. Ann Neurol, 26: 321–330.

Szabo G, Katarova Z, Greenspan R. (1994). Distinct protein forms are produced from alternatively spliced bicistronic glutamic acid decarboxylase mRNAs during development. Mol Cell Biol, 14: 7535–7545.

Szatkowski M, Attwell D. (1994). Triggering and execution of neuronal death in brain ischemia: two phases of glutamate release by different mechanisms. Trends Neurosci, 17: 359–365.

Takahashi T, Kajikawa Y, Tsujimoto T. (1998). G–protein–coupled modulation of presynaptic calcium currents and transmitter release by a GABAB receptor. J Neurosci, 18: 3138–3146.

Takayama C, Inoue Y. (2003). Normal formation of the postsynaptic elements of GABAergic synapses in the reeler cerebellum. Brain Res Dev Brain Res, 145: 197–211.

Takayama C, Inoue Y. (2004). Extrasynaptic localization of GABA in the developing mouse cerebellum. Neurosci Res, 50: 447–458.

Taniguchi H, Murakami K, Yoshioka M, Ejiri K, lshihara K, Baba S, Okada Y. (1982). GABA and insulin in pancreatic islets. in Problems in GABA Research From Brain to Bacteriu. (Edited by Okada Y. and Roberts E.) Excerpta Med, Amsterdam. P: 406–412.

Taniguchi H, Okada Y, Seguchi H, Shimada C, Seki M, Tsutou A, Baba S. (1979). High concentration of gamma–aminobutyric acid in pancreatic beta cells. Diabetes, 28: 629–633.

Thilmann R, Xie Y, Kleihues P, Kiessling M. (1986). Persistent inhibition of protein synthesis precedes delayed neuronal death in postischemic gerbil hippocampus, Acta Neuropathol, 71: 88–93.

Thoenen H. (1995). Neurotrophins and neuronal plasticity. Science, 270: 593–598.

Thomas G, Rojas MC, Epstein SK, Balk EM, Liangos O, Jaber BL. (2007). Insulin therapy and acute kidney injury in critically ill patients a systematic review. Nephrol Dial Transplant, 22: 2849–2855.

Thouvarecq R, Protais P, Jouen F, Caston J. (2001). Influence of cholinergic system on motor learning during aging in mice. Behav Brain Res, 118: 209–218.

Tkacs NC, Pan Y, Raghupathi R, Dunn–Meynell AA, Levin BE. (2005). Cortical Fluoro–Jade staining and blunted adrenomedullary response to hypoglycemia after noncoma hypoglycemia in rats. J Cereb Blood Flow Metab, 25: 1645–1655.

Torrao AS, Lindstrom JM, Britto LR. (2003). Nicotine and alpha–bungarotoxin modify the dendritic growth of cholinoceptive neurons in the developing chick tectum. Dev Brain Res, 143: 115–118.

Treiman DM. (2001). GABAergic mechanisms in epilepsy. Epilepsia, 42: 8–12.

Tzavara ET, Bymaster FP, Felder CC, Wade M, Gomeza J, Wess J, McKinzie DL, Nomikos GG. (2003). Dysregulated hippocampal acetylcholine neurotransmission and impaired cognition in M2, M4, and M2/M4 muscarinic receptor knockout mice. Mol Psychiatry, 8: 673–679.

Ueki K, Okada T, Hu J, Liew CW, Assmann A, Dahlgren GM, Peters JL, Shackman JG, Zhang M, Artner I, Satin LS, Stein R, Holzenberger M, Kennedy RT, Kahn CR, Kulkarni RN. (2006). Total insulin and IGF–I resistance in pancreatic beta cells causes overt diabetes. Nat Genet, 38: 583–588.

Uemura E, Greenlee HW. (2001). Amyloid beta–peptide inhibits neuronal glucose uptake by preventing exocytosis. Exp Neurol, 170: 270–276.

Uemura E, Greenlee HW. (2006). Insulin regulates neuronal glucose uptake by promoting translocation of glucose transporter GLUT3. Experimental Neurology, 198: 48–53.

Unger J, McNeill TH, Moxley RT 3rd, White M, Moss A, Livingston JN. (1989). Distribution of insulin receptor–like immunoreactivity in the rat forebrain. Neuroscience, 31: 143–157.

Unger JW, Livingston JN, Moss AM. (1991). Insulin receptors in the central nervous system: localization, signalling mechanisms and functional aspects. Prog Neurobiol, 36: 343–362.

Unger RH, Orci L. (1975). The essential role of glucagon in the pathogenesis of diabetes mellitus. Lancet, 1: 14–16.

Unger RH, Orci L. (1977). Possible roles of the pancreatic D–cell in the normal and diabetic states. Diabetes, 26: 241–244.

Valentin A, Pavlov, Mahendar O, Margot Gallowitsch–Puerta, Kanta O, Jared M, Huston, Christopher J, Czura, Yousef Al–Abed, Kevin J. (2006). Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. Proc Natl Acad Sci, 103: 5219–5223.

Van den Berghe G, Schoonheydt K, Becx P, Bruyninckx F, Wouters PJ. (2005). Insulin therapy protects the central and peripheral nervous system of intensive care patients. Neurology, 64: 1348–1353.

Van der Zee EA, Buwalda B, Strubbe JH, Strosberg AD, Luiten PG. (1992). Immunocytochemical localization of muscarinic acetylcholine receptors in the rat endocrine pancreas. Cell Tissue Res, 269: 99–106.

Van Der Zee EA, Luiten GM. (1999). Muscarinic acetylcholine receptors in the Hippocampus, neocortex and amygdala: a Review of immunocytochemical

localization In relation to learning and memory. Progress in Neurobiology, 58: 409–471.

Van Dort CJ, Baghdoyan HA, Lydic R. (2008). Neurochemical modulators of sleep and anesthetic states. Int Anesthesiol Clin, 46: 75–104.

Vander Heiden MG, Chandel NS, Williamson EK, Schumacker PT, Thompson CB. (1997). Bcl–xL regulates the membrane potential and volume homeostasis of mitochondria. Cell, 91: 627–637.

Vannucci RC, Vannucci SJ. (2001). Hypoglycemic brain injury. Semin. Neonatol, 6: 147–155.

Vannucci Susan J, Rebekah R, Clark, Ellen Koehler–Stec, Kang Lib, Carolyn B, Smith, Peter Davies, Fran Maher, Ian A, Simpson. (1998). Glucose Transporter Expression in Brain: Relationship to Cerebral Glucose Utilization. Dev Neurosci, 20: 369–379.

Velazquez–Moctezuma J, Gillin JC, Shiromani PJ. (1989). Effect of specific M1, M2 muscarinic receptor agonists on REM sleep generation. Brain Res, 503: 128–131.

Veneman T, Mitrakou A, Mokan M, Cryer PE, Gerich J. (1993). Induction of hypoglycaemia unawareness by asymptomatic nocturnal hypoglycaemia. Diabetes, 42: 1233–1237.

Viberg Henrik, Anders Fredriksson, Per Eriksson. (2008). Deranged spontaneous behaviour and decrease in cholinergic muscarinic receptors in hippocampus in the adult rat, after neonatal exposure to the brominated flame–retardant, 2,2_,4,4_, 5–

pentabromodiphenyl ether (PBDE 99). Behavioural Brain Research, 188: 255–262.

Vijayaraghavan S, Pugh P, Zhang ZW, Rathouz M, Berg D. (1992). Nicotinic receptors that bind alpha–bungarotoxin on neurons raise intracellular free Ca. Neuron, 8: 353–362.

Volpicelli LA, Levey AI. (2004). Muscarinic acetylcholine receptor subtypes in cerebral cortex and hippocampus, Prog Brain Res, 145: 59–66.

Waldvogel HJ, Billinton A, White JH, Emson PC, Faull RL. (2004). Comparative cellular distribution of GABAA and GABAB receptors in the human basal ganglia: immunohistochemical colocalization of the alpha 1 subunit of the GABAA receptor, and the GABABR1 and GABABR2 receptor subunits. J Comp Neurol, 470: 339–356.

Walter S, Zawalich, Kathleen C, Zawalich, Gregory J, Tesz, Makoto M, Taketo, John Sterpka, William Philbrick, Minoru Matsui. (2004). Effects of muscarinic receptor type 3 knockout on mouse islet secretory responses. Biochemical and Biophysical Research Communications, 315: 872–876.

Wang J, Zheng H, Berthoud HR. (1999). Functional vagal input to chemically identified neurons in pancreatic ganglia as revealed by Fos expression. Am J Physiol, 277: 958–964.

Wang YT, Linden DJ. (2000). Expression of cerebellar long–term depression requires postsynaptic clathrin–mediated endocytosis. Neuron, 25: 635–647.

Wang HL, Morales M. (2009). Pedunculopontine and laterodorsal tegmental nuclei contain distinct populations of cholinergic, glutamatergic and GABAergic neurons in the rat. Eur J Neurosci, 29: 340–358.

Washburn MS, Moises HC. (1992). Muscarinic responses of rat basolateral amygdaloid neurons recorded in vitro. J. Physiol, 449: 121–154.

Wassmer E, Davies P, William P, Whitehouse, Stuart H. (2003). Clinical spectrum associated with cerebellar hypoplasia. Pediatr Neurol, 28: 347–351.

Watari N. (1968). Fine structure of nervous elements in the pancreas of some vertebrates. Z Zellforsch Mikrosk Anat, 85: 291–314.

Watson GS, Craft S. (2003). The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. CNS Drugs, 17: 27–45.

Watson RT, Pessin JE. (2001). Subcellular compartmentalization and trafficking of the insulin–responsive glucose transporter, GLUT4. Exp Cell Res, 271: 75–83.

Wayne EA, Dean HJ, Booth F, Tenenbein M. (1990). Focal neurological deficits associated with hypoglycemia in children with diabetes. J Pediatr, 117: 575–577.

Wehr M, Zador AM. (2003). Balanced inhibition underlies tuning and sharpens spike timing in auditory cortex. Nature, 426: 442–446.

Weihua X, Judith AS, Arnaud C, Philip JW, Angie R, Rodney DM, Palmer T, Steven HH, Oksana L. (2000). Postnatal developmental delay and supersensitivity to organophosphate in gene–targeted mice lacking acetylcholineesterase. Pharmacology, 293: 896–902.

Weiner DM, Levey AI, Brann MR. (1990). Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. Proc Natl Acad Sci, 87: 7050–7054.

Wess J, Brann MR, Bonner TI. (1989). Identification of a small intracellular region of the muscarinic m3 receptor as a determinant of selective coupling to PI turnover. FEBS Lett, 258: 133–136.

White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM, Marshall FH. (1998). Heterodimerization is required for the formation of a functional GABAB receptor. Nature, 396: 679–682.

Whiting PJ, Bonnert TP, McKernan RM, Farrar S, Le Bourdelles B, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghji DJ, Thompson SA, Wafford KA. (1999). Molecular and functional diversity of the expanding GABA–A receptor gene family. Ann N Y Acad Sci, 868: 645–653.

Wickelgren I. (1998). Tracking insulin to the mind. Science, 280: 517–519.

Widom B, Simonson DC. (1992). Intermittent hypoglycaemia impairs glucose counterregulation. Diabetes, 41: 1597–1602.

Wieloch T, Engelsen B, Westerberg E, Auer R. (1985). Lesions of the glutamatergic cortico–striatal projections in the rat ameliorate hypoglycaemic brain damage in the striatum. Neurosci Lett, 58: 25–30.

Wieloch T, Harris RJ, Symon L, Siesjo BK. (1984). Influence of severe hypoglycaemia on brain extracellular calcium and potassium activities, energy– and phospholipid metabolism. J Neurochem, 43: 160–168.

Wing RR, Klein R, Moss SE. (1990). Weight gain associated with improved glycemic control in population–based sample of subjects with type I diabetes. Diabetes Care, 13: 1106–1109.

Winks JS, Hughes S, Filippov AK, Tatulian L, Abogadie FC, Brown DA. (2005). Relationship between membrane phosphatidylinositol –4, 5–bisphosphate and receptor–mediated inhibition of native neuronal M channels. J Neurosci, 25: 3400–3413.

Woo RJ, Hong GK, Kil LK. (2008). Ganglioside GQ1b improves spatial learning and memory of rats as measured by the Y–maze and the Morris water maze tests. Neuroscience Letters, 439: 220–225.

Woods SC, Porte Jr D. (1974). Neural control of the endocrine pancreas. Physiol Rev, 54: 596–619.

Woolf NJ, Butcher LL. (1986). Cholinergic systems in the rat brain: III. Projections from the pontomesencephalic tegmentum to the thalamus, tectum, basal ganglia, and basal forebrain. Brain Res Bull, 16: 603–637.

Woolf NJ. (2006). Acetylcholine, cognition, and consciousness. J Mol Neurosci, 30: 219–222.

Woolf NJ, Butcher LL. (2010). Cholinergic systems mediate action from movement to higher consciousness. Behav. Brain Res, doi:10.1016/j.bbr.2009.12.046.

Wozniak M, Rydzewski B, Baker SP, Raizada MK. (1993). The cellular and physiological actions of insulin in the central nervous system. Neurochem Int, 22: 1–10.

Wredling R, Levander S, Adamson U, Lins PE. (1990). Permanent neuropsychological impairment after recurrent episodes of severe hypoglycaemia in man. Diabetologia, 33: 152–157.

Wredling RA, Theorell PG, Roll HM, Lins PE, Adamson UK. (1992). Psychosocial state of patients with IDDM prone to recurrent episodes of severe hypoglycemia. Diabetes Care, 15: 518–521.

Wu Y, Wang W, Richerson GB. (2001). GABA transaminase inhibition induces spontaneous and enhances depolarization–evoked GABA efflux via reversal of the GABA transporter. J Neurosci, 21: 2630–2639.

Xu SH, Honda E, Ono K, Inenaga K. (2001). Muscarinic modulation of GABAergic transmission to neurons in the rat subfornical organ, Am J Physiol Regul Integr Comp Physiol. 280: 657–664.

Xu ZC. (1995). Neurophysiological changes of spiny neurons in rat neostriatum after transient forebrain ischemia: an in vivo intracellular recording and staining study. Neuroscience, 67: 823–36.

Yamada M, Lamping KG, Duttaroy A, Zhang W, Cui Y, Bymaster FP. (2001). Cholinergic dilation of cerebral blood vessels is abolished in M5 muscarinic acetylcholine receptor knockout mice. Proc Natl Acad Sci USA, 98: 14096–14101.

Yamaguchi N. (1992). Sympathoadrenal system in neuroendocrine control of glucose: mechanisms involved in the liver, pancreas, and adrenal gland under hemorrhagic and hypoglycemic stress. Can J Physiol. Pharmacol, 70: 167–206.

Yamamura HI, Synder G. (1981). Binding of [3H] QNB in rat brain. Proc Natl. Acad Sci U S A, 71: 1725–1729.

Yang XJ, Kow LM, Pfaff DW, Mobbs CV. (2004). Metabolic pathways that mediate inhibition of hypothalamic neurons by glucose. Diabetes, 53: 67–73.

Yoshida M, Nagatsuka Y, Muramatsu S, Niijima K. (1991). Differential roles of the caudate nucleus and putamen in motor behavior of the cat as investigated by local injection of GABA antagonists. Neurosci Res, 10: 34–51.

Zawalich WS, Rasmussen H. (1990). Control of insulin secretion: a model involving Ca2+, cAMP and diacylglycerol. Mol Cell Endocrinol, 70:119–137.

Zeller KR, Duelli J, Vogel H, Schrock, Kuschinsky W. (1995). Autoradiographic analysis of the regional distribution of Glut3 glucose transporters in the rat brain. Brain Res, 698: 175–179.

Z'Graggen WJ, Metz GA, Kartje GL, Thallmair M, Schwab ME. (1998). Functional recovery and enhanced corticofugal plasticity after unilateral pyramidal tract lesion and blockade of myelin–associated neurite growth inhibitors in adult rats. J Neurosci, 18: 4744–4757.

Zhang L, Warren RA. (2002). Muscarinic and nicotinic presynaptic modulation of EPSCs in the nucleus accumbens during postnatal development. J Neurophysiol, 88: 3315–3330.

Zhank W, Basile AS, Gomeza J, Volpicelli LA, Levey AI, Wess JJ. (2002). Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock–out mice. Neurosci, 22: 1709–1717.

Zhao W, Chen H, Xu H, Moore E, Meiri N, Quon MJ, Alkon DL. (1999). Brain insulin receptors and spatial memory. Correlated changes in gene expression, tyrosine phosphorylation, and signaling molecules in the hippocampus of water maze trained rats. J Biol Chem, 274: 34893–34902.

Zhao WQ, Alkon DL. (2001). Role of insulin and insulin receptors in learning and memory. Mol Cell Endocrinol, 177: 125–134.

Ziegler D, Hubinger A, Muhlen H, Gries FA. (1992). Effects of previous glycaemic control on the onset and magnitude of cognitive dysfunction during hypoglycaemia in type 1 (insulin–dependent) diabetic patients. Diabetologia, 35:828–834.

Zimmet P, Alberti KGMM, Shaw J. (2001). Global and societal implications of the diabetes epidemic. Nature, 414: 782–787.

Papers Published

1. **Sherin Antony**, Peeyush Kumar T, Jobin Mathew, Anju T.R and C. S. Paulose. (2010). Hypoglycemia Induced Changes in Cholinergic receptor expression in the cerebellum of diabetic rats. Journal of Biomedical sciences. Journal of Biomedical Science, 17:7.

2. Anu Joseph, **Sherin Antony**, C. S. Paulose. (2008). Increased glutamate receptor gene expression in the cerebral cortex of insulin induced hypoglycemic and streptozotocin-induced diabetic rats. Neuroscience, 156: 298-304

3. Savitha, B., Jobin Mathew., **Sherin, Antony**., and Paulose, C. S. (2009). Muscarinic M1, M3 receptors function in the brainstem of streptozotocin induced diabetic rats: their role in insulin secretion from the pancreatic islets as a function of age. European Journal of Pharmacology, 608: 14-22.

4. Peeyush Kumar T, Savitha Balakrishnan, **Sherin Antony**, Anju T R, and Jes Paul. 2010. Cholinergic, Dopaminergic and Insulin Receptors Gene Expression in the Cerebellum of Streptozotocin Induced Diabetic Rats: Functional Regulation with Vitamin D3 Supplementation. Pharmacology Biochemistry and behavior. Pharmacology Biochemistry and Behaviour, 95: 216-222.

5. Peeyush Kumar T., **Sherin Antony**., Nandhu M S, Jayanarayanan S, Naijil George & Paulose C. S. (2010). Vitamin D3 Restores Altered Cholinergic and Insulin Receptor expression in the Cerebral Cortex and Muscarinic M3 receptor expression in Pancreatic Islets of Streptozotocin Induced Diabetic Rats. Journal of Nutritional Biochemistry doi: 01.2045/j.nb.2010.03.0435 (in press).

6. Anju T R, Pretty Mary Abraham, **Sherin Antony**; Paulose CS. (2010). Alterations in cortical GABA B receptors in neonatal rats exposed to hypoxic stress: Role of glucose, oxygen and epinephrine resuscitation. Molecular and Cellular Biochemistry doi: 10.1007/s11010-010-0491-9 (in press).

Abstracts/Presentations

1. **Sherin Antony**, Anu Joseph, C. S. Paulose. Kinetic parameters of acetylcholine esterase in the heart of Streptozotocin Induced Diabetic and Insulin Induced Hypoglycemic rats. Meeting of Society of Biological Chemists (India) conducted in Sri Venkiteswara University, Thirupathi (2007).

2. Anu Joseph, **Sherin Antony**, C. S. Paulose. Decreased α-$_{2a}$ adrenergic receptor gene expression in the Hypothalamus and Brainstem of Insulin Induced Hypoglycaemic and Streptozotocin Induced Diabetic Rats. Annual meeting of Society for Biotechnologists, India and National Symposium on Current Trends in Stem Cell Biology, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram (2007).

3. **Sherin Antony**, Anu Joseph, C. S. Paulose. Decreased [^{3}H] QNB binding to muscarinic M1 receptors in the cerebral cortex of diabetic and insulin induced hypoglycemic rats. 8th Biennial meeting of Asia Pacific Society for Neurochemistry Symposium organized by International Society for Neurochemistry/Asia Pacific Society for Neurochemistry/International Brain Research Organization held in Shanghai, China (2008).

4. **Sherin Antony**, Anu Joseph, Peeyush Kumar T, C. S. Paulose. Enhanced Muscarinic M3 receptor gene expression in the cerebral cortex of Diabetic

and Insulin Induced Hypoglycemic rats. 77th Annual Meeting of the Society of Biological Chemists (India) IIT Madras, Chennai. (2008).

5. **Sherin Antony**, Nandhu M.S, C. S. Paulose. Alterations in the muscarinic m1 and m3 receptor gene expression in the corpus striatum of diabetic and insulin induced hypoglycemic rats. International Conference on Advances in Neurosciences & XXVI Annual Meeting of Indian Academy of Neurosciences, Kochi (2008).

6. Anu Joseph, **Sherin Antony**, C. S. Paulose. Enhanced Glutamate and IP3 receptor gene expression in the Cerebral Cortex of Insulin Induced Hypoglycemic and Streptozotocin Induced Diabetic Rats. International Symposium on Regenerative Neuroscience and Annual Meeting of Society for Neurochemistry (India), NIMHANS, Bangalore, INDIA (2008).

7. Anu Joseph, **Sherin Antony**, C. S. Paulose. Enhanced NMDA Receptors Gene Expression and Neurodegeneration in the Cerebellum of Insulin Induced Hypoglycaemic and Streptozotocin Induced Diabetic Old Rats. 21st Kerala science Congress –Young Scientist award contest paper, Kollam (2009).

Figure-1

Body weight of control and experimental rats

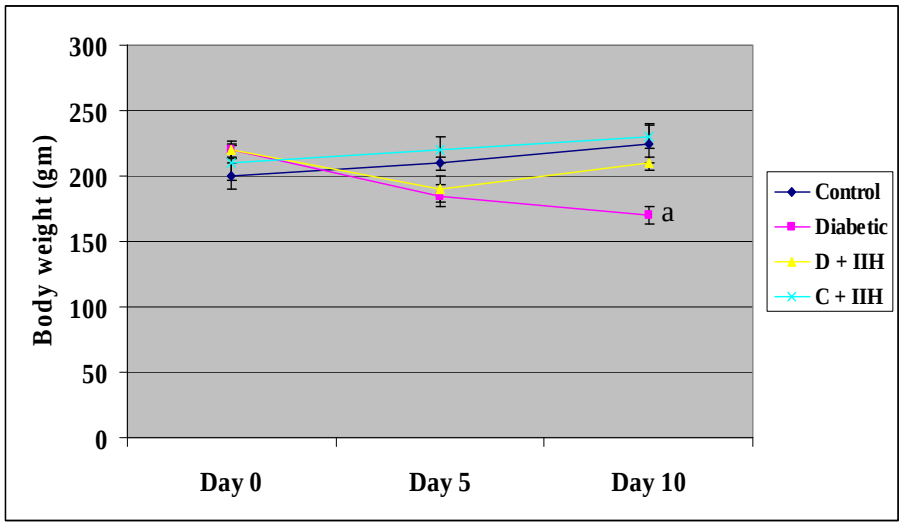


Table-1

Body weight of control and experimental rats

Experimental groups	Body Weight (gm)		
	Day 0	Day 5	Day 10
Control	200 ± 20	235 ± 0	240 ± 15
Diabetic	220 ± 08	190 ± 08	170 ± 10 [a]
D + IIH	220 ± 10	215 ± 10	210 ± 05 [b]
C + IIH	200 ± 12	220 ± 10	230 ± 16 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.01$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-2

Blood glucose level of control and experimental rats

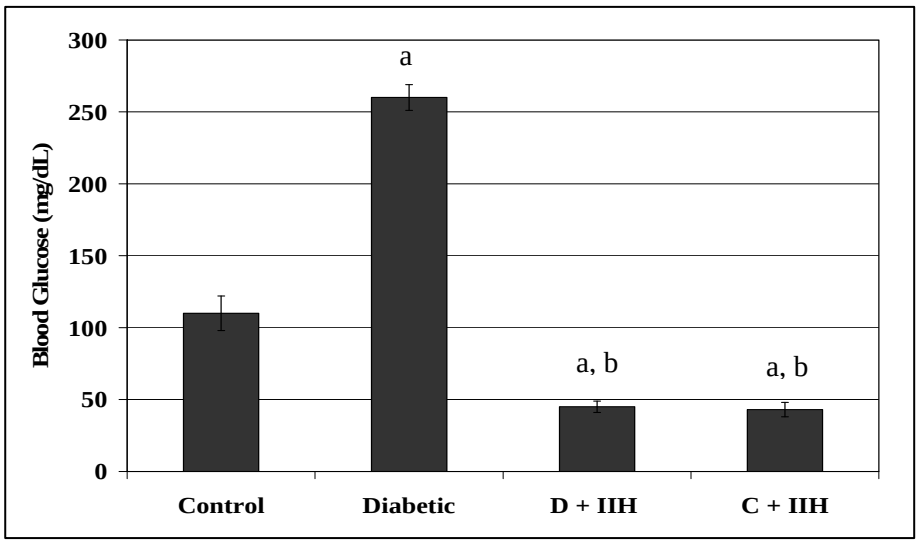


Table-2

Blood glucose level of control and experimental rats

Experimental groups	Blood Glucose (mg/dL)
Control	110 ± 12
Diabetic	260 ± 9 [a]
D + IIH	45 ± 4 [a, b]
C + IIH	42 ± 5 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-3

Blood glucose levels at different time intervals after insulin treatment in control and experimental rats

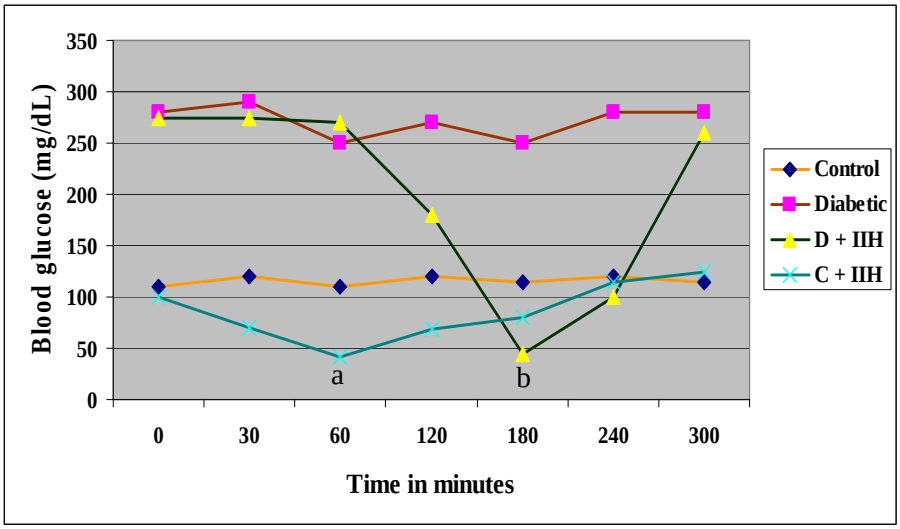


Table-3

Blood glucose levels at different time intervals after insulin treatment in control and experimental rats

Experimental groups	Time in minutes						
	0	30	60	120	180	240	300
Control	110 ± 5	120 ± 6	110 ± 3	120 ± 3	115 ± 9	120 ± 8	115 ± 5
Diabetic	280 ± 2	290 ± 4	250 ± 5	270 ± 5	250 ± 4	280 ± 2	280 ± 4
D + IIH	275 ± 3	275 ± 7	270 ± 5	180 ± 3	45 ± 4 [b]	100 ± 5	260 ± 6
C + IIH	100 ± 5	70 ± 5	42 ± 5 [a]	69 ± 4	80 ± 2	115 ± 6	105 ± 5

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic. IIH-Insulin Induced Hypoglycemia

Figure-4

Circulating insulin level in the plasma of control and experimental rats

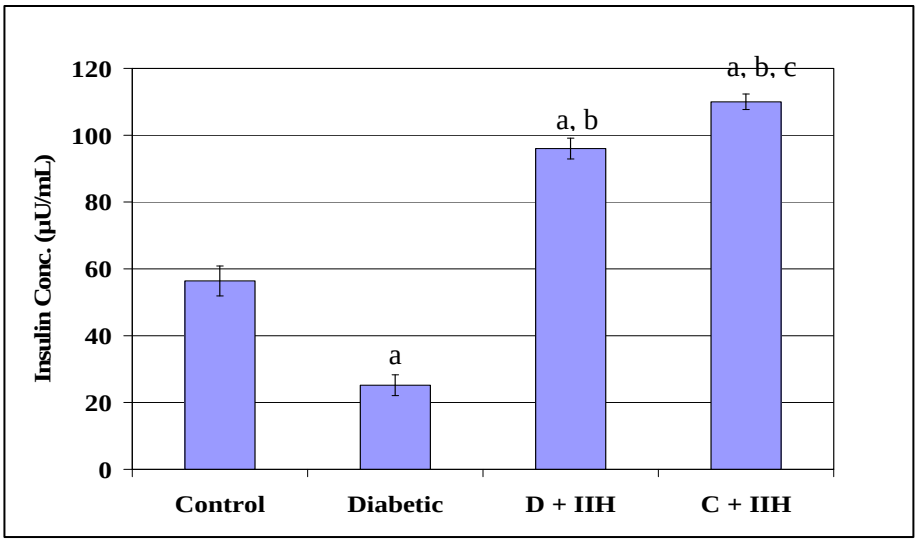


Table-4

Circulating insulin level in the plasma of control and experimental rats

Experimental groups	Insulin Concentration (µU/mL)
Control	56.4 ± 4.51
Diabetic	25.2 ± 3.10 [a]
D + IIH	96.0 ± 3.20 [a, b]
C + IIH	110.8 ± 2.34 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic, **c** $p<0.01$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-5

Behavioural response of control and experimental rats on Y maze test

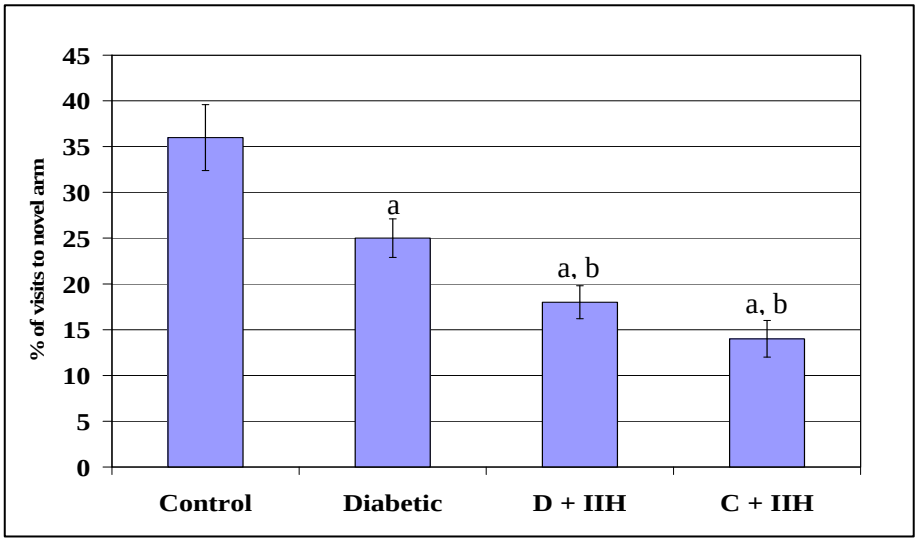


Table-5

Behavioural response of control and experimental rats on Y maze test

Experimental Group	% of visits to novel arm
Control	36.8 ± 3.2
Diabetic	25.6 ± 2.1 [a]
D + IIH	18.4 ± 3.8 [a, b]
C + IIH	14.2 ± 3.2 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-6

Behavioural response of control and experimental rats on grid walk test

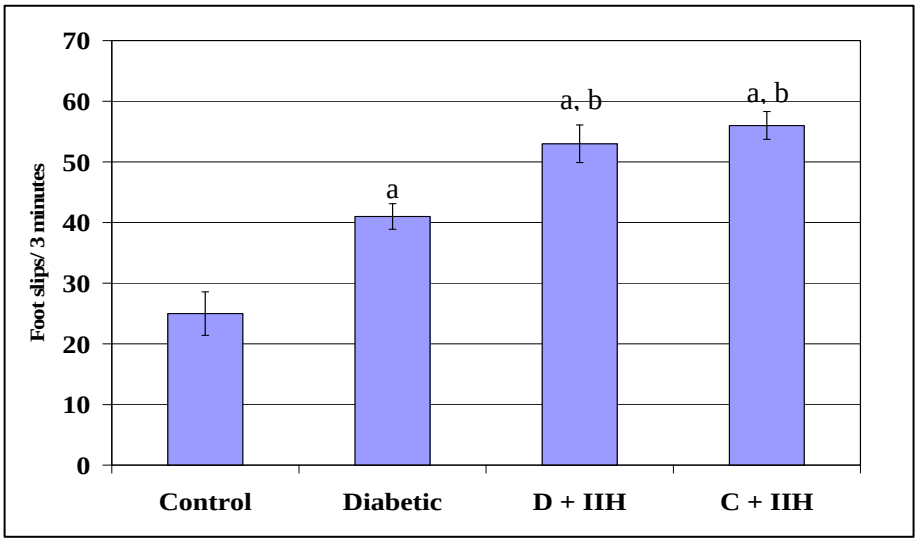


Table-6

Behavioural response of control and experimental rats on grid walk test

Experimental Group	Foot slips/ 3 minutes
Control	23.5 ± 3.60
Diabetic	38.2 ± 2.10 [a]
D + IIH	49.6 ± 3.10 [a, b]
C + IIH	52.1 ± 2.30 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-7

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebral cortex of control and experimental rats

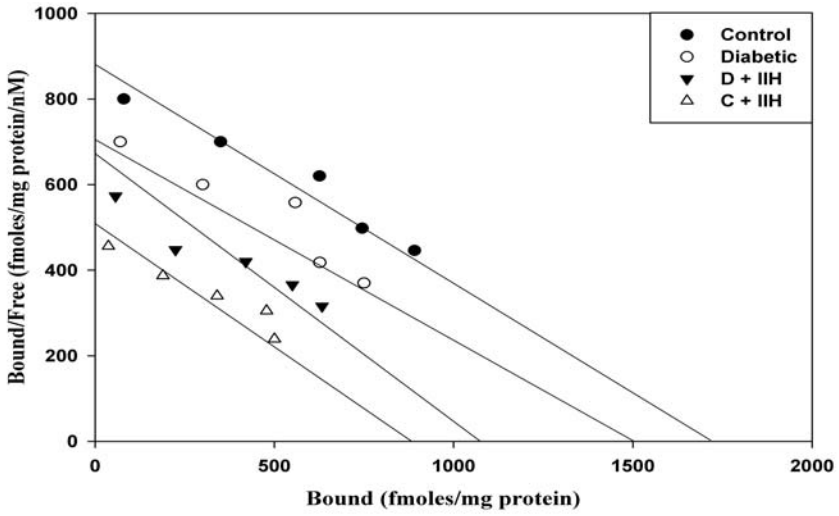


Table-7

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	1716 ± 17.7	1.96 ± 0.8
Diabetic	1500 ± 15.3 [a]	1.68 ± 0.7 [d]
D + IIH	1066 ± 14.4 [a, b]	1.60 ± 1.0 [d]
C + IIH	874 ± 17.0 [a, b, c]	1.70 ± 1.2 [d]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$, **d** $p<0.01$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH . IIH- Insulin Induced Hypoglycemia

Figure-8

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebral cortex of control and experimental rats

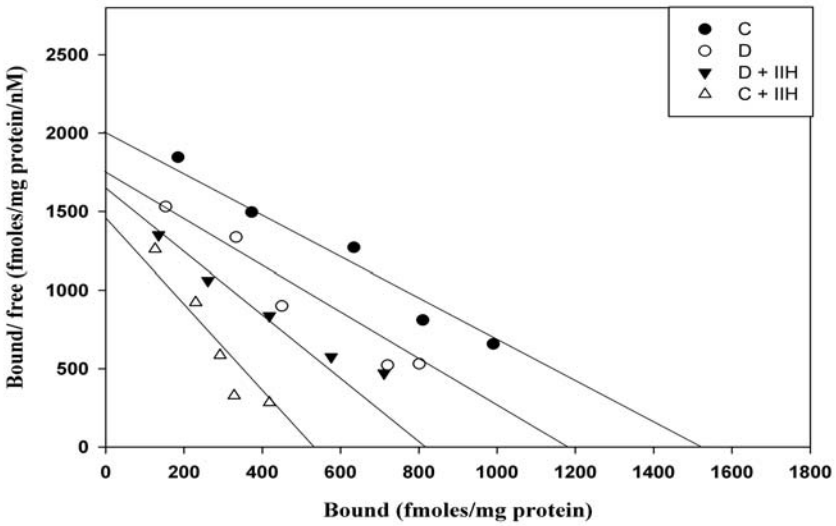


Table-8

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	1522 ± 12.3	0.76 ± 0.09
Diabetic	1181 ± 11.6 [a]	0.67 ± 0.07
D + IIH	817 ± 15.4 [a, b]	0.49 ± 0.05 [d, e]
C + IIH	532 ± 10.2 [a, b, c]	0.36 ± 0.06 [d, e]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [d] $p<0.01$ when compared to control, [b] $p<0.001$, [e] $p<0.01$ when compared to diabetic, [c] $p<0.001$ when compared to D + IIH . IIH- Insulin Induced Hypoglycemia

Figure-9

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebral cortex of control and experimental rats

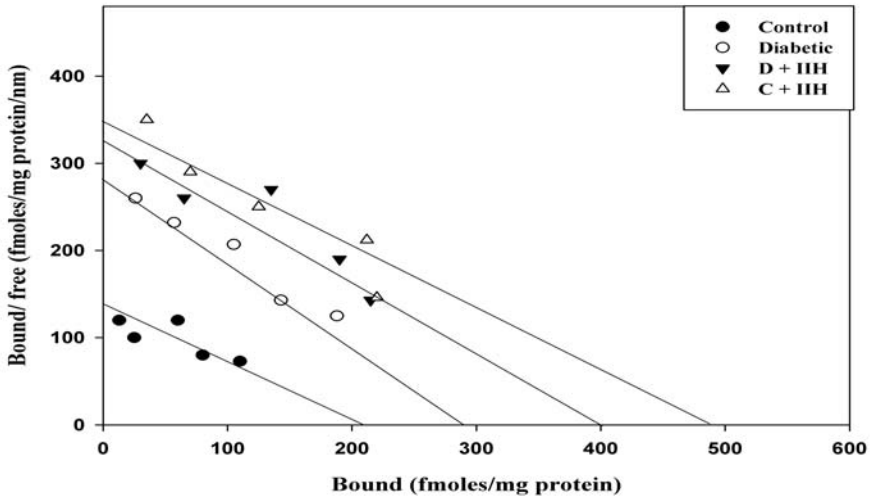


Table-9

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	208 ± 10.7	1.50 ± 0.03
Diabetic	289 ± 14.5 [a]	1.05 ± 0.02 [d]
D + IIH	400 ± 12.4 [a, b]	1.25 ± 0.05 [d, e]
C + IIH	487 ± 11.8 [a, b, c]	1.40 ± 0.04 [e, f]

Values are Mean ± S.E.M. of 3-5 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$, **d** $p<0.01$ when compared to control, **b** $p<0.001$, **e** $p<0.01$ when compared to diabetic, **c** $p<0.001$, **f** $p<0.01$ when compared to D + IIH . IIH- Insulin Induced Hypoglycemia

Figure-10

Scatchard analysis of [^{3}H] GABA binding against GABA in the cerebral cortex of control and experimental rats

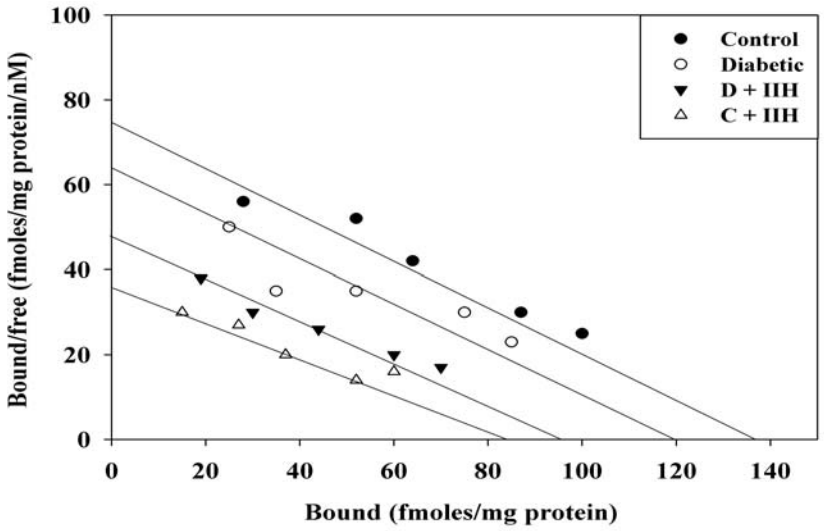


Table-10

Scatchard analysis of [^{3}H] GABA binding against GABA in the cerebral cortex of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	136 ± 3.2	1.85 ± 0.10
Diabetic	125 ± 3.5 [a]	1.98 ± 0.12
D + IIH	95 ± 2.8 [a, b]	2.00 ± 0.15
C + IIH	83 ± 1.9 [a, b, c]	2.10 ± 0.13

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. [a] P<0.01 when compared to control, [b] P<0.01 when compared to diabetic, [c] P<0.01 when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-11

Real Time PCR amplification of AChE mRNA in the cerebral cortex of control and experimental rats

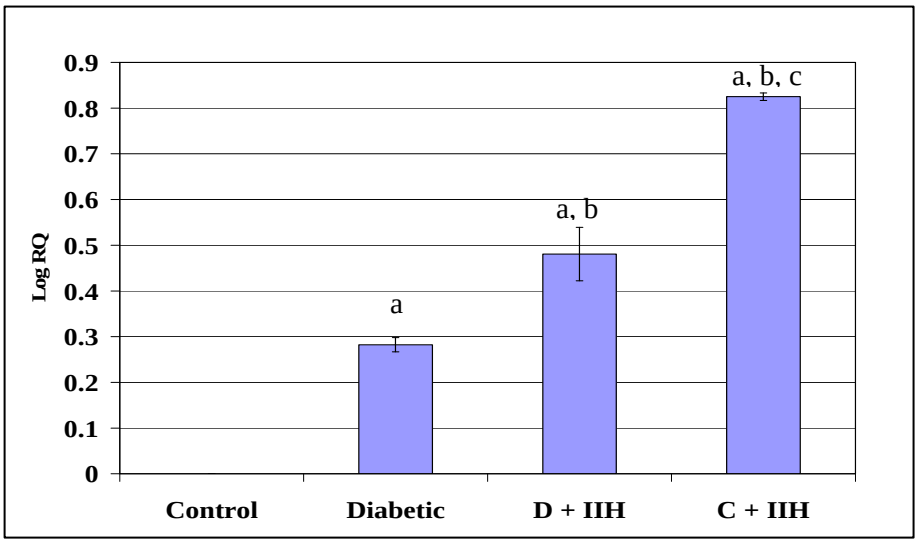


Table-11

Real Time PCR amplification of AChE mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.28 ± 0.01^{a}
D + IIH	$0.48 \pm 0.05^{a, b}$
C + IIH	$0.82 \pm 0.01^{a, b, c}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic, **c** $p<0.001$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-12

Real Time PCR amplification of ChAT mRNA in the cerebral cortex of control and experimental rats

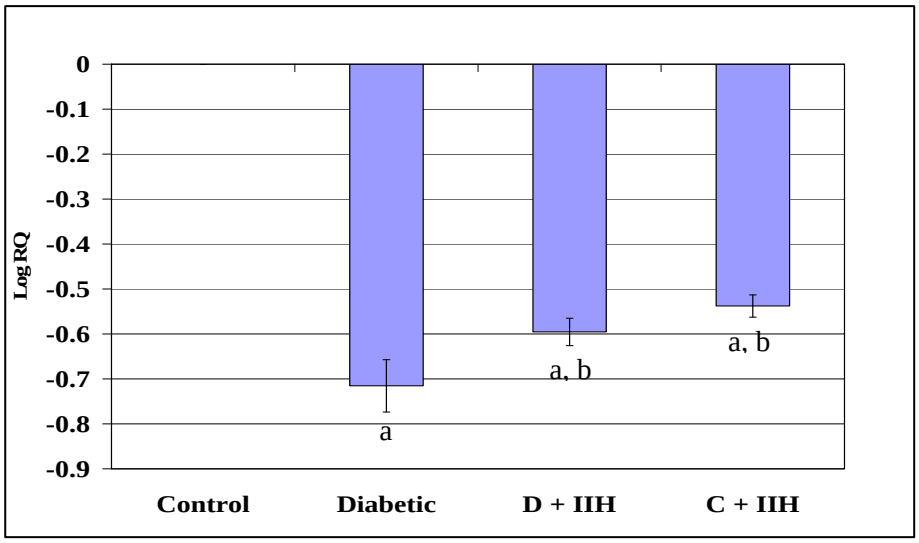


Table-12

Real Time PCR amplification of ChAT mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	- 0.71 ± 0.05 [a]
D + IIH	- 0.59 ± 0.03 [a, b]
C + IIH	- 0.53 ± 0.02 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-13

Real Time PCR amplification of muscarinic M1 receptor mRNA in the cerebral cortex of control and experimental rats

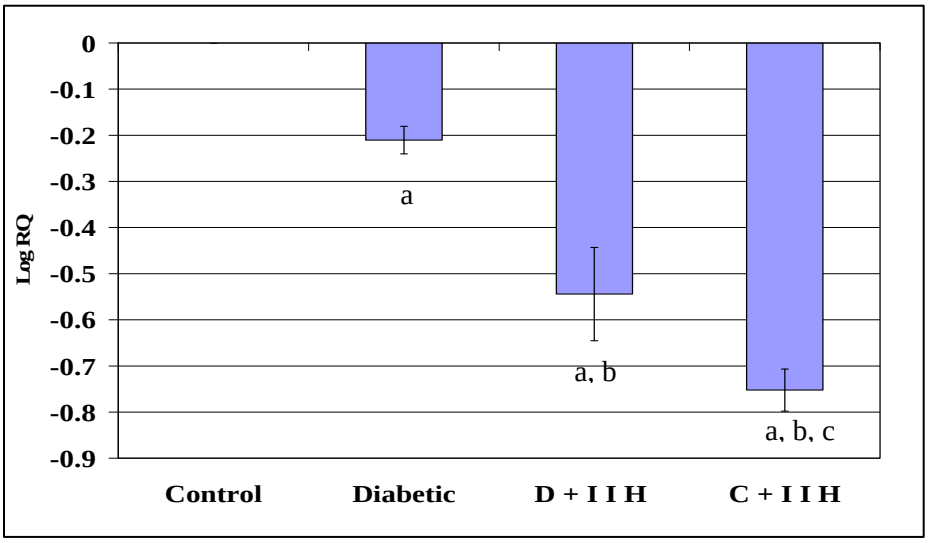


Table-13

Real Time PCR amplification of muscarinic M1 receptor mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	- 0.21 ± 0.02 [a]
D + IIH	- 0.54 ± 0.07 [a, b]
C + IIH	- 0.75 ± 0.04 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic, **c** $p<0.001$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-14

Real Time PCR amplification of muscarinic M3 receptor mRNA in the cerebral cortex of control and experimental rats

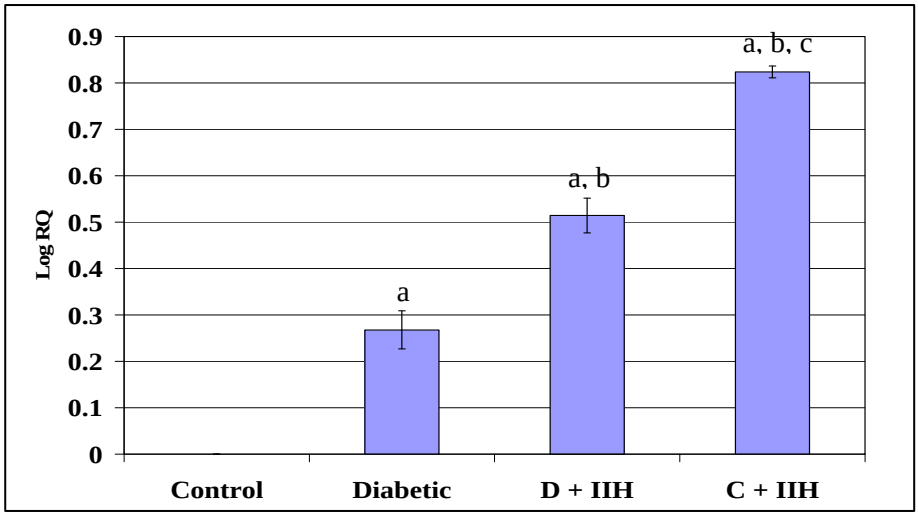


Table-14

Real Time PCR amplification of muscarinic M3 receptor mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.28 ± 0.04 [a]
D + IIH	0.51 ± 0.03 [a, b]
C + IIH	0.82 ± 0.01 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic, **c** $p<0.001$ when compared with D + IIH . IIH - Insulin Induced Hypoglycemia

Figure-15

Real Time PCR amplification of α7 nicotinic acetylcholine receptor mRNA in the cerebral cortex of control and experimental rats

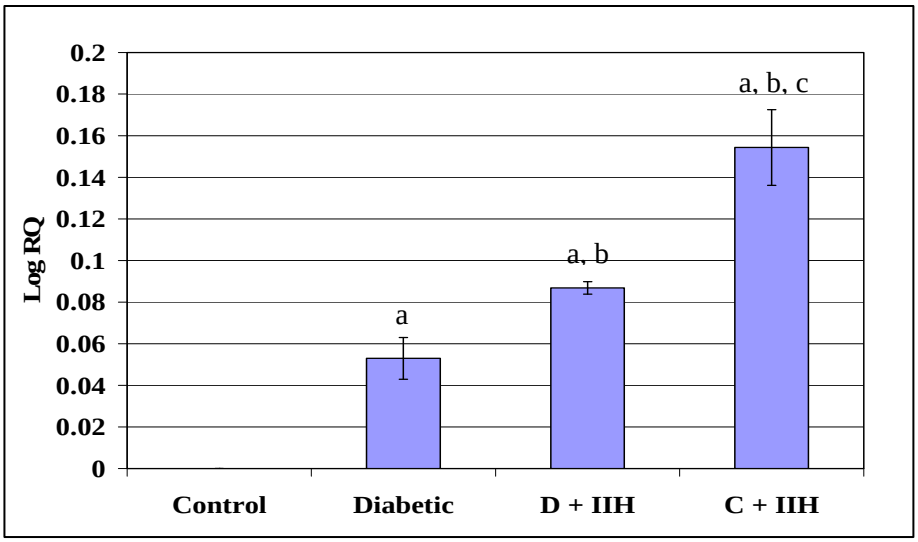


Table-15

Real Time PCR amplification of α7 nicotinic acetylcholine receptor mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.05 ± 0.01 [a]
D + IIH	0.08 ± 0.02 [a, b]
C + IIH	0.15 ± 0.01 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic, **c** $p<0.001$ when compared with D + IIH rats. IIH - Insulin Induced Hypoglycemia

Figure-16

Real Time PCR amplification of GAD mRNA in the cerebral cortex of control and experimental rats

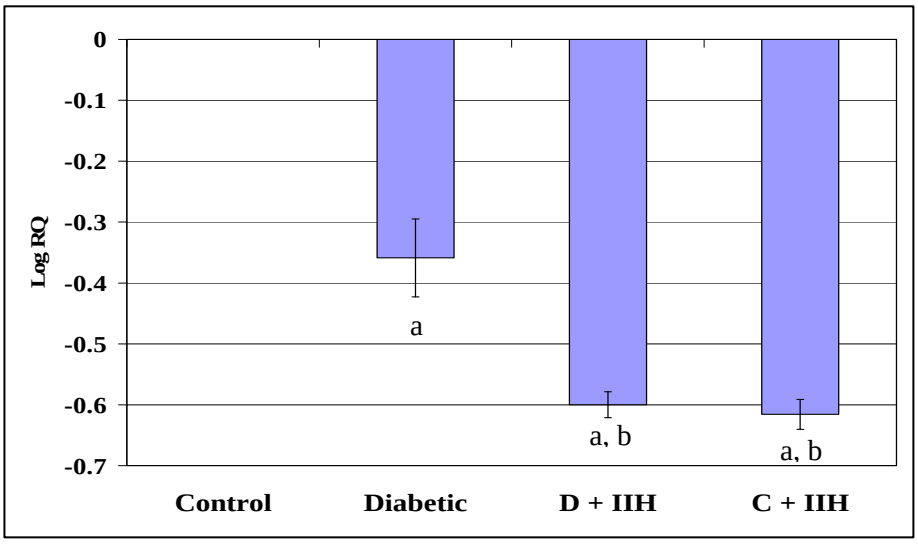


Table-16

Real Time PCR amplification of GAD mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.36 ± 0.10 [a]
D + IIH	-0.60 ± 0.10 [a, b]
C + IIH	-0.60 ± 0.09 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-17

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the cerebral cortex of control and experimental rats

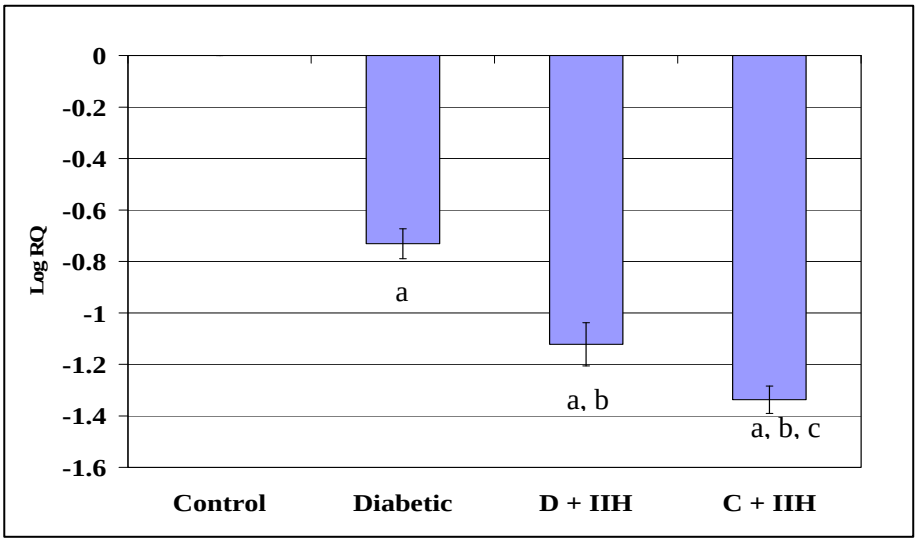


Table-17

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	- 0.73 ± 0.035 [a]
D + IIH	- 1.10 ± 0.04 [a, b]
C + IIH	-1.30 ± 0.03 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic, **c** $p<0.01$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-18

Real Time PCR amplification of $GABA_B$ mRNA in the cerebral cortex of control and experimental rats

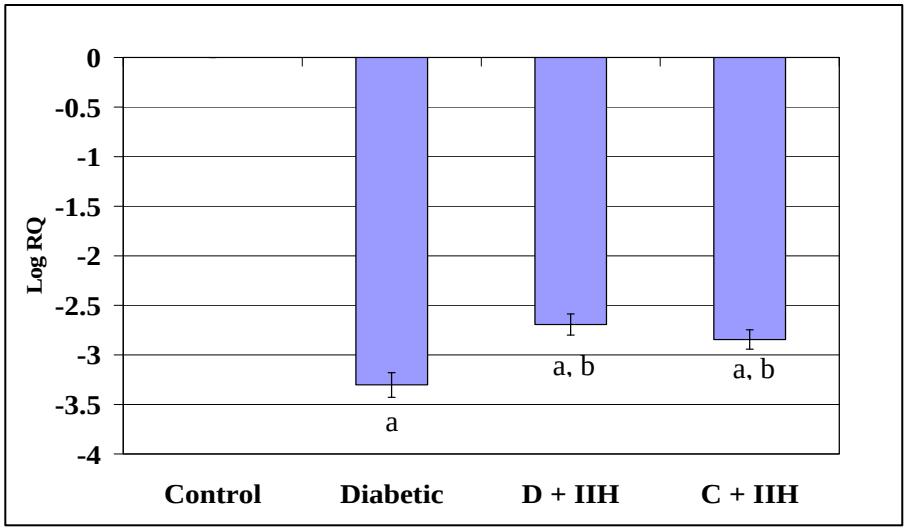


Table-18

Real Time PCR amplification of $GABA_B$ mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	- 3.30 ± 0.05 [a]
D + IIH	- 2.60 ± 0.05 [a, b]
C + IIH	-2.80 ± 0.04 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.01$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-19

Real Time PCR amplification of GLUT3 mRNA in the cerebral cortex of control and experimental rats

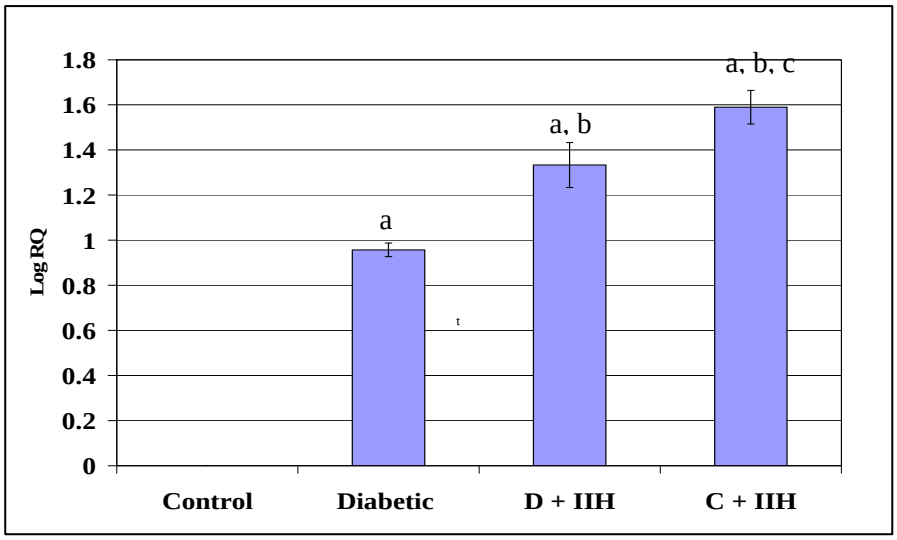


Table-19

Real Time PCR amplification of GLUT3 mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.95 ± 0.01^{a}
D + IIH	$1.33 \pm 0.03^{a, b}$
C + IIH	$1.60 \pm 0.02^{a, b, c}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic, **c** $p<0.01$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-20

Real Time PCR amplification of insulin receptor mRNA in the cerebral cortex of control and experimental rats

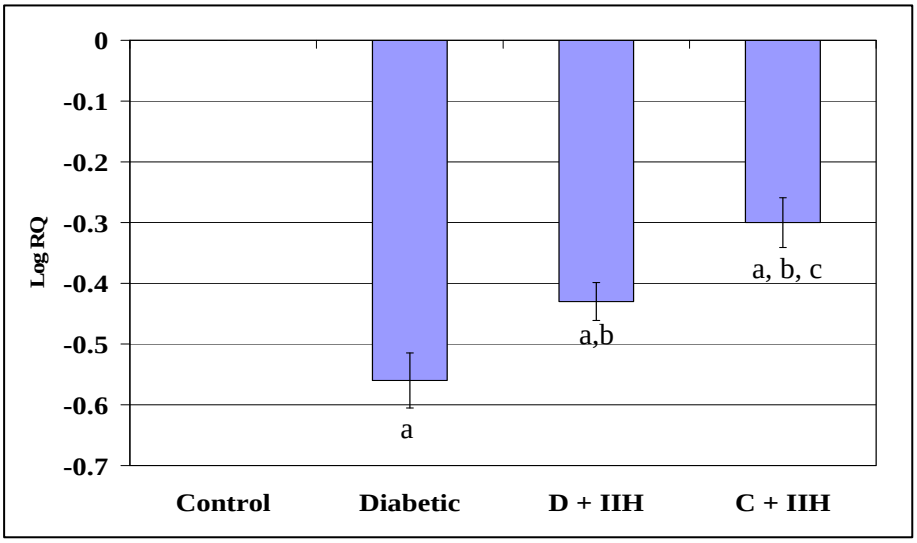


Table-20

Real Time PCR amplification of insulin receptor mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.56 ± 0.04 [a]
D + IIH	-0.43 ± 0.03 [a, b]
C + IIH	-0.30 ± 0.04 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. a $p<0.001$ when compared with control, b $p<0.001$ when compared with diabetic, c $p<0.01$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia.

Figure-21

Real Time PCR amplification of SOD mRNA in the cerebral cortex of control and experimental rats

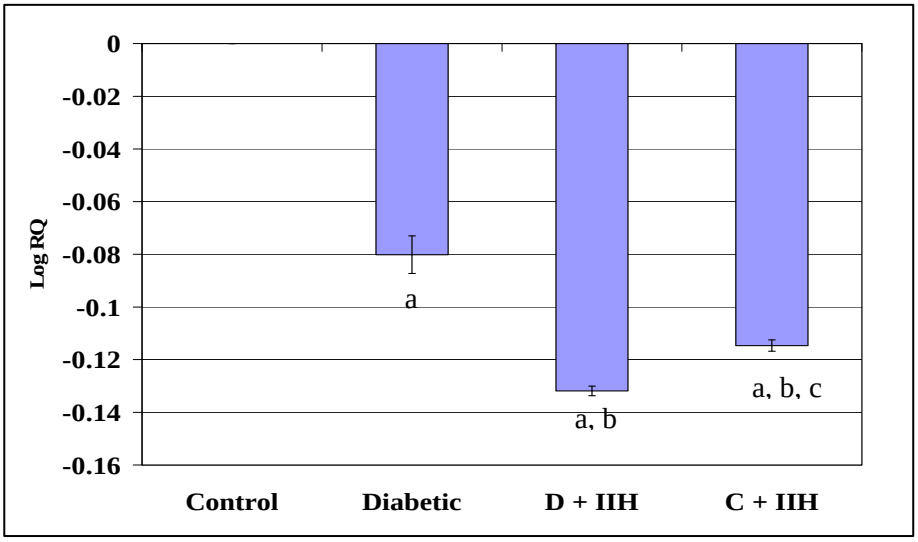


Table-21

Real Time PCR amplification of SOD mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.08 ± 0.007 [a]
D + IIH	-0.13 ± 0.008 [a, b]
C + IIH	-0.11 ± 0.002 [a,b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.01$ when compared with diabetic, **c** $p<0.01$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-22

Real Time PCR amplification of Bax mRNA in the cerebral cortex of control and experimental rats

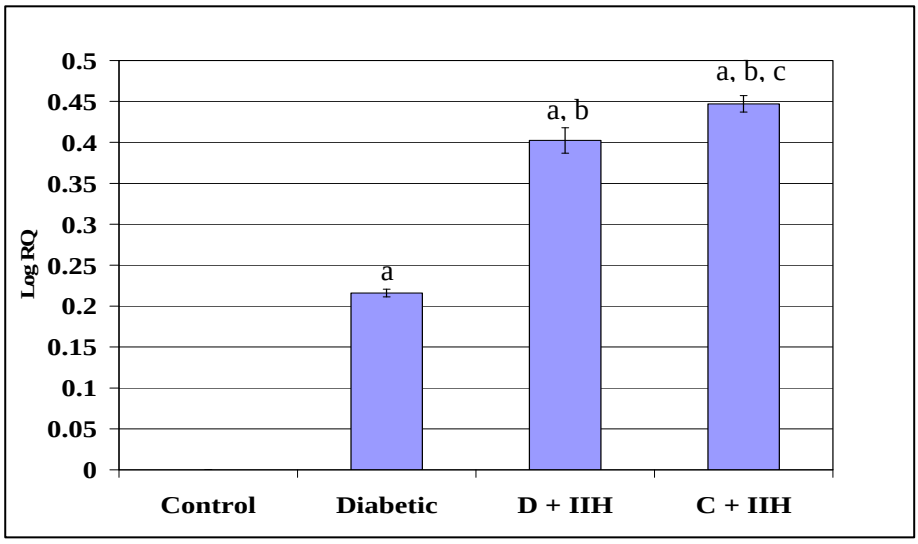


Table-22

Real Time PCR amplification of Bax mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.21 ± 0.007 [a]
D + IIH	0.40 ± 0.008 [a, b]
C + IIH	0.44 ± 0.002 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic, **c** $p<0.01$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-23

Real Time PCR amplification of phospholipase C mRNA in the cerebral cortex of control and experimental rats

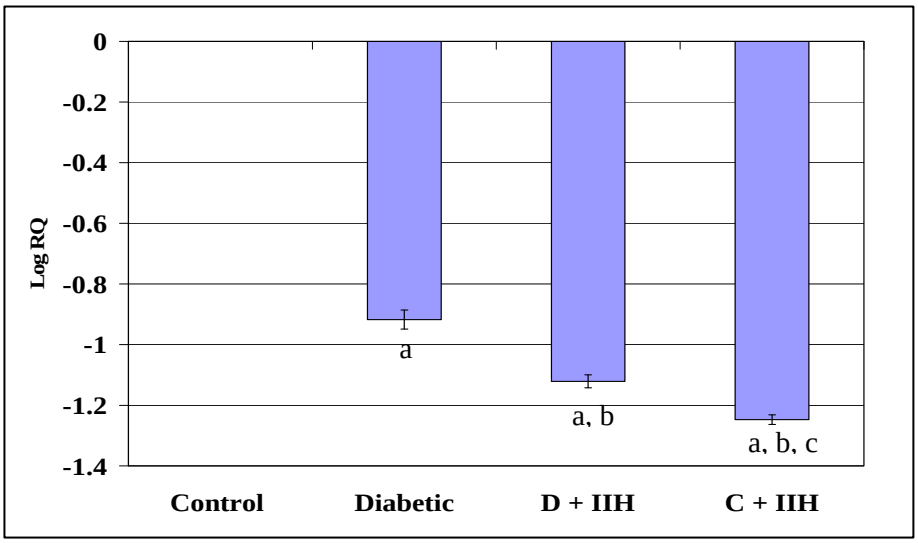


Table-23

Real Time PCR amplification of phospholipase C mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	- 0.91 ± 0.03 [a]
D + IIH	- 1.10 ± 0.02 [a, b]
C + IIH	-1.25 ± 0.01 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.01$ when compared with diabetic, **c** $p<0.01$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-24

Real Time PCR amplification of CREB mRNA in the cerebral cortex of control and experimental rats

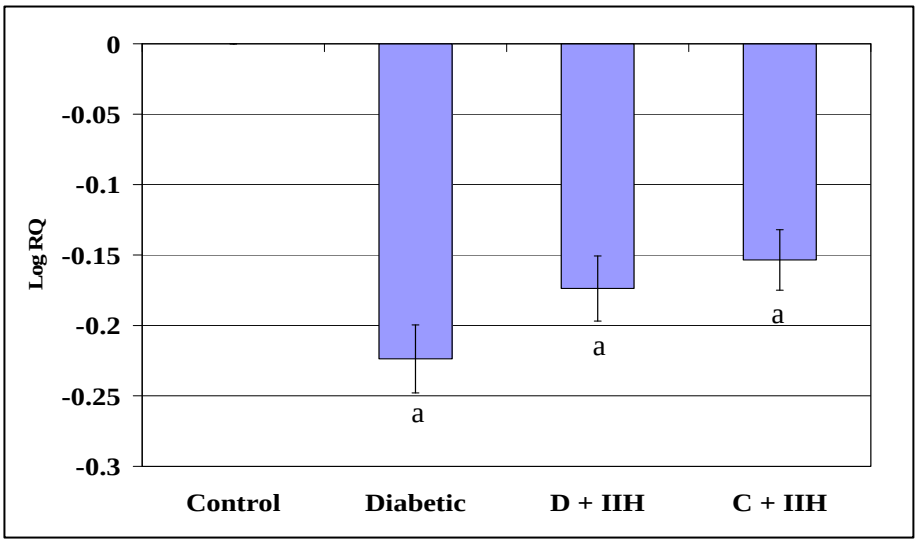


Table-24

Real Time PCR amplification of CREB mRNA in the cerebral cortex of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.22 ± 0.02 [a]
D + IIH	-0.17 ± 0.02 [a]
C + IIH	-0.15 ± 0.02 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control. IIH - Insulin Induced Hypoglycemia

Figure--25

Muscarinic M1 receptor expression in the cerebral cortex of control and experimental rats

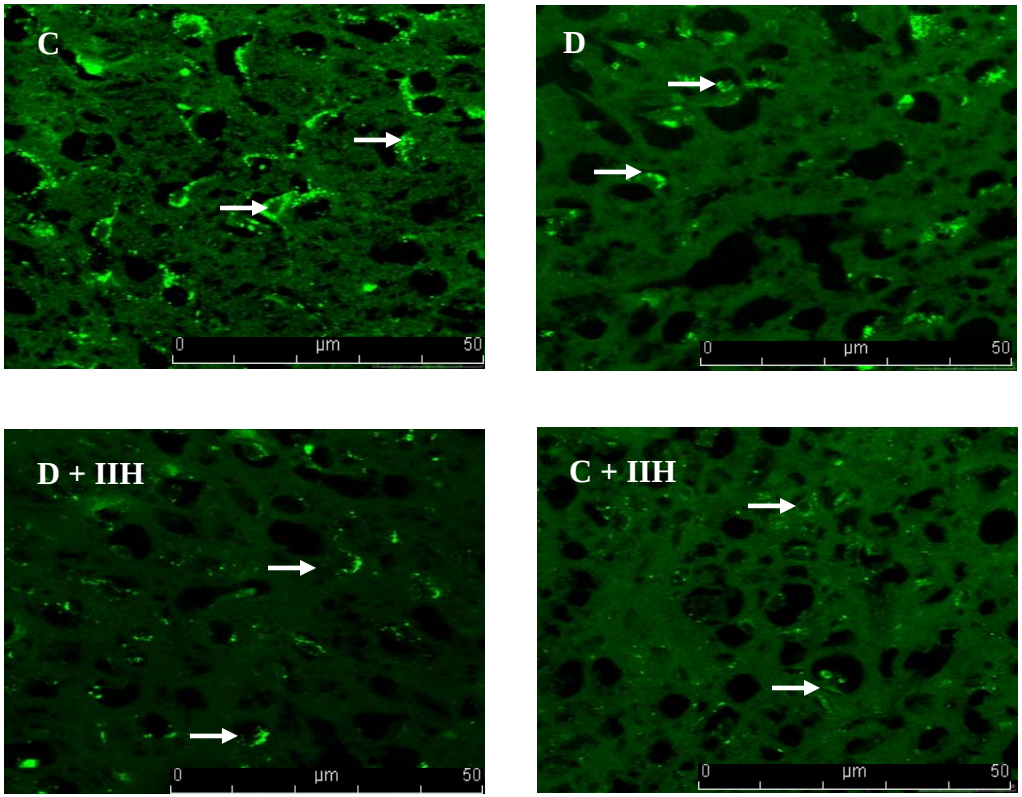


Table-25

Condition	Mean Pixel Value
Control	48 ± 5.6
Diabetic	32 ± 2.9 [a]
D+IIH	26 ± 3.8 [a, b]
C + IIH	17 ± 3.4 [a, b, c]

Confocal image of muscarinic M1 receptors in the cerebral cortex of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M1 receptors. Scale bar = 50 µm. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic, [c] $p<0.01$ when compared to D + IIH.

Figure--26

Muscarinic M3 receptor expression in the cerebral cortex of control and experimental rats

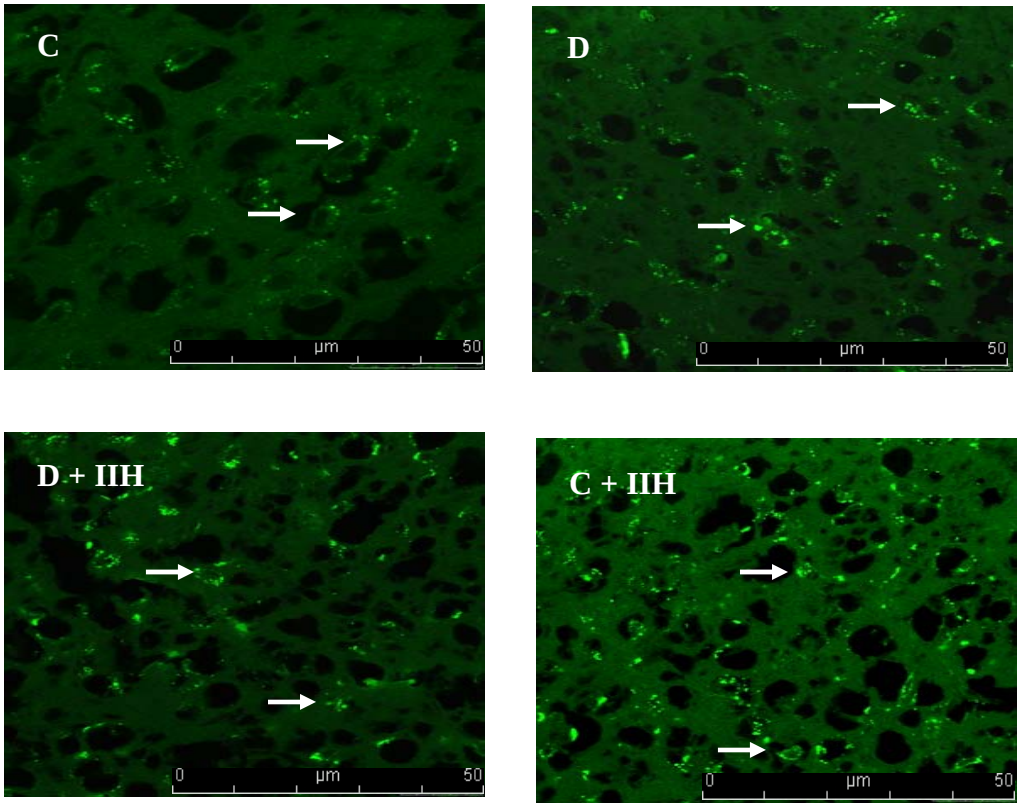


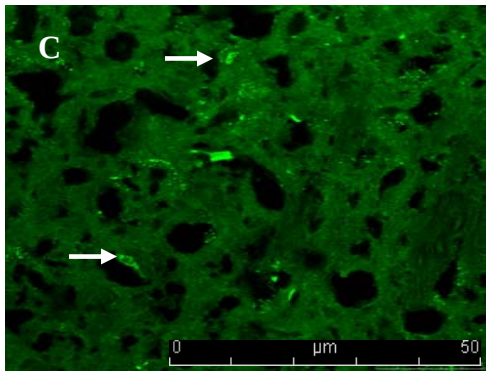
Table-26

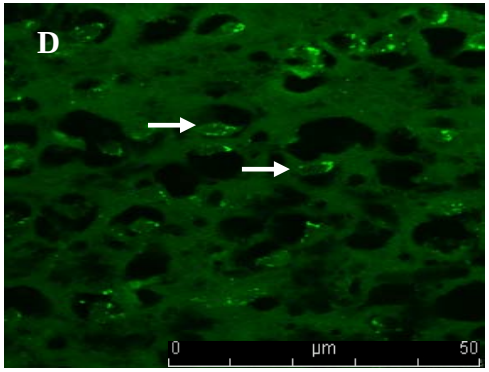
Condition	Mean Pixel Value
Control	16 ± 2.4
Diabetic	35 ± 3.1 [a]
D + IIH	42 ± 2.7 [a, b]
C + IIH	56 ± 4.2 [a, b, c]

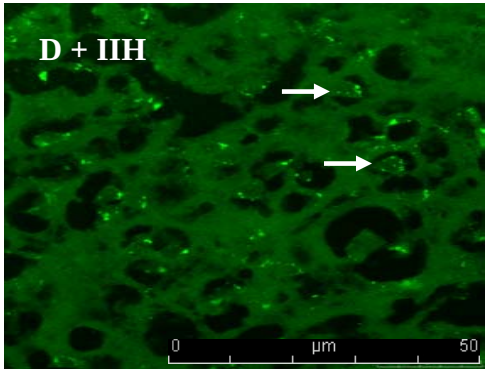
Confocal image of muscarinic M3 receptors in the cerebral cortex of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M3 receptors. Scale bar = 50 µm. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH.

Figure--27

α 7 nACh receptor expression in the cerebral cortex of control and experimental rats







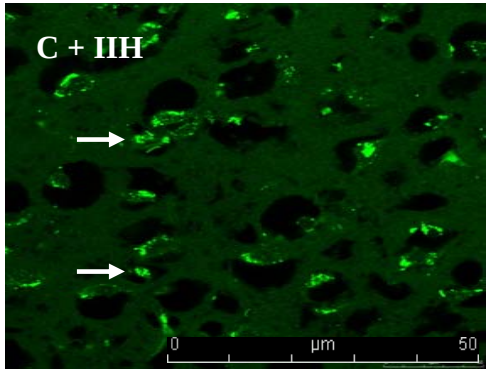


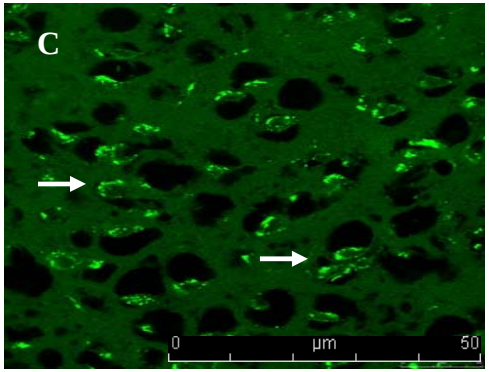
Table-27

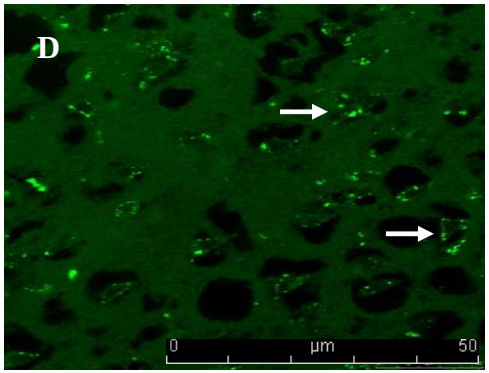
Condition	Mean Pixel Value
Control	23 ± 4.5
Diabetic	38 ± 3.1 [a]
D + IIH	46 ± 4.7 [a, b]
C + IIH	58 ± 3.8 [a, b, c]

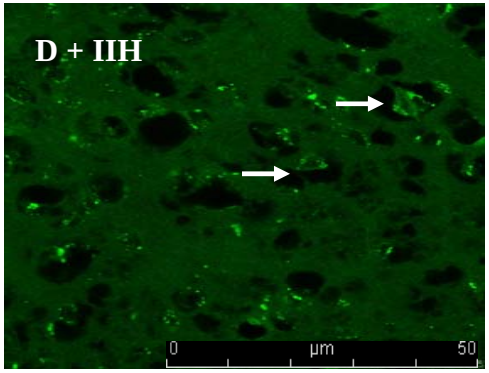
Confocal image of α7 nACh receptors in the cerebral cortex of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent α7 nACh receptor specific primary antibody and FITC as secondary antibody. (→) in white shows α7 nACh receptors. Scale bar = 50 µm. [a] $p<0.001$when compared to control, [b] $p<0.001$ when compared to diabetic, [c] $p<0.01$ when compared to D + IIH.

Figure--28

$GABA_{A\alpha1}$ receptor expression in the cerebral cortex of control and experimental rats







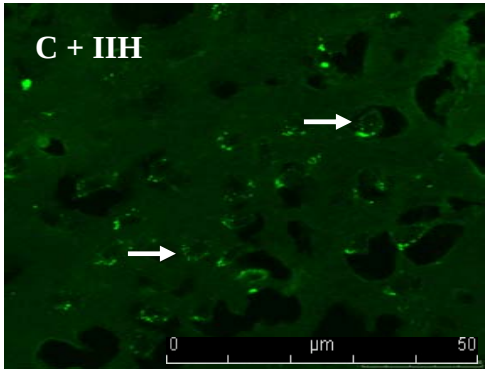


Table-28

Condition	Mean Pixel Value
Control	54 ± 5.6
Diabetic	33 ± 2.9 [a]
D+IIH	25 ± 3.8 [a, b]
C + IIH	14 ± 3.4 [a, b, c]

Confocal image of $GABA_{A\alpha1}$ receptors in the cerebral cortex of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent $GABA_{A\alpha1}$ receptor specific primary antibody and FITC as secondary antibody. (→) in white shows $GABA_{A\alpha1}$ receptors. Scale bar = 50 µm. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH.

Figure-29

Scatchard analysis of [^{3}H]QNB binding against total muscarinic receptor antagonist, atropine in the cerebellum of control and experimental rats

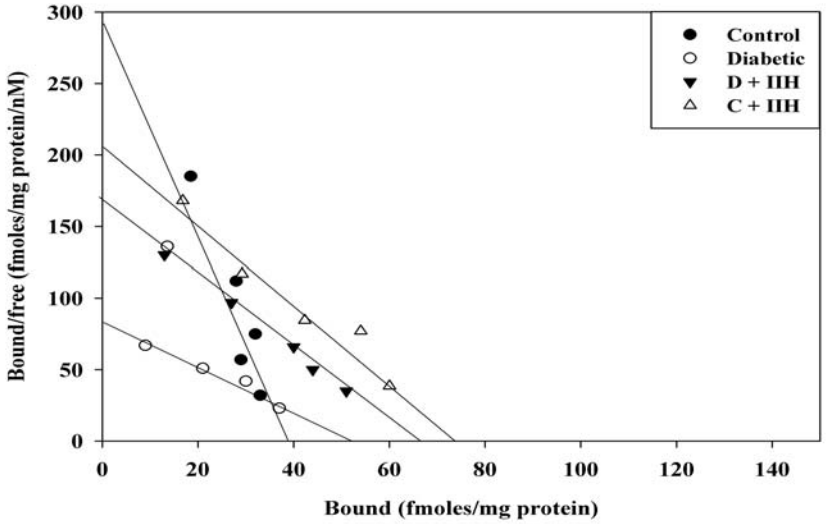


Table-29

Scatchard analysis of [3H]QNB binding against total muscarinic receptor antagonist, atropine in the cerebellum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	39 ± 9.4	0.13 ± 0.03
Diabetic	57 ± 8.5 [a]	0.65 ± 0.02 [a]
D + IIH	68 ± 5.0 [a, b]	0.39 ± 0.03 [a, b]
C + IIH	73 ± 6.5 [a, b]	0.35 ± 0.02 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.01$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-30

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebellum of control and experimental rats

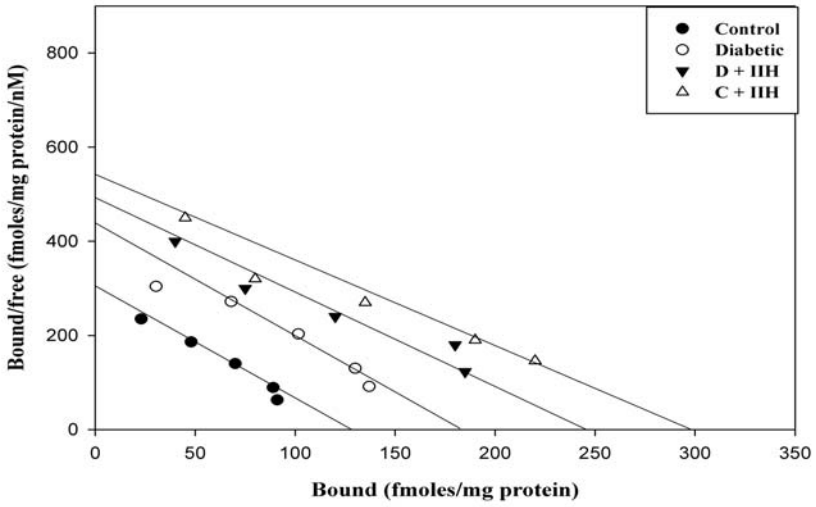


Table-30

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the cerebellum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	127 ± 12.4	0.40 ± 0.03
Diabetic	183 ± 11.5 [a]	0.41 ± 0.02
D + IIH	245 ± 10.5 [a,b]	0.50 ± 0.03 [a, b]
C + IIH	296 ± 9.8 [a, b, c]	0.54 ± 0.02 [a, b]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p< 0.01$ when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-31

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP in the cerebellum of control and experimental rats

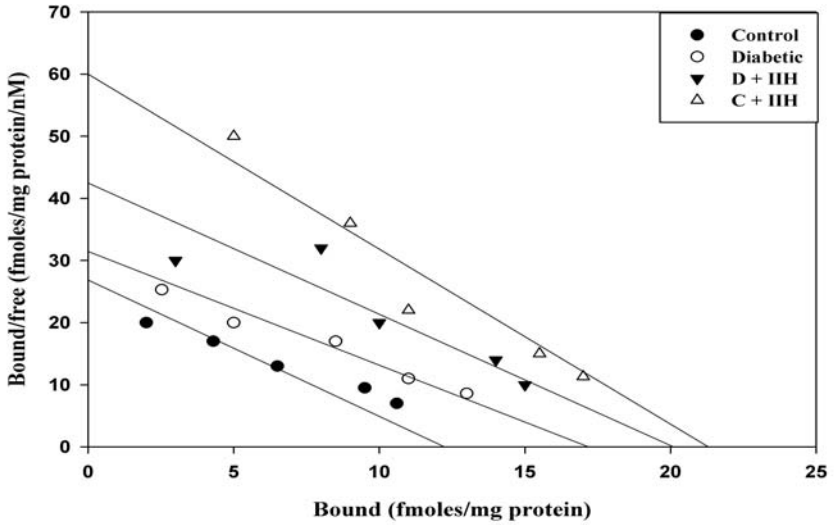


Table-31

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP in the cerebellum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	12 ± 1.5	0.46 ± 0.04
Diabetic	17 ± 0.5 [a]	0.45 ± 0.05
D + IIH	20 ± 1.2 [a, b]	0.43 ± 0.06
C + IIH	22 ± 1.5 [a, b]	0.36 ± 0.07

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.01$ when compared to control, **b** $p<0.01$ when compared to diabetic. IIH- Insulin Induced Hypoglycemia

Figure-32

Scatchard analysis of [^{3}H] GABA binding against GABA in the cerebellum of control and experimental rats

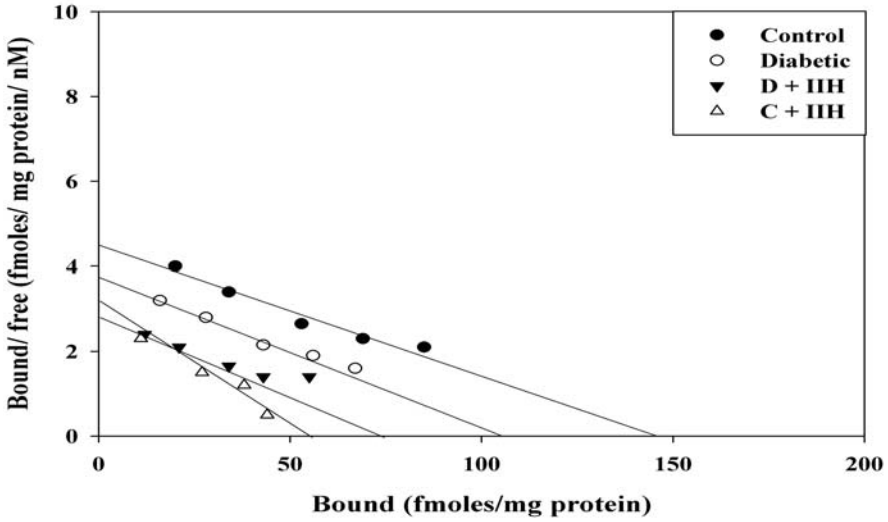


Table-32

Scatchard analysis of [^{3}H] GABA binding against GABA in the cerebellum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	146 ± 9.8	33.0 ± 1.3
Diabetic	105 ± 8.2 [a]	28.3 ± 1.9 [d]
D + IIH	74 ± 7.0 [a, b]	27.1 ± 1.0 [d]
C + IIH	55 ± 9.3 [a, b, c]	17.1 ± 1.2 [d, e]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$, [d] $p< 0.01$ when compared to control, [b] $p<0.001$ when compared to diabetic, [c] $p< 0.01$, [e] $p< 0.01$ when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-33

Real Time PCR amplification of AChE mRNA in the cerebellum of control and experimental rats

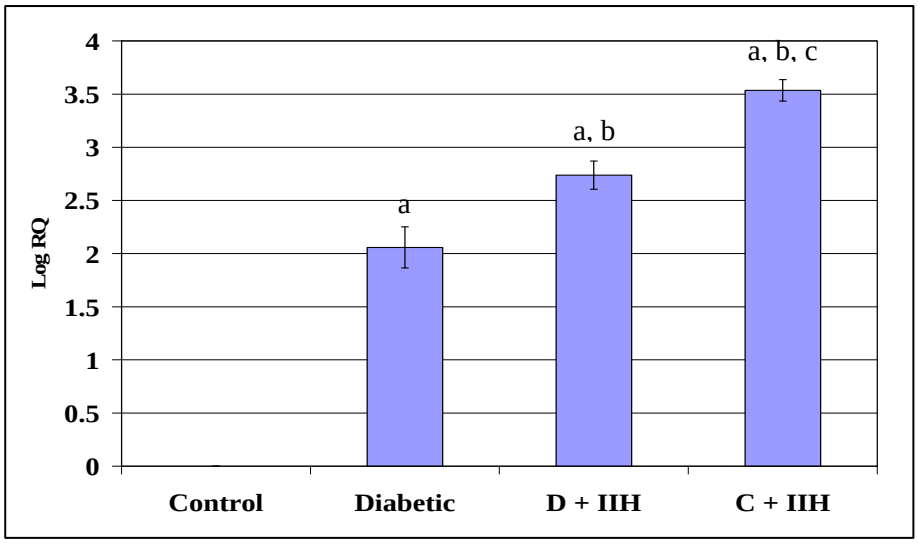


Table-33

Real Time PCR amplification of AChE mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	2.05 ± 0.19 [a]
D + IIH	2.70 ± 0.13 [a, b]
C + IIH	3.50 ± 0.10 [a, b, c]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p< 0.001$ when compared to D + IIH. IIH- Insulin Induced Hypoglycemia.

Figure-34

Real Time PCR amplification of ChAT mRNA in the cerebellum of control and experimental rats

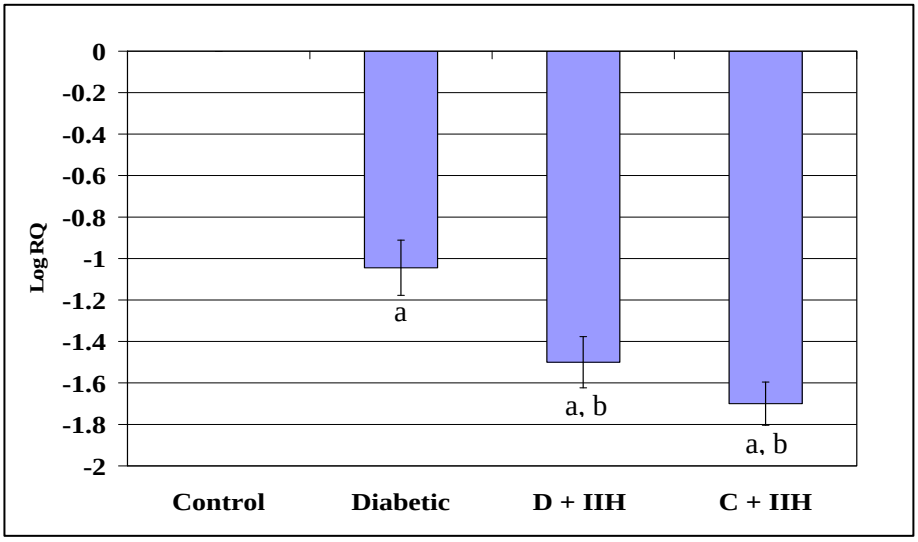


Table-34

Real Time PCR amplification of ChAT mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.04 ± 0.13 [a]
D + IIH	-1.50 ± 0.12 [a, b]
C + IIH	-1.70 ± 0.10 [a, b]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic. IIH- Insulin Induced Hypoglycemia

Figure-35

Real Time PCR amplification of muscarinic M1 receptor mRNA in the cerebellum of control and experimental rats

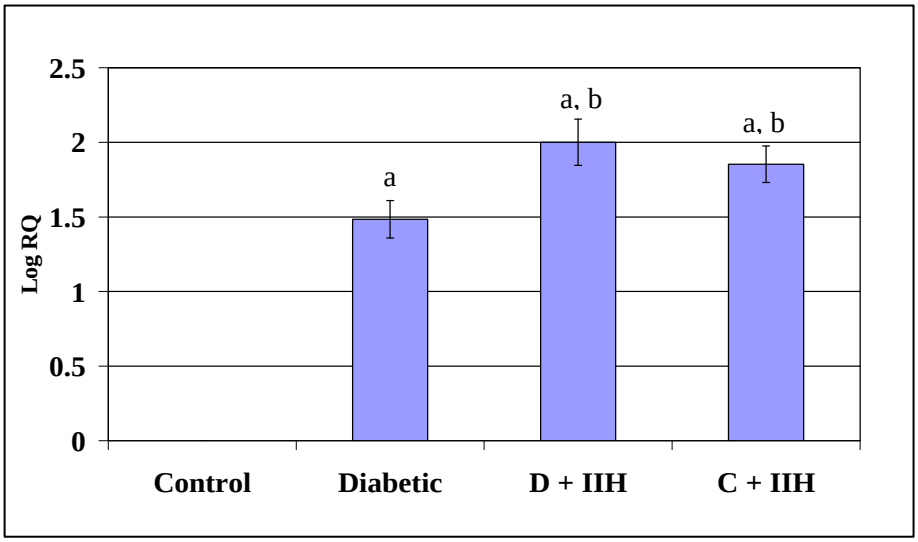


Table-35

Real Time PCR amplification of muscarinic M1 receptor mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.48 ± 0.12 [a]
D + IIH	2.00 ± 0.15 [a, b]
C + IIH	1.85 ± 0.12 [a, b]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic. IIH- Insulin Induced Hypoglycemia

Figure-36

Real Time PCR amplification of muscarinic M3 receptor mRNA in the cerebellum of control and experimental rats

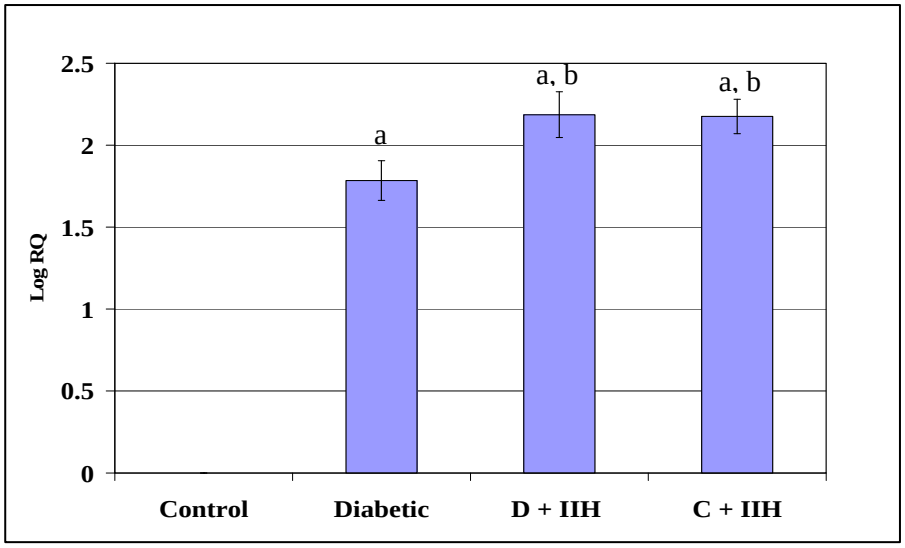


Table-36

Real Time PCR amplification of muscarinic M3 receptor mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.78 ± 0.03 [a]
D + IIH	2.18 ± 0.03 [a, b]
C + IIH	2.17 ± 0.03 [a, b]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.01$ when compared to diabetic. IIH- Insulin Induced Hypoglycemia

Figure-37

Real Time PCR amplification of α7 nicotinic acetylcholine receptor mRNA in the cerebellum of control and experimental rats

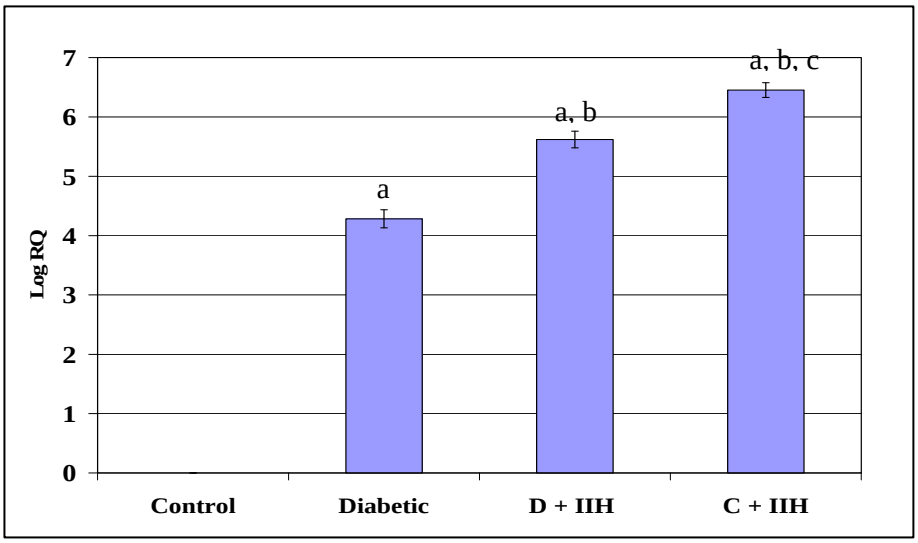


Table-37

Real Time PCR amplification of α7 nicotinic acetylcholine receptor mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	4.28 ± 0.05 [a]
D + IIH	5.6 ± 0.04 [a, b]
C + IIH	6.4 ± 0.04 [a, b, c]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.01$ when compared to diabetic, **c** $p< 0.01$ when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-38

Real Time PCR amplification of GAD mRNA in the cerebellum of control and experimental rats

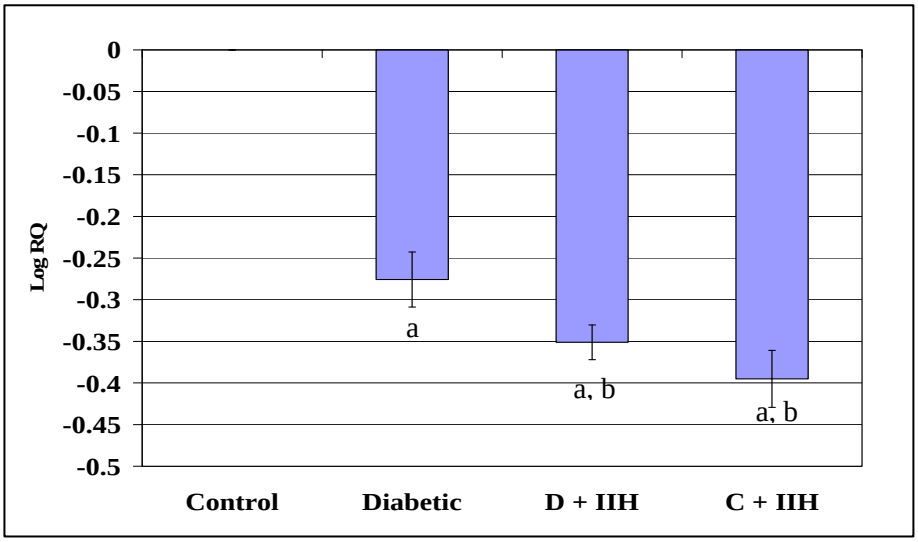


Table-38

Real Time PCR amplification of GAD mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.28 ± 0.04 [a]
D + IIH	-0.35 ± 0.02 [a, b]
C + IIH	-0.39 ± 0.04 [a, b]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **[a]** $p<0.001$ when compared to control, **[b]** $p<0.001$ when compared to diabetic. IIH- Insulin Induced Hypoglycemia

Figure-39

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the cerebellum of control and experimental rats

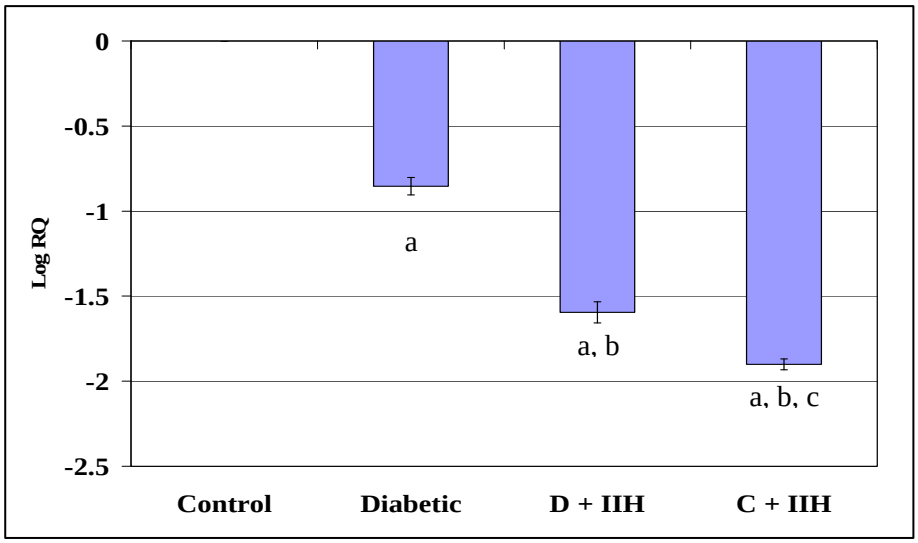


Table-39

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.85 ± 0.053 [a]
D + IIH	-1.60 ± 0.06 [a, b]
C + IIH	-1.90 ± 0.03 [a, b, c]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH . IIH- Insulin Induced Hypoglycemia

Figure-40

Real Time PCR amplification of $GABA_B$ mRNA in the cerebellum of control and experimental rats

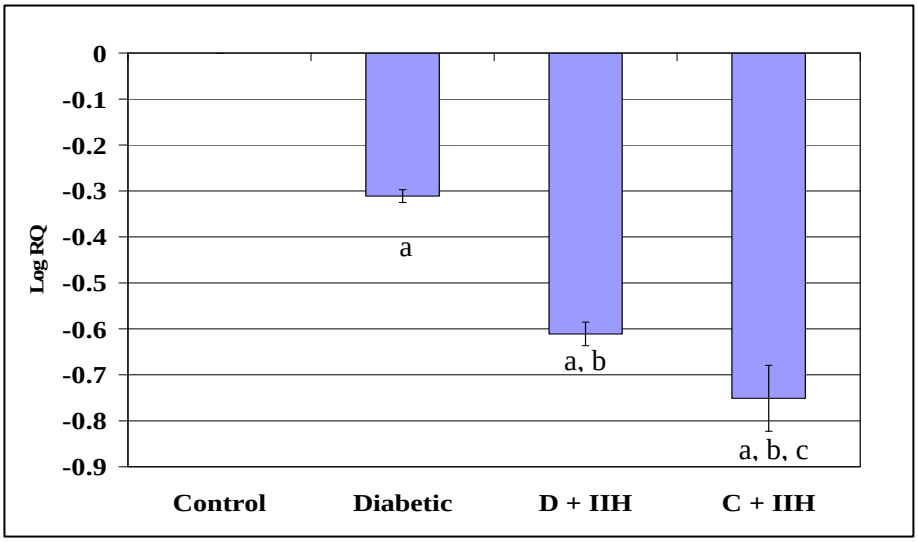


Table-40

Real Time PCR amplification of $GABA_B$ mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.31 ± 0.01^{a}
D + IIH	$-0.61 \pm 0.02^{a, b}$
C + IIH	$-0.75 \pm 0.07^{a, b, c}$

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats.
a $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH . IIH- Insulin Induced Hypoglycemia

Figure-41

Real Time PCR amplification of GLUT3 mRNA in the cerebellum of control and experimental rats

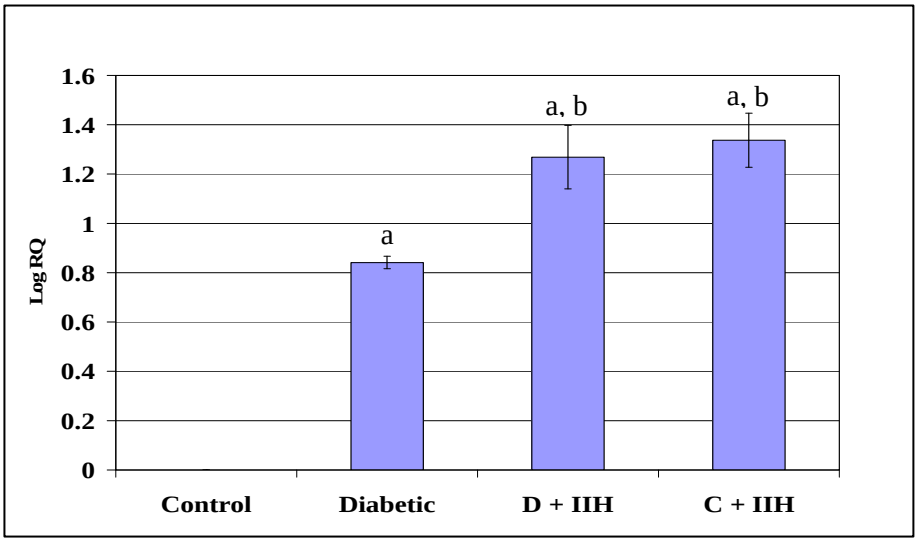


Table-41

Real Time PCR amplification of GLUT3 mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.04 ± 0.03 [a]
D + IIH	-1.50 ± 0.09 [a, b]
C + IIH	-1.70 ± 0.07 [a, b]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **[a]** $p<0.001$ when compared to control, **[b]** $p<0.001$ when compared to diabetic. IIH- Insulin Induced Hypoglycemia

Figure-42

Real Time PCR amplification of insulin receptor mRNA in the cerebellum of control and experimental rats

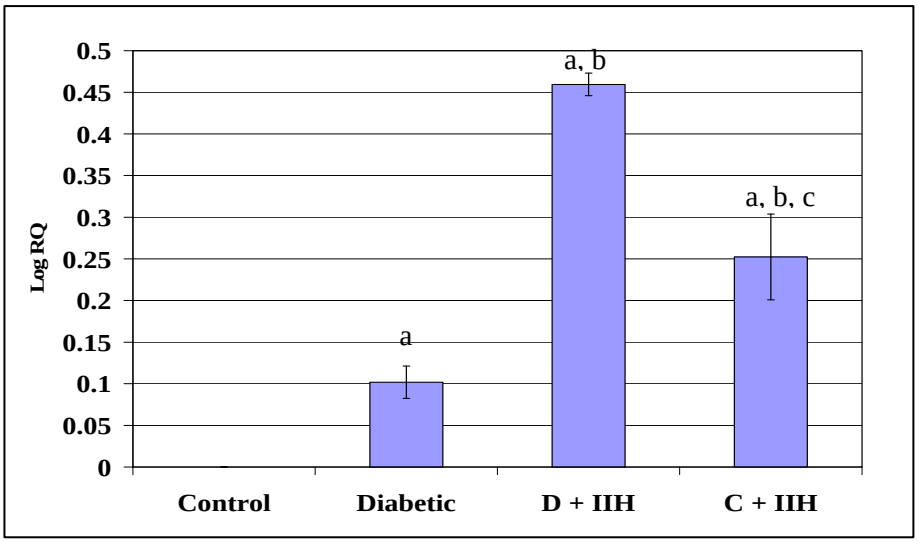


Table-42

Real Time PCR amplification of insulin receptor mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.10 ± 0.02^{a}
D + IIH	$0.45 \pm 0.01^{a, b}$
C + IIH	$0.25 \pm 0.05^{a, b, c}$

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p< 0.01$ when compared to D + IIH, IIH- Insulin Induced Hypoglycemia

Figure-43

Real Time PCR amplification of SOD mRNA in the cerebellum of control and experimental rats

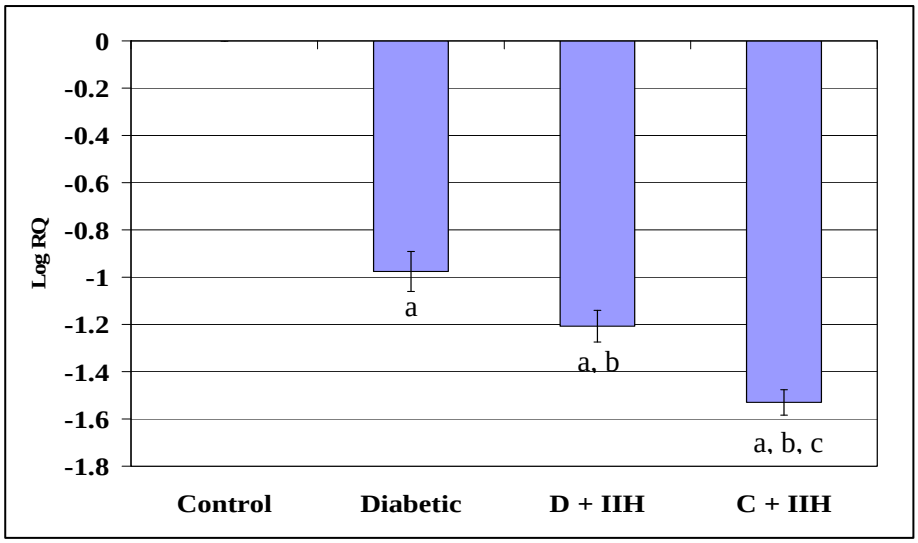


Table-43

Real Time PCR amplification of SOD mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.97 ± 0.08 [a]
D + IIH	-1.20 ± 0.06 [a, b]
C + IIH	-1.54 ± 0.05 [a, b, c]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-44

Real Time PCR amplification of Bax mRNA in the cerebellum of control and experimental rats

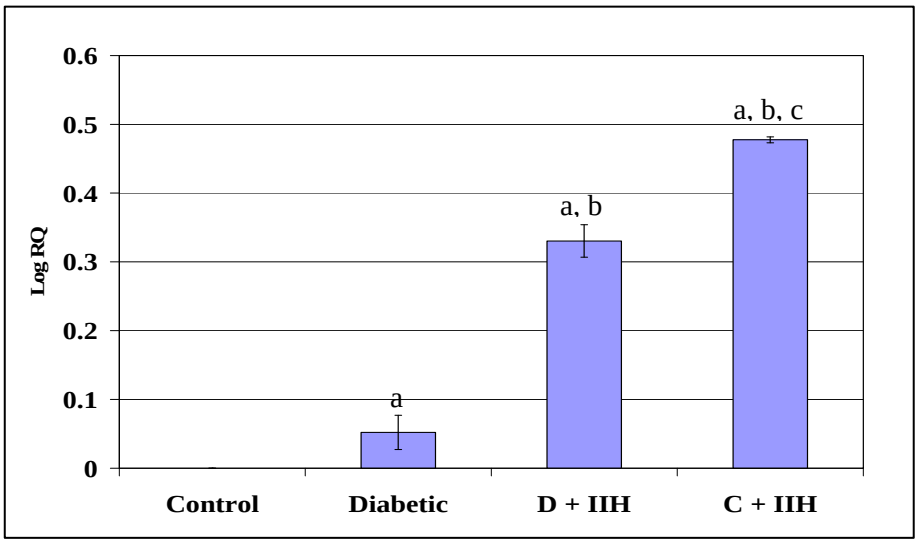


Table-44

Real Time PCR amplification of Bax mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.05 ± 0.02 [a]
D + IIH	0.33 ± 0.02 [a, b]
C + IIH	0.47 ± 0.01 [a, b, c]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-45

Real Time PCR amplification of phospholipase C mRNA in the cerebellum of control and experimental rats

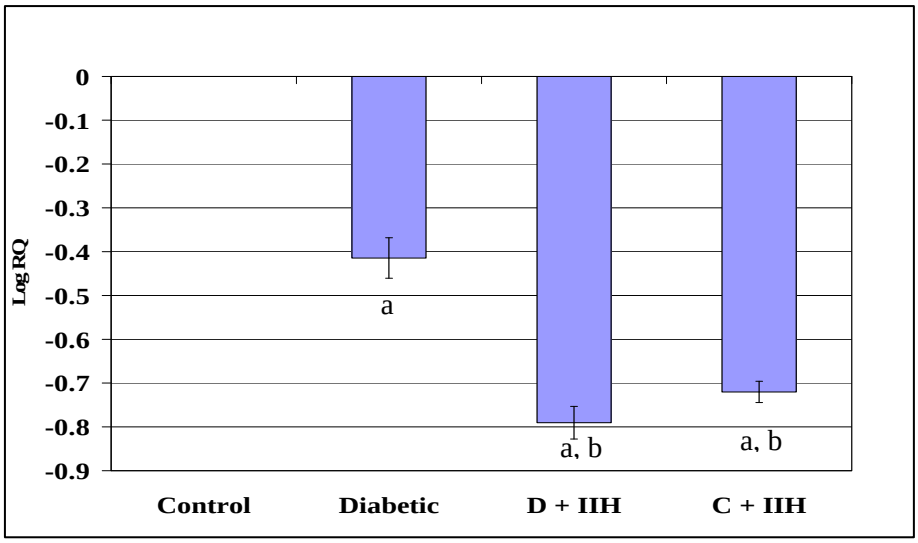


Table-45

Real Time PCR amplification of phospholipase C mRNA in the cerebellum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.40± 0.04 [a]
D + IIH	-0.79 ± 0.03 [a, b]
C + IIH	-0.72 ± 0.02 [a, b]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic. IIH- Insulin Induced Hypoglycemia

Figure-46

Real Time PCR amplification of CREB mRNA in the cerebellum of control and experimental rats

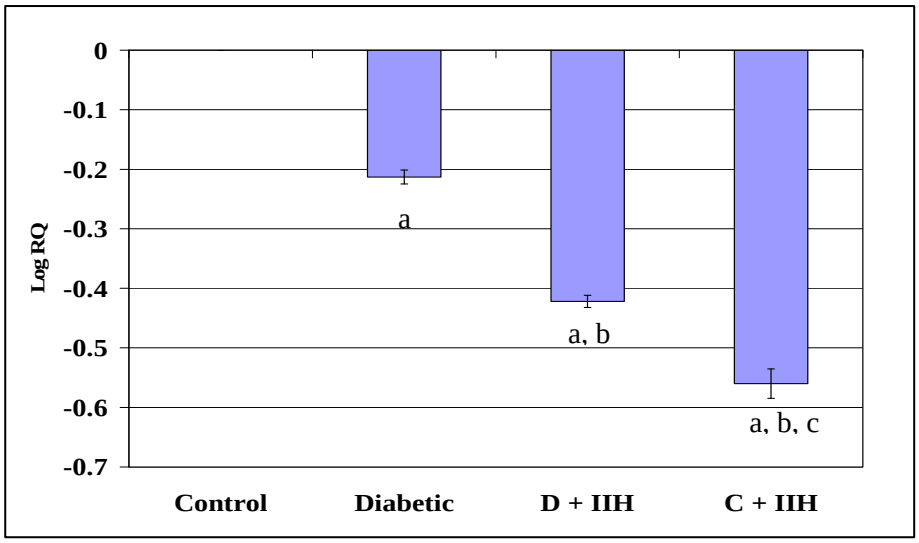


Table-46

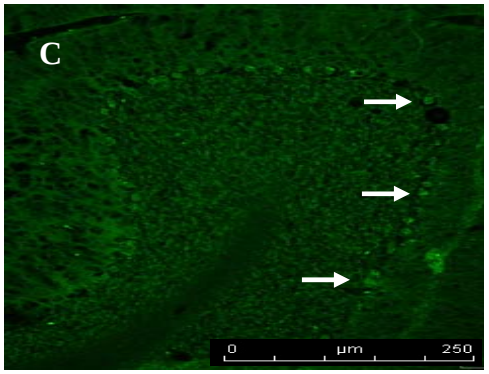
Real Time PCR amplification of CREB mRNA in the cerebellum of control and experimental rats

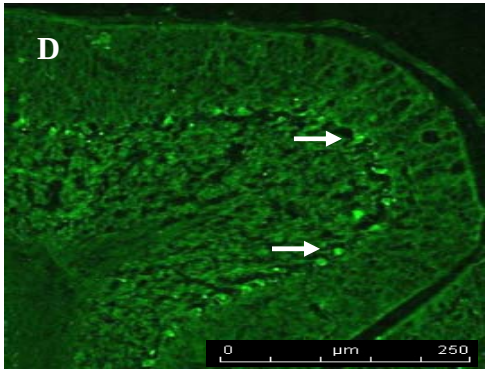
Experimental groups	Log RQ
Control	0
Diabetic	-0.21 ± 0.02 [a]
D + IIH	-0.42 ± 0.02 [a, b]
C + IIH	-0.57 ± 0.04 [a, b, c]

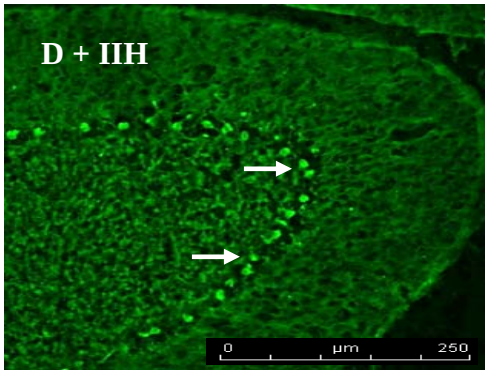
Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p< 0.001$ when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure--47

Muscarinic M1 receptor expression in the cerebellum of control and experimental rats







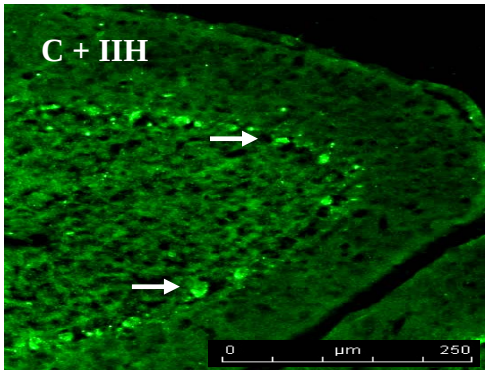


Table-47

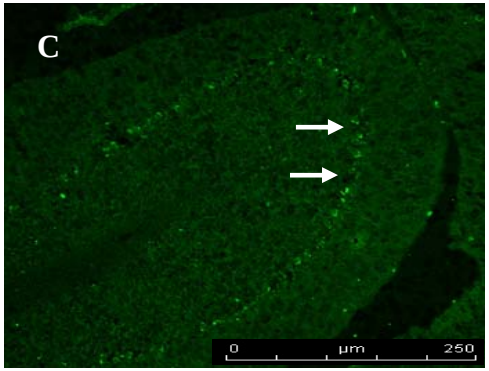
Condition	Mean Pixel Value
Control	17.5 ± 5.6
Diabetic	26.8 ± 2.9 [a]
D + IIH	33.2 ± 3.8 [a, b]
C + IIH	42.7 ± 3.4 [a, b, c]

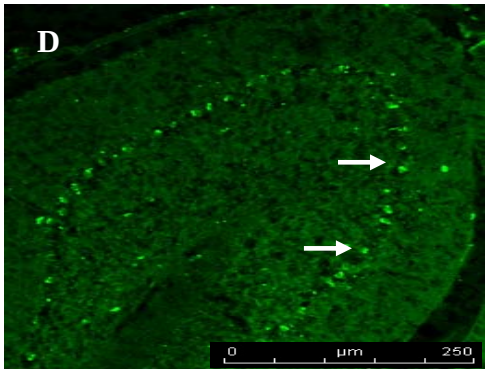
Confocal image of muscarinic M1 receptors in the cerebellum of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M1 receptors. Scale bar = 250 µm. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH.

Figure--48

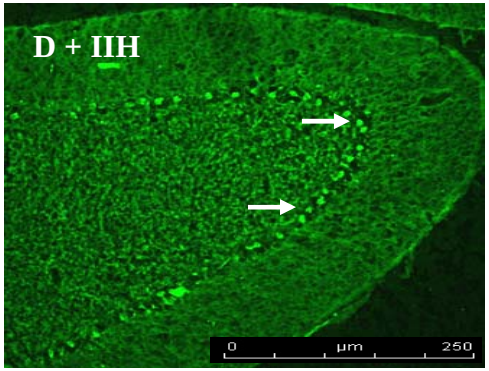
Muscarinic M3 receptor expression in the cerebellum of control and experimental rats

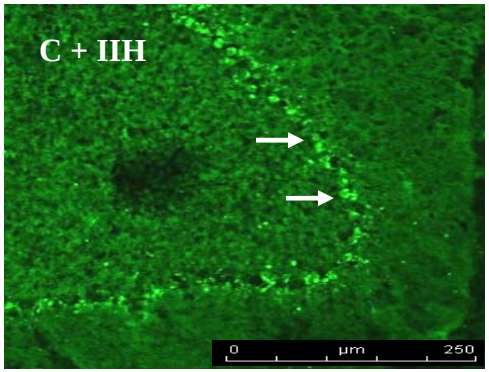
Table-48





Condition	Mean Pixel Value
Control	15 ± 3.3
Diabetic	32 ± 4.1 [a]
D+IIH	46 ± 4.8 [a, b]
C + IIH	59 ± 2.9 [a, b, c]

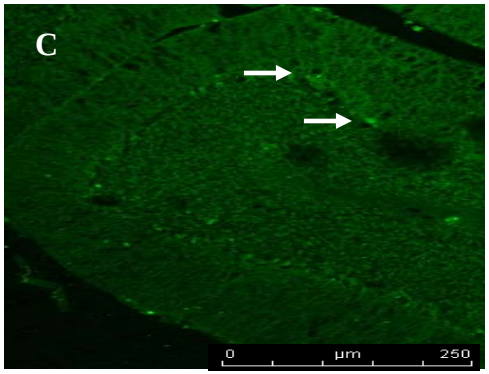


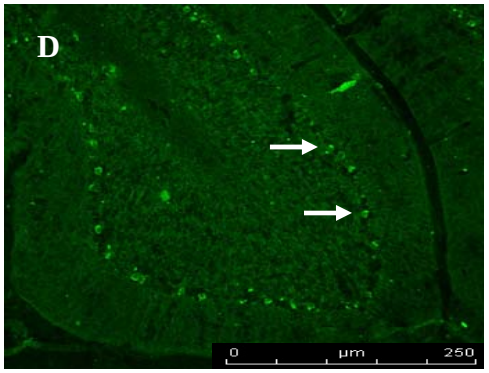


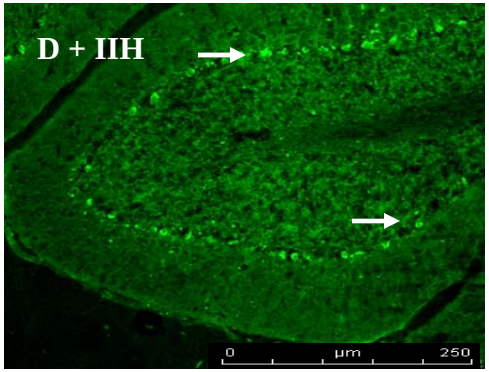
Confocal image of muscarinic M3 receptors in the cerebellum of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M3 receptors. Scale bar = 250 µm. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic, [c] $p<0.001$ when compared to D + IIH. .

Figure--49

α7 nicotinic acetylcholine receptor expression in the cerebellum of control and experimental rats







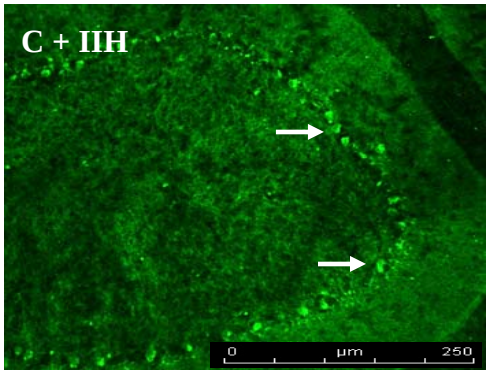


Table-49

Condition	Mean Pixel Value
Control	23 ± 4.5
Diabetic	38 ± 3.1 [a]
D+IIH	46 ± 5.1 [a, b]
C + IIH	58 ± 3.8 [a, b, c]

Confocal image of α7 nACh receptors in the cerebellum of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent α7 nACh receptor specific primary antibody and FITC as secondary antibody. (→) in white shows α7 nACh receptors. Scale bar = 250 µm. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH.

Figure--50

$GABA_{A\alpha1}$ receptor expression in the cerebellum of control and experimental rats

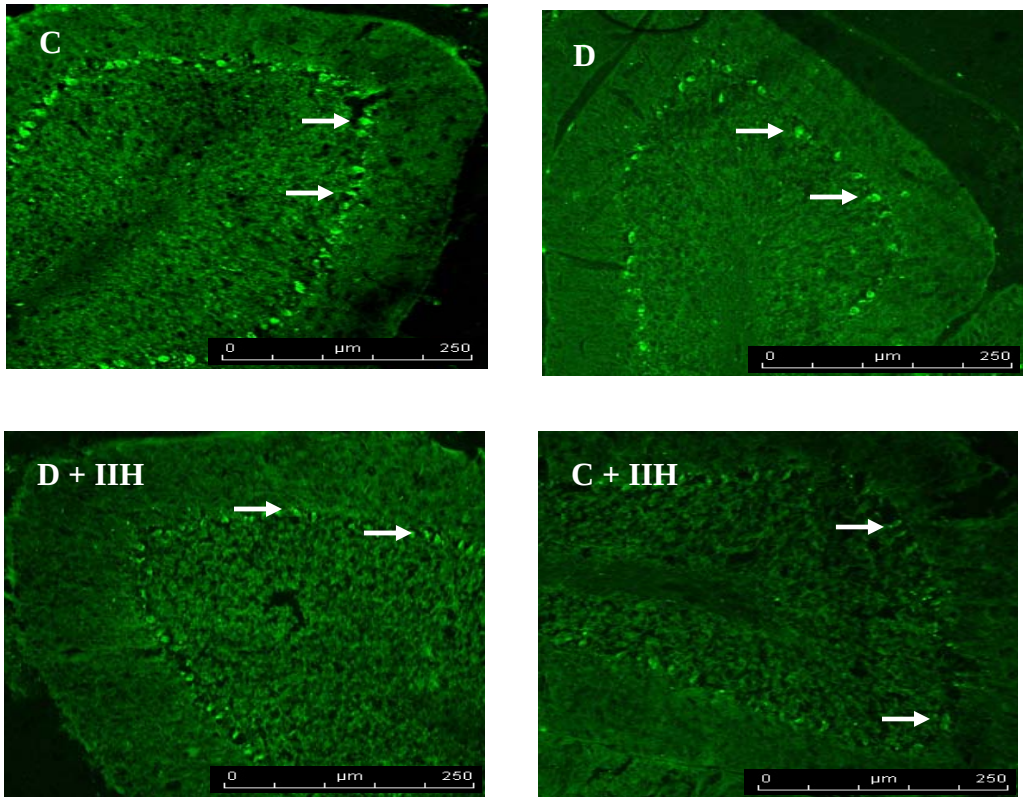


Table-50

Condition	Mean Pixel Value
Control	58 ± 5.7
Diabetic	40 ± 4.3 [a]
D + IIH	29 ± 4.5 [a, b]
C + IIH	18 ± 3.6 [a, b, c]

Confocal image of $GABA_{A\alpha1}$ receptors in the cerebellum of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent $GABA_{A\alpha1}$ receptor specific primary antibody and FITC as secondary antibody. (→) in white shows $GABA_{A\alpha1}$ receptors. Scale bar = 250 µm. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH.

Figure-51

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the brainstem of control and experimental rats

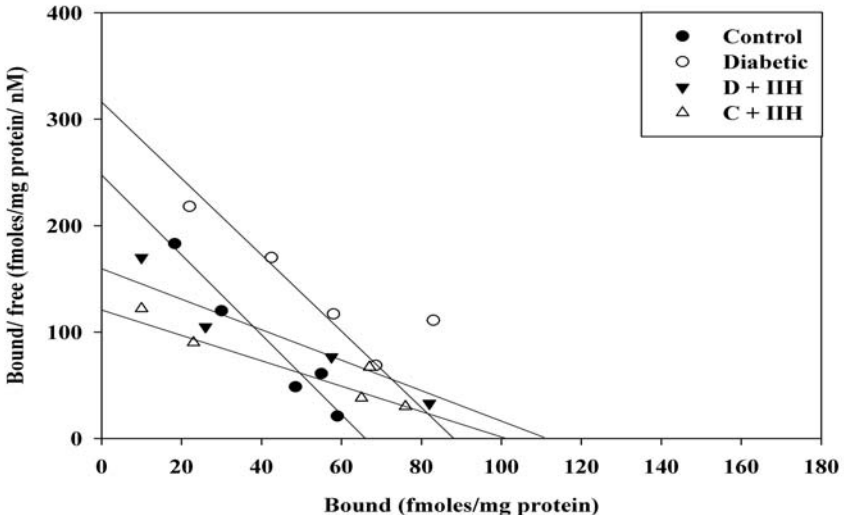


Table-51

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the brainstem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	62 ± 4.0	0.26 ± 0.8
Diabetic	88 ± 5.3 [a]	0.28 ± 0.7
D + IIH	110 ± 4.4 [a, b]	0.70 ± 1.0 [d, e]
C + IIH	100 ± 7.0 [a, b, c]	0.84 ± 1.2 [d, e]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$, **d** $p<0.01$ when compared with control, **b** $p<0.001$, **e** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-52

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the brainstem of control and experimental rats

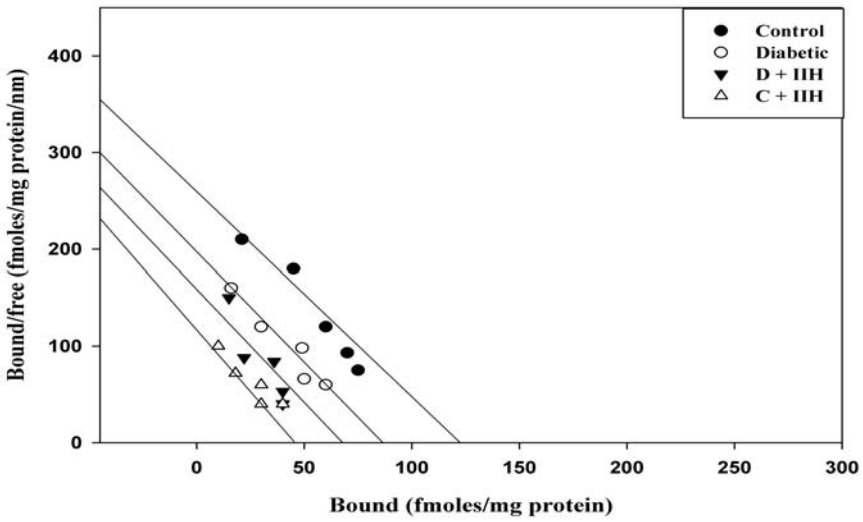


Table-52

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the brainstem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	121 ± 5.7	0.33 ± 0.08
Diabetic	85 ± 4.3 [a]	0.28 ± 0.07
D + IIH	67 ± 3.4 [a, b]	0.25 ± 0.03
C + IIH	42 ± 2.3 [a, b, c]	0.20 ± 0.06

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-53

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the brainstem of control and experimental rats

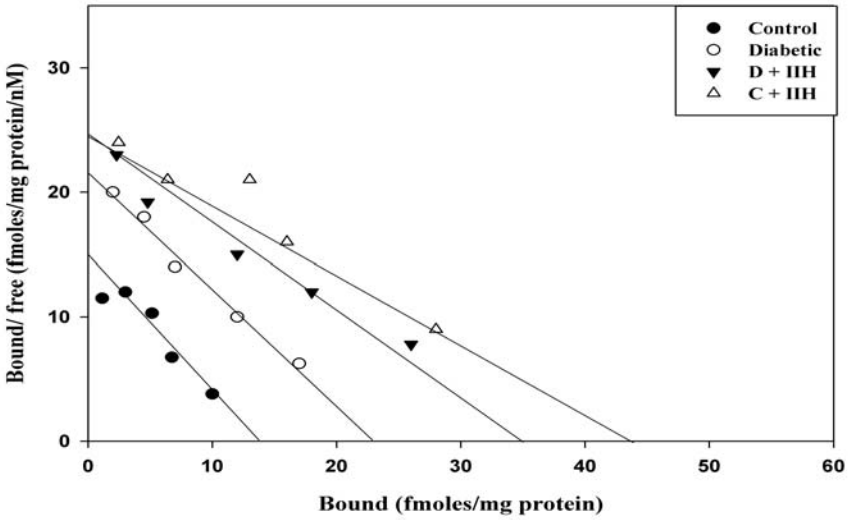


Table-53

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4 DAMP mustard in the brainstem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	13.8 ± 2.2	0.92 ± 0.03
Diabetic	22.8 ± 3.0 [a]	1.06 ± 0.05
D + IIH	35.0 ± 3.1 [a, b]	1.4 ± 0.01 [d, e]
C + IIH	43.7 ± 3.6 [a, b, c]	1.8 ± 0.02 [d, e]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$, **d** $p<0.01$ when compared with control, **b** $p<0.001$, **e** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-54

Scatchard analysis of [^{3}H] GABA binding against GABA in the brainstem of control and experimental rats

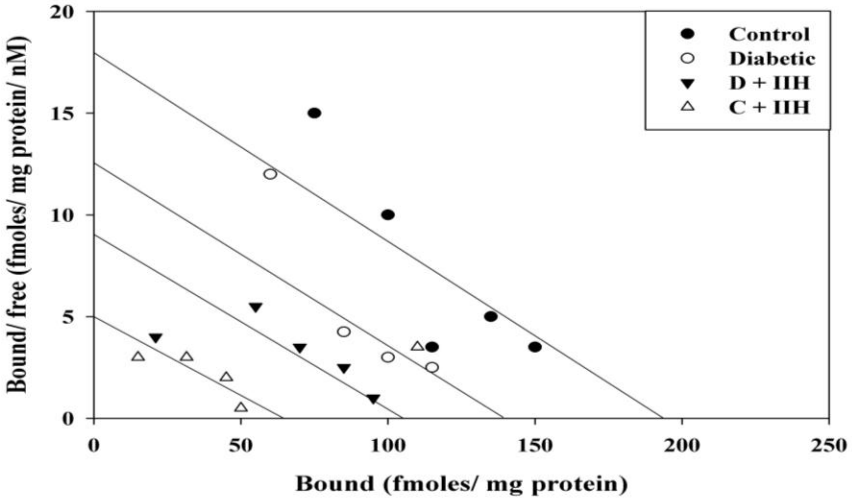


Table-54

Scatchard analysis of [^{3}H] GABA binding against GABA in the brainstem of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	194 ± 2.4	11.4 ± 1.2
Diabetic	140 ± 5.3 [a]	11.6 ± 1.5
D + IIH	105 ± 4.8 [a, b]	11.9 ± 2.4
C + IIH	64 ± 6.4 [a, b, c]	12.8 ± 1.8

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control, [b] $p<0.001$ when compared to diabetic, [c] $p<0.001$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-55

Real Time PCR amplification of AChE mRNA in the brainstem of control and experimental rats

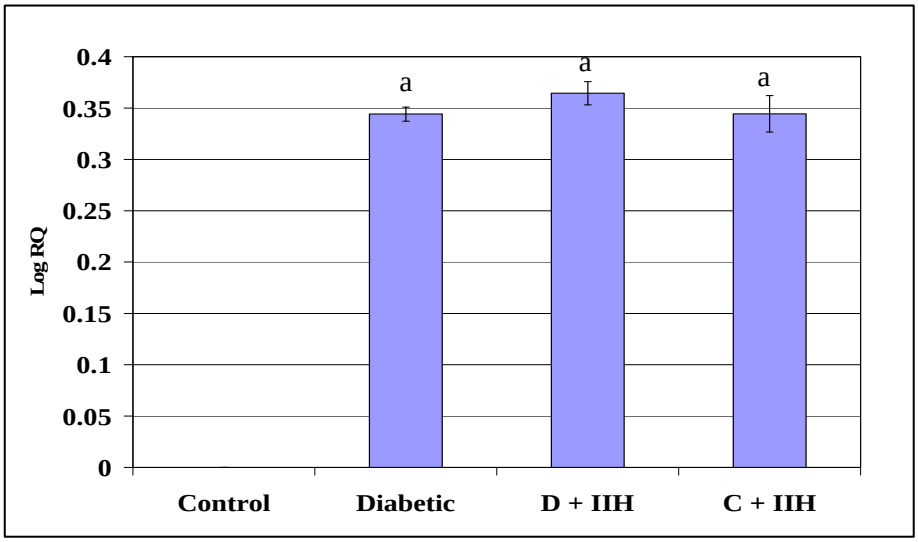


Table-55

Real Time PCR amplification of AChE mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.34 ± 0.01 [a]
D + IIH	0. 36 ± 0.01 [a]
C + IIH	0.34 ± 0.03 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control. IIH - Insulin Induced Hypoglycemia

Figure-56

Real Time PCR amplification of ChAT mRNA in the brainstem of control and experimental rats

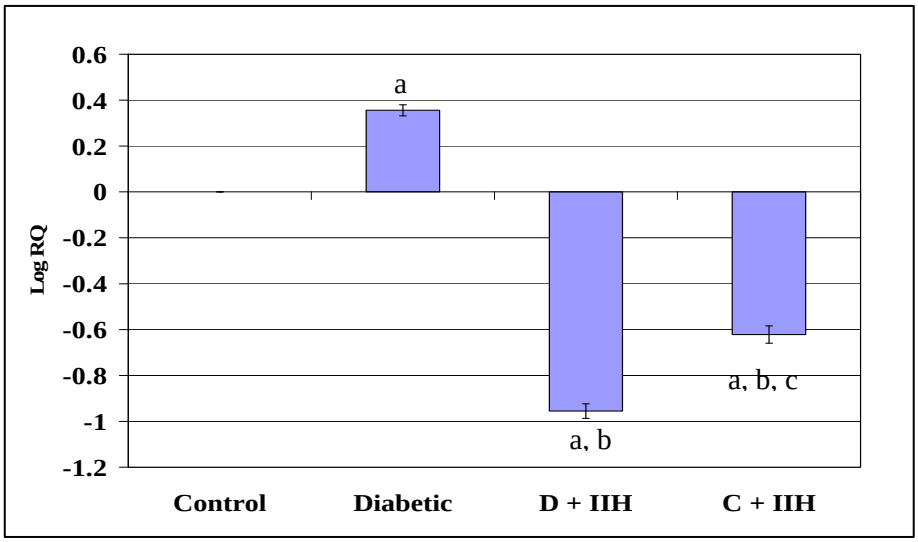


Table-56

Real Time PCR amplification of ChAT mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.34 ± 0.01 [a]
D + IIH	-0.96 ± 0.01 [a, b]
C + IIH	-0.62 ± 0.01 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-57

Real Time PCR amplification of muscarinic M1 receptor mRNA in the brainstem of control and experimental rats

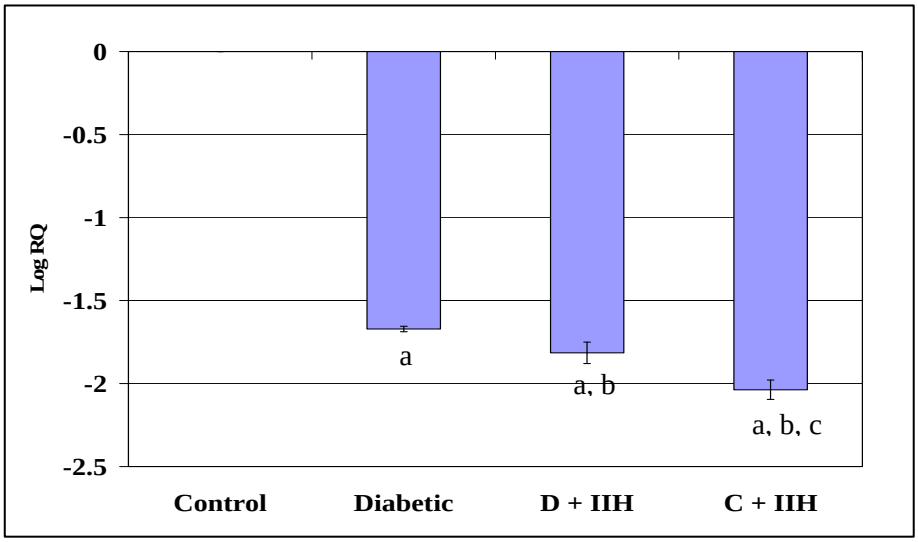


Table-57

Real Time PCR amplification of muscarinic M1 receptor mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.67 ± 0.01 [a]
D + IIH	-1.80 ± 0.06 [a, b]
C + IIH	-2.03 ± 0.05 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-58

Real Time PCR amplification of muscarinic M3 receptor mRNA in the brainstem of control and experimental rats

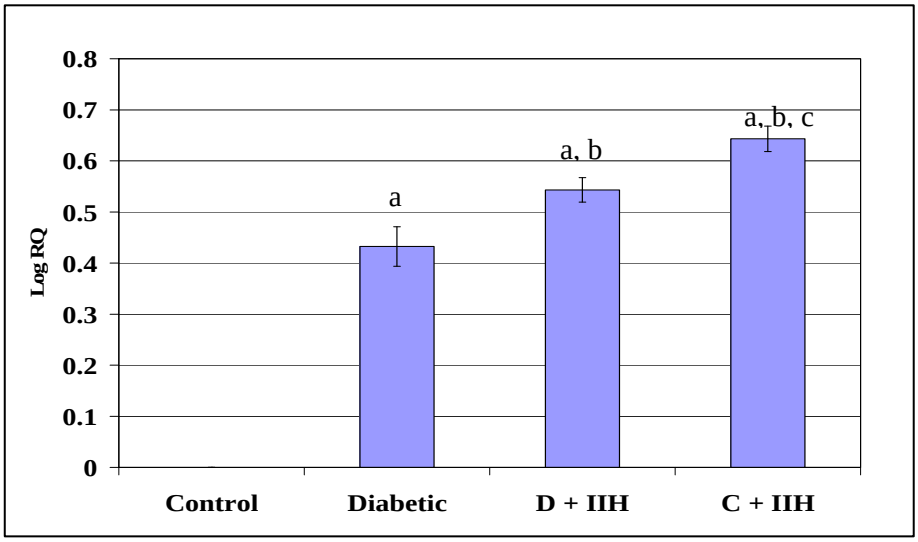


Table-58

Real Time PCR amplification of muscarinic M3 receptor mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.43 ± 0.03 [a]
D + IIH	0.54 ± 0.02 [a, b]
C + IIH	0.64 ± 0.02 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-59

Real Time PCR amplification of α7 nicotinic acetylcholine receptor mRNA in the brainstem of control and experimental rats

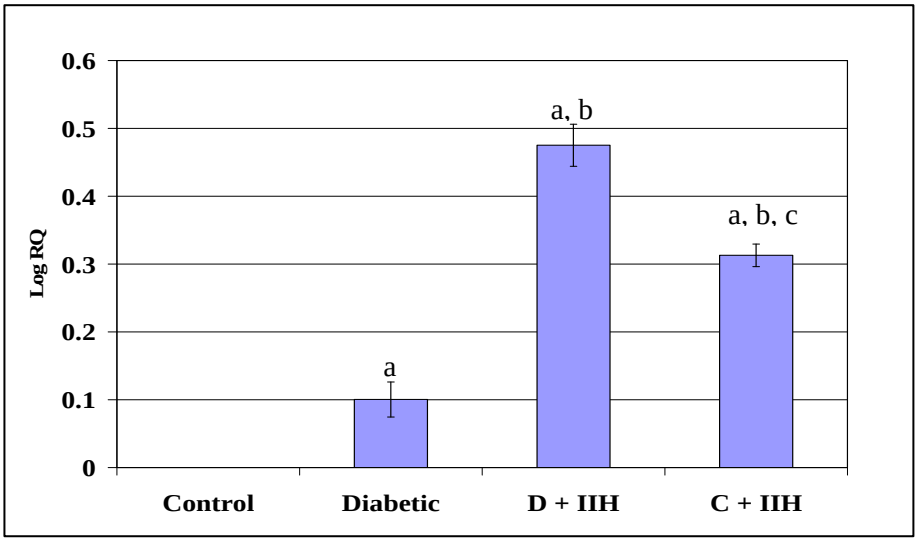


Table-59

Real Time PCR amplification of α7 nicotinic acetylcholine receptor mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.10 ± 0.04 [a]
D + IIH	0.48 ± 0.04 [a, b]
C + IIH	0.31 ± 0.02 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-60

Real Time PCR amplification of GAD mRNA in the brainstem of control and experimental rats

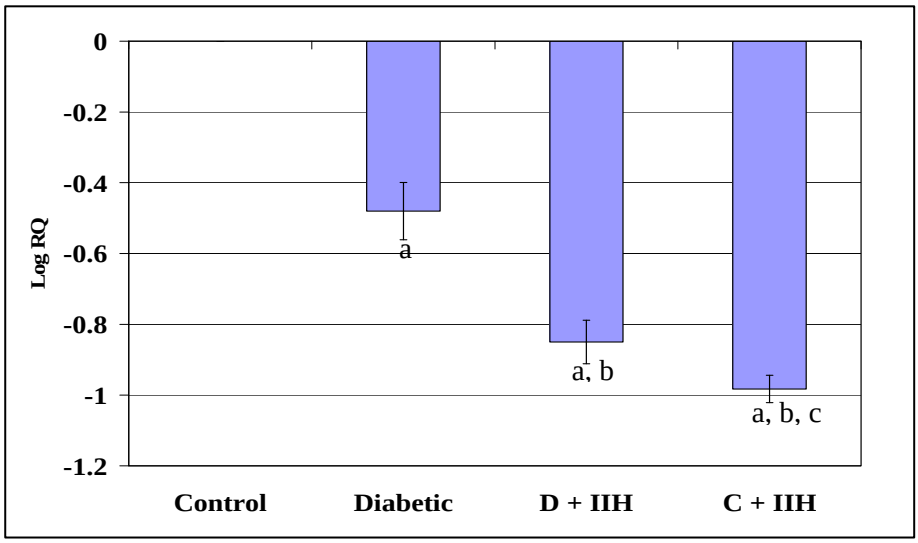


Table-60

Real Time PCR amplification of GAD mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.45 ± 0.08 [a]
D + IIH	-0.85 ± 0.06 [a, b]
C + IIH	-0.98 ± 0.03 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-61

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the brainstem of control and experimental rats

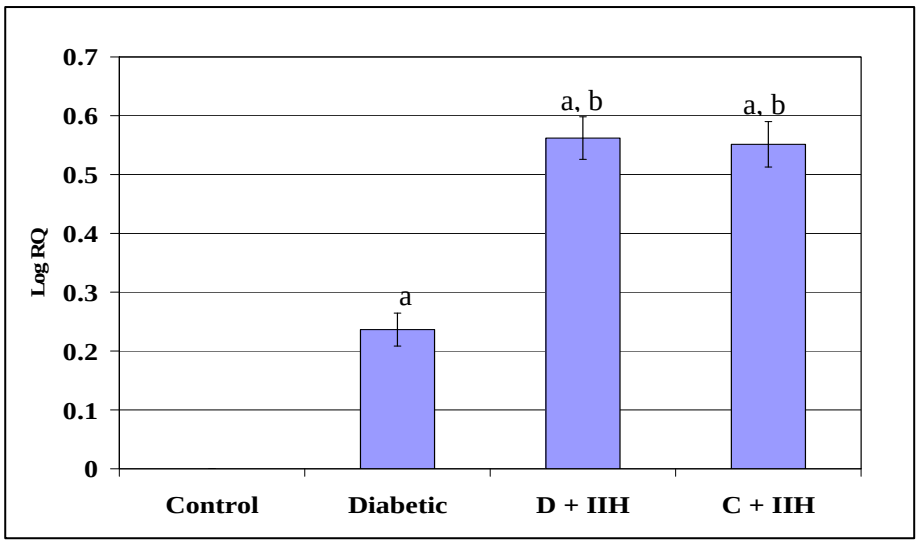


Table-61

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.23 ± 0.02 [a]
D + IIH	0.56 ± 0.03 [a, b]
C + IIH	0.55 ± 0.03 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic. IIH - Insulin Induced Hypoglycemia

Figure-62

Real Time PCR amplification of $GABA_B$ mRNA in the brainstem of control and experimental rats

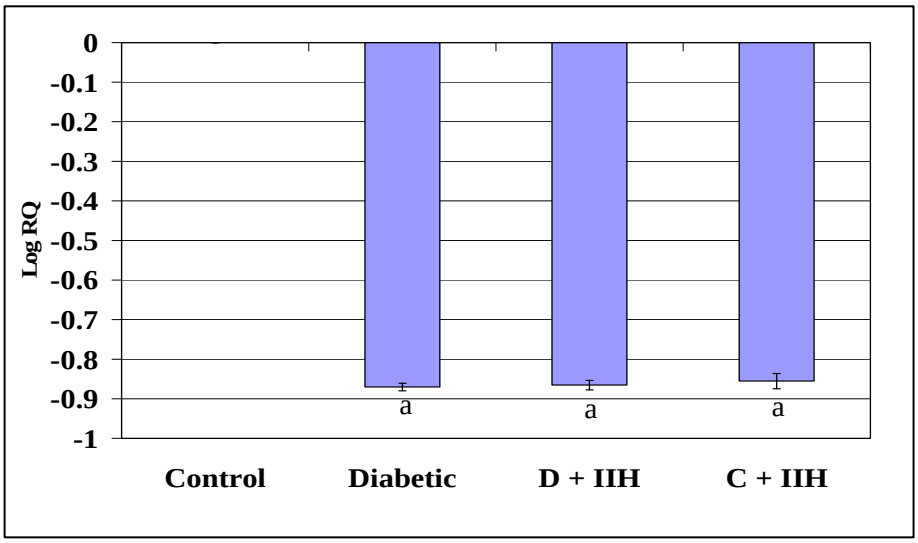


Table-62

Real Time PCR amplification of $GABA_B$ mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.87 ± 0.01 [a]
D + IIH	-0.86 ± 0.01 [a]
C + IIH	-0.85 ± 0.02 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control. IIH - Insulin Induced Hypoglycemia

Figure-63

Real Time PCR amplification of GLUT3 mRNA in the brainstem of control and experimental rats

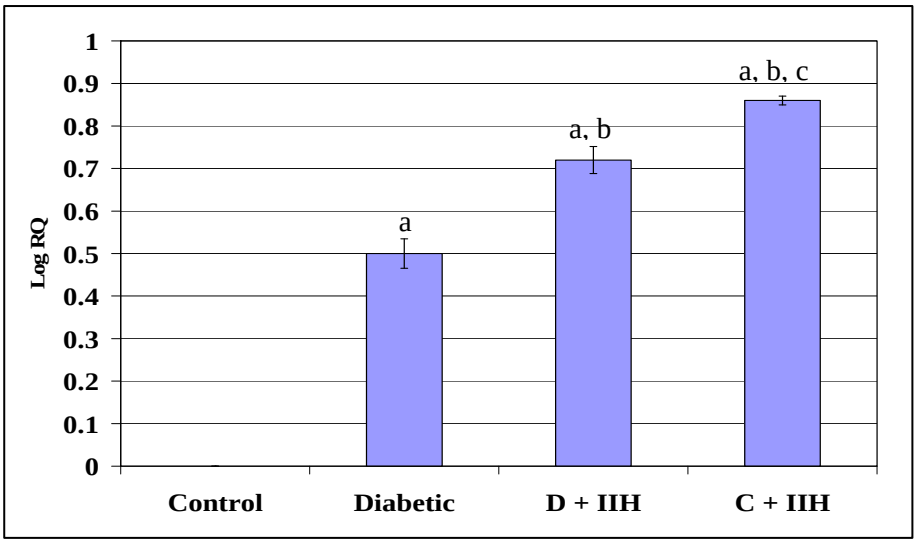


Table-63

Real Time PCR amplification of GLUT3 mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.50 ± 0.03 [a]
D + IIH	0.73 ± 0.03 [a, b]
C + IIH	0.85 ± 0.01 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **[a]** $p<0.001$ when compared with control, **[b]** $p<0.001$ when compared to diabetic, **[c]** $p<0.01$ when compared to D + IIH . IIH - Insulin Induced Hypoglycemia

Figure-64

Real Time PCR amplification of insulin receptor mRNA in the brainstem of control and experimental rats

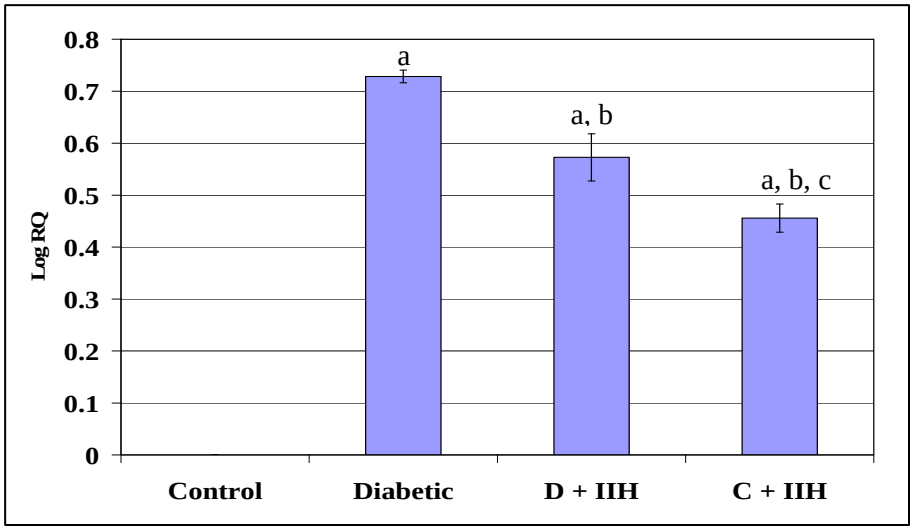


Table-64

Real Time PCR amplification of insulin receptor mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.72 ± 0.01 [a]
D + IIH	0.57 ± 0.04 [a, b]
C + IIH	0.45 ± 0.02 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control, [b] $p<0.01$ when compared to diabetic, [c] $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-65

Real Time PCR amplification of SOD mRNA in the brainstem of control and experimental rats

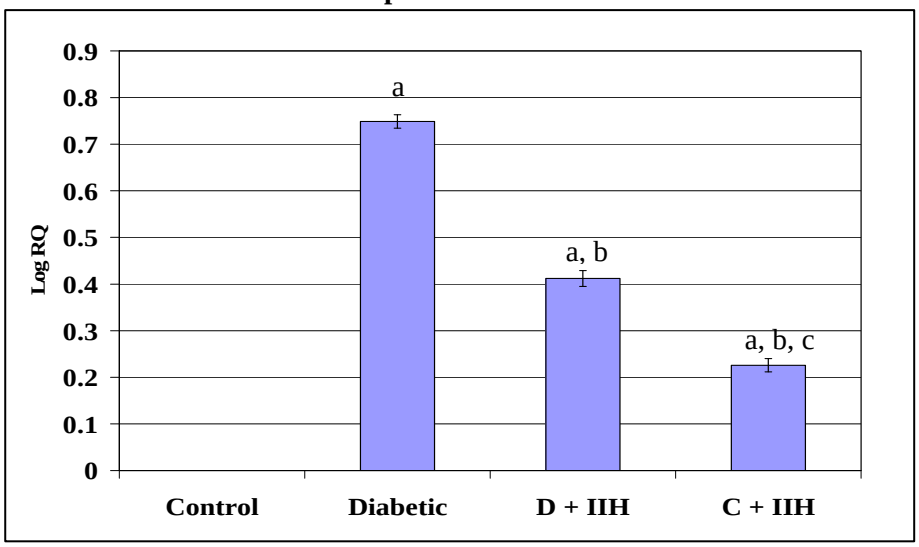


Table-65

Real Time PCR amplification of SOD mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.74 ± 0.01 [a]
D + IIH	0.41 ± 0.02 [a, b]
C + IIH	0.22 ± 0.03 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-66

Real Time PCR amplification of Bax mRNA in the brainstem of control and experimental rats

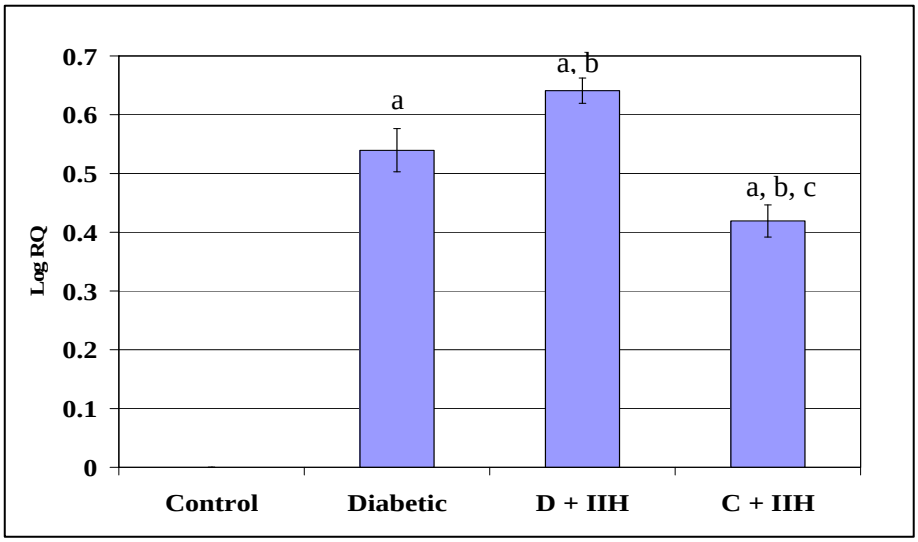


Table-66

Real Time PCR amplification of Bax mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.53 ± 0.03 [a]
D + IIH	0.64 ± 0.02 [a, b]
C + IIH	0.41 ± 0.03 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-67

Real Time PCR amplification of phospholipase C mRNA in the brainstem of control and experimental rats

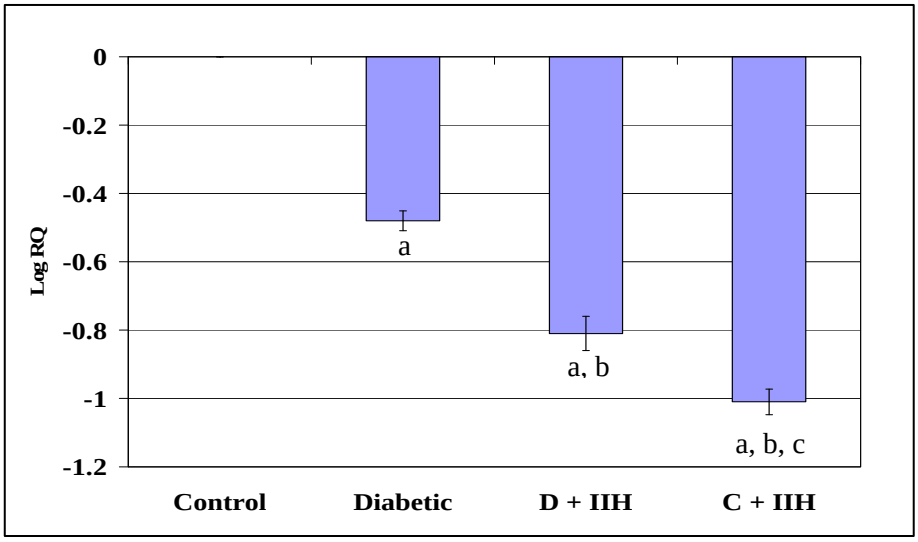


Table-67

Real Time PCR amplification of phospholipase C mRNA in the brainstem of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.48 ± 0.02 [a]
D + IIH	-0.81 ± 0.05 [a, b]
C + IIH	-1.01 ± 0.04 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-68

Real Time PCR amplification of CREB mRNA in the brainstem of control and experimental rats

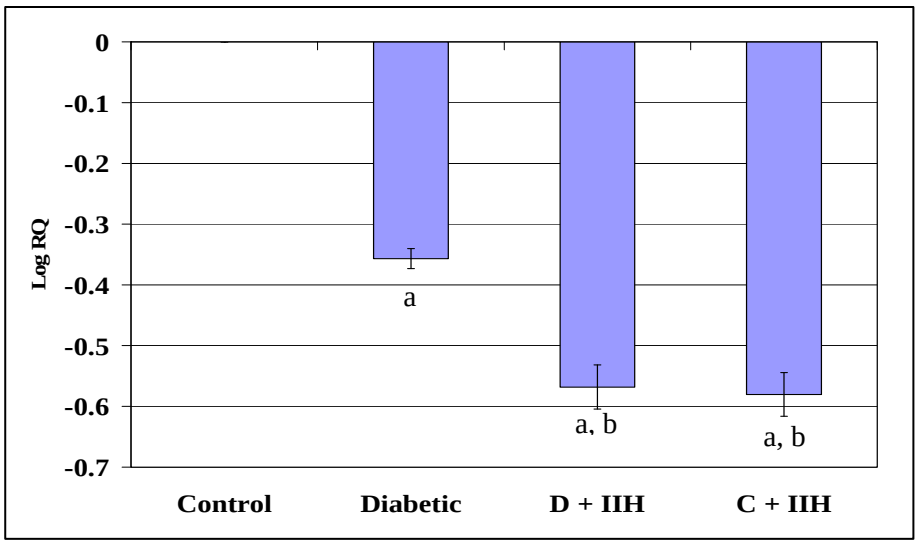


Table-68

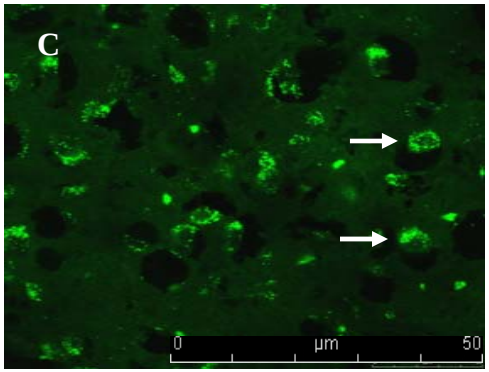
Real Time PCR amplification of CREB mRNA in the brainstem of control and experimental rats

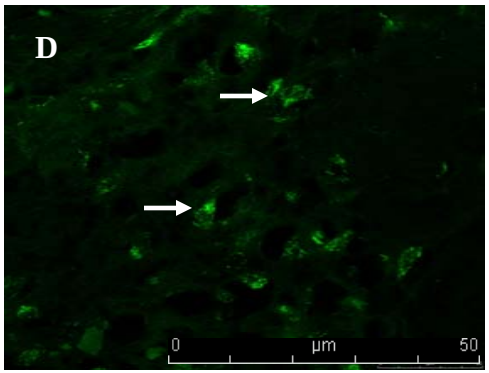
Experimental groups	Log RQ
Control	0
Diabetic	-0.35 ± 0.01 [a]
D + IIH	-0.56 ± 0.03 [a, b]
C + IIH	-0.58 ± 0.03 [a, b]

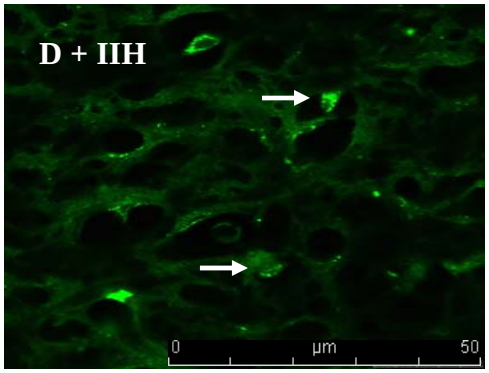
Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **[a]** $p<0.001$ when compared with control, **[b]** $p<0.001$ when compared to diabetic. IIH - Insulin Induced Hypoglycemia

Figure--69

Muscarinic M1 receptor expression in the brainstem of control and experimental rats







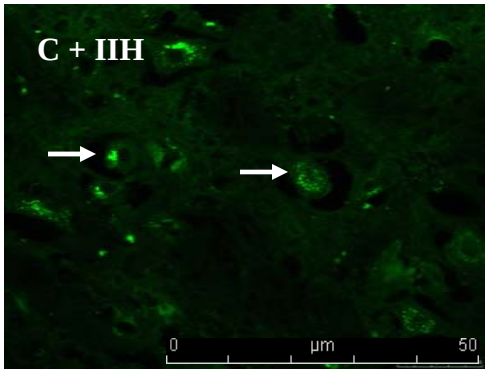


Table-69

Condition	Mean Pixel Value
Control	39.8 ± 3.6
Diabetic	25.4 ± 4.5 [a]
D + IIH	18.7 ± 4.6 [a, b]
C + IIH	15.0 ± 3.4 [a, b]

Confocal image of muscarinic M1 receptors in the brainstem of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M1 receptors. Scale bar = 50 µm. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic.

Figure--70

Muscarinic M3 receptor expression in the brainstem of control and experimental Rats

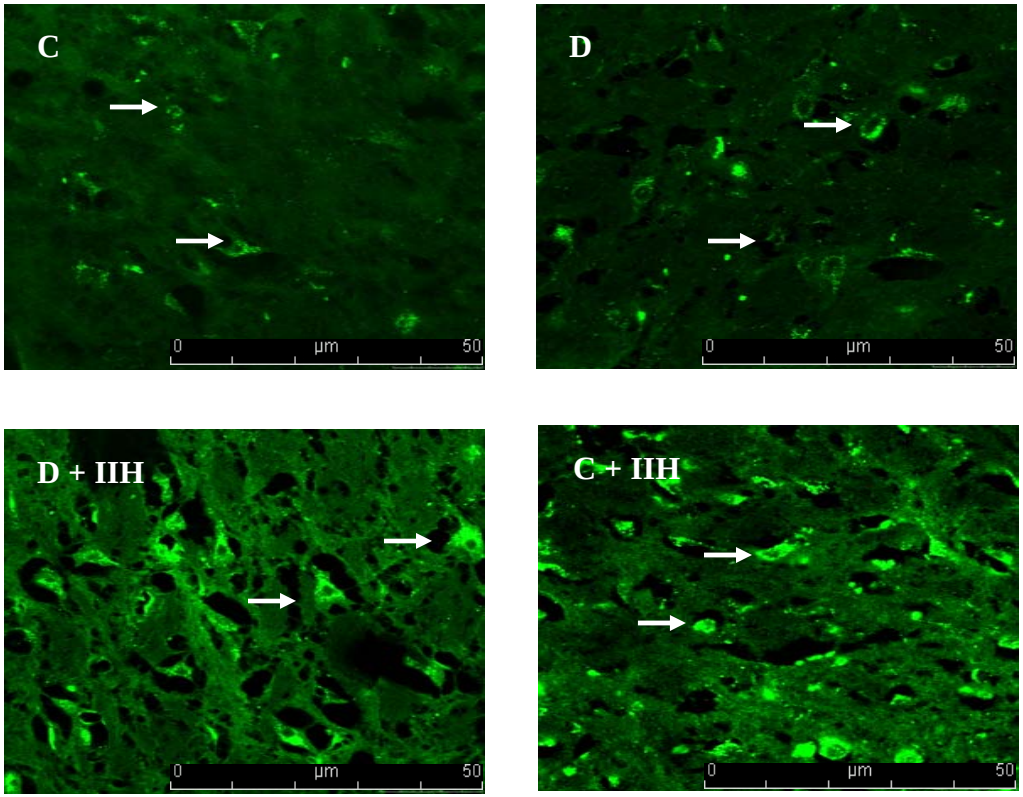


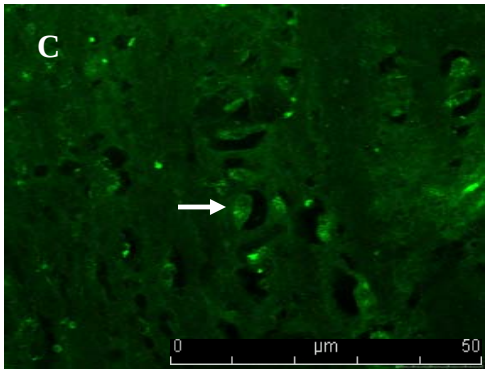
Table-70

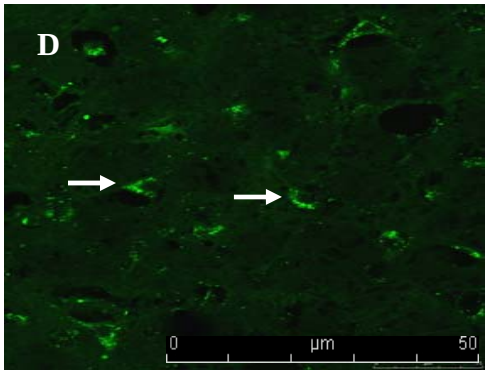
Condition	Mean Pixel Value
Control	18.6 ± 4.6
Diabetic	32.8 ± 3.5 [a]
D + IIH	45.7 ± 2.5 [a, b]
C + IIH	54.6 ± 3.4 [a, b, c]

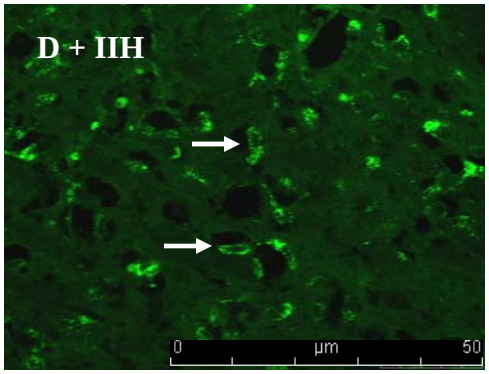
Confocal image of muscarinic M3 receptors in the brainstem of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M3 receptors. Scale bar = 50 µm. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH.

Figure--71

α7 nicotinic acetylcholine receptor expression in the brainstem of control and experimental rats







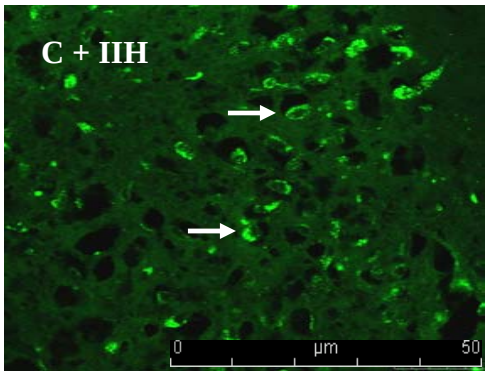


Table-71

Condition	Mean Pixel Value
Control	21.5 ± 5.6
Diabetic	34.6 ± 2.9 [a]
D + IIH	48.7 ± 4.2 [a, b]
C + IIH	55.8 ± 4.4 [a, b]

Confocal image of α7 nACh receptors in the brainstem of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent α7 nACh receptor specific primary antibody and FITC as secondary antibody. (→) in white shows α7 nACh receptors. Scale bar = 50 µm. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic.

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Figure--72

$GABA_{A\alpha1}$ receptor expression in the brainstem of control and experimental rats

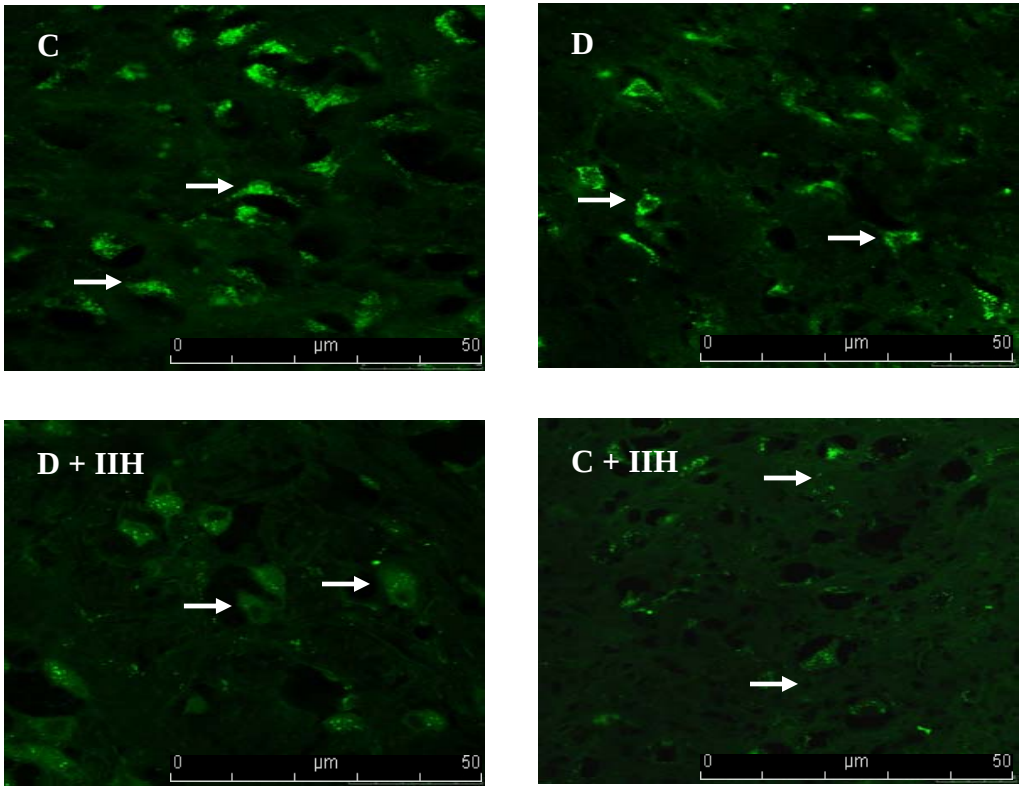


Table-72

Condition	Mean Pixel Value
Control	62.3 ± 4.4
Diabetic	42.5 ± 4.9 [a]
D + IIH	30.7 ± 5.5 [a, b]
C + IIH	19.3 ± 4.7 [a, b, c]

Confocal image of $GABA_{A\alpha1}$ receptors in the brainstem of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent $GABA_{A\alpha1}$ receptor specific primary antibody and FITC as secondary antibody. (→) in white shows $GABA_{A\alpha1}$ receptors. Scale bar = 50 µm. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic, [c] $p<0.001$ when compared to D + IIH.

Figure-73

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the corpus striatum of control and experimental rats

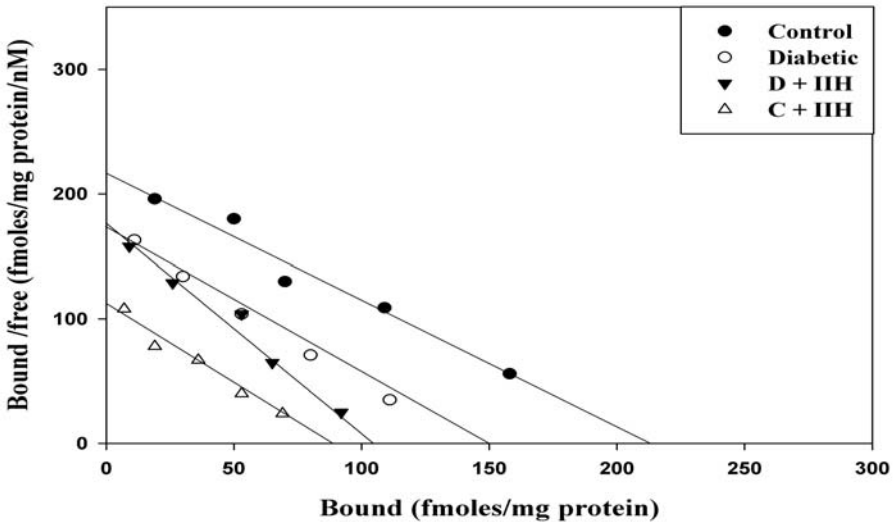


Table-73

Scatchard analysis of [^{3}H]QNB binding against total muscarinic receptor antagonist, atropine in the corpus striatum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	213 ± 10.1	0.98 ± 0.02
Diabetic	150 ± 9.3 [a]	0.87± 0.05
D + IIH	105 ± 11.8 [a,b]	0.60 ± 0.03 [a,b]
C + IIH	88 ± 6.3 [a,b]	0.80 ± 0.04

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.01$ when compared with control, **b** $p<0.01$ when compared to diabetic. IIH - Insulin Induced Hypoglycemia

Figure-74

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the corpus striatum of control and experimental rats

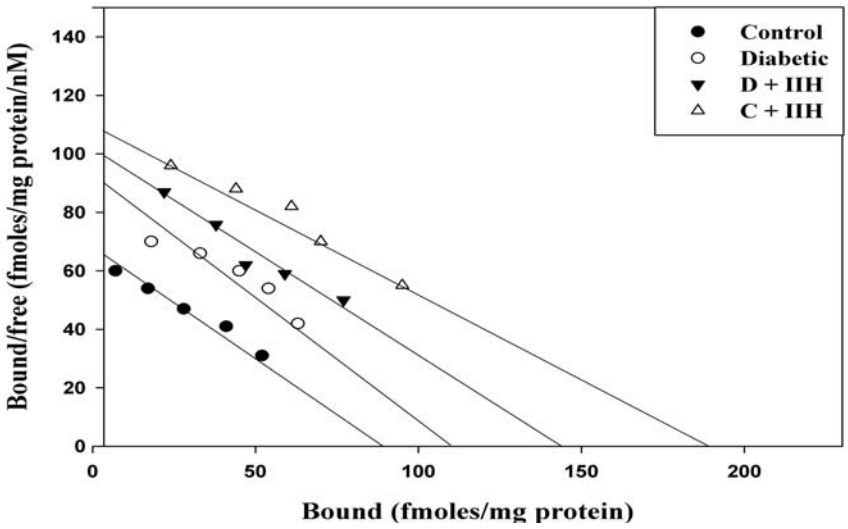


Table-74

Scatchard analysis of [^{3}H]QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the corpus striatum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	89 ± 3.9	1.40 ± 0.02
Diabetic	111 ± 6.1 [a]	1.30 ± 0.05
D + IIH	143 ± 5.2 [a, b]	1.40 ± 0.03
C + IIH	188 ± 4.8 [a, b, c]	1.50 ± 0.04

Values are Mean ± S.E.M. of 3-5 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic , [c] $p<0.01$ when compared to D + IIH . IIH- Insulin Induced Hypoglycemia

Figure-75

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4- DAMP mustard in the corpus striatum of control and experimental rats

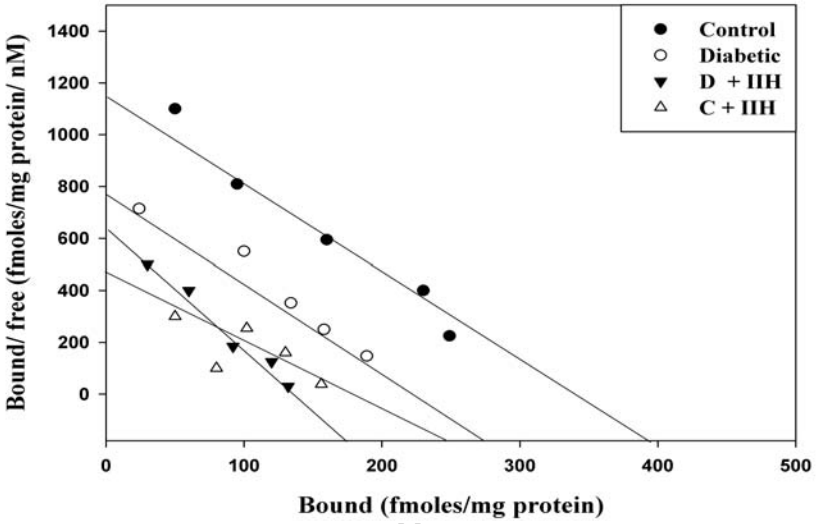


Table- 75

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4- DAMP mustard in the corpus striatum of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	393 ± 10.1	0.34 ± 0.02
Diabetic	274 ± 9.3 [a]	0.35± 0.05
D + IIH	175 ± 11.8 [a,b]	0.27 ± 0.03
C + IIH	246 ± 6.3 [a,b,c]	0.54 ± 0.04 [a, b,c]

Values are Mean ± S.E.M. of 3-5 separate experiments. Each group consists of 6-8 rats. **a** $p<0.01$ when compared to control, **b** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH . IIH- Insulin Induced Hypoglycemia

Figure-76

Real Time PCR amplification of AChE mRNA in the corpus striatum of control and experimental rats

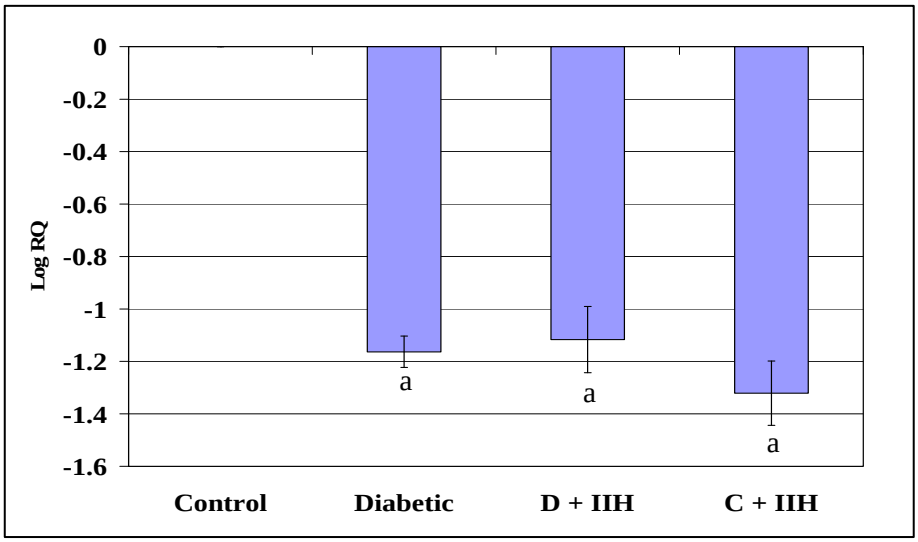


Table-76

Real Time PCR amplification of AChE mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.16 ± 0.05 [a]
D + IIH	-1.11 ± 0.12 [a]
C + IIH	-1.32 ± 0.12 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control. IIH - Insulin Induced Hypoglycemia

Figure-77

Real Time PCR amplification of ChAT mRNA in the corpus striatum of control and experimental rats

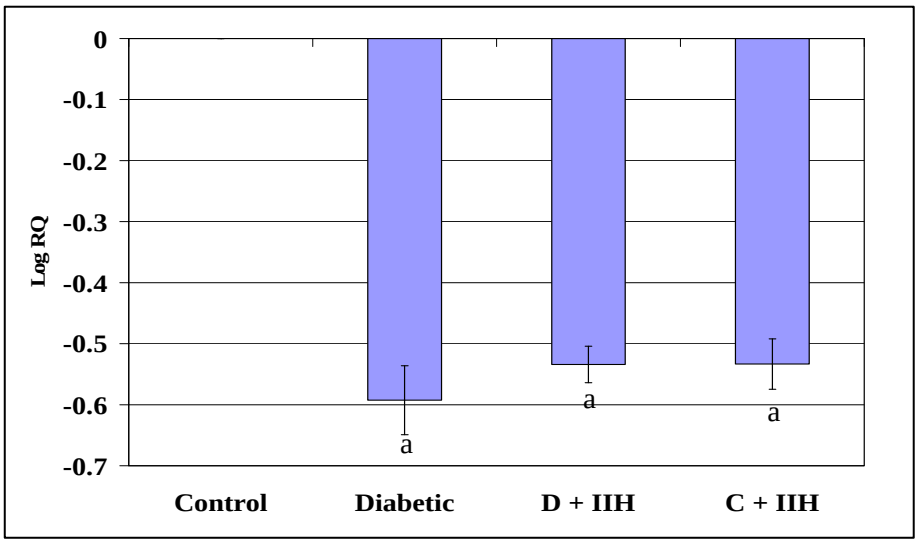


Table-77

Real Time PCR amplification of ChAT mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.59 ± 0.05 [a]
D + IIH	-0.53 ± 0.02 [a]
C + IIH	-0.53 ± 0.02 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control. IIH - Insulin Induced Hypoglycemia

Figure-78

Real Time PCR amplification of muscarinic M1 receptor mRNA in the corpus striatum of control and experimental rats

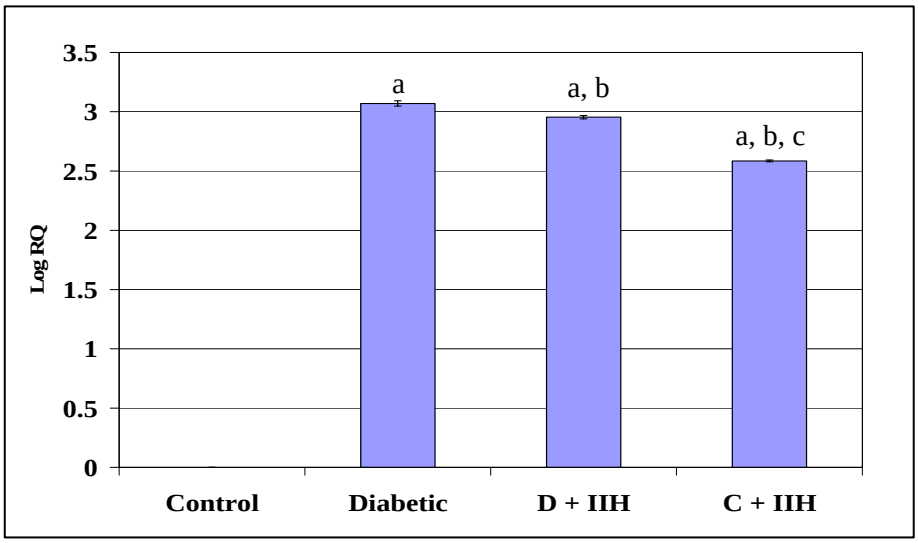


Table-78

Real Time PCR amplification of muscarinic M1 receptor mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	3.20 ± 0.001 [a]
D + IIH	2.90 ± 0.002 [a, b]
C + IIH	2.60 ± 0.001 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **[a]** $p<0.001$ when compared with control, **[b]** $p<0.01$ when compared to diabetic, **[c]** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-79

Real Time PCR amplification of muscarinic M3 receptor mRNA in the corpus striatum of control and experimental rats

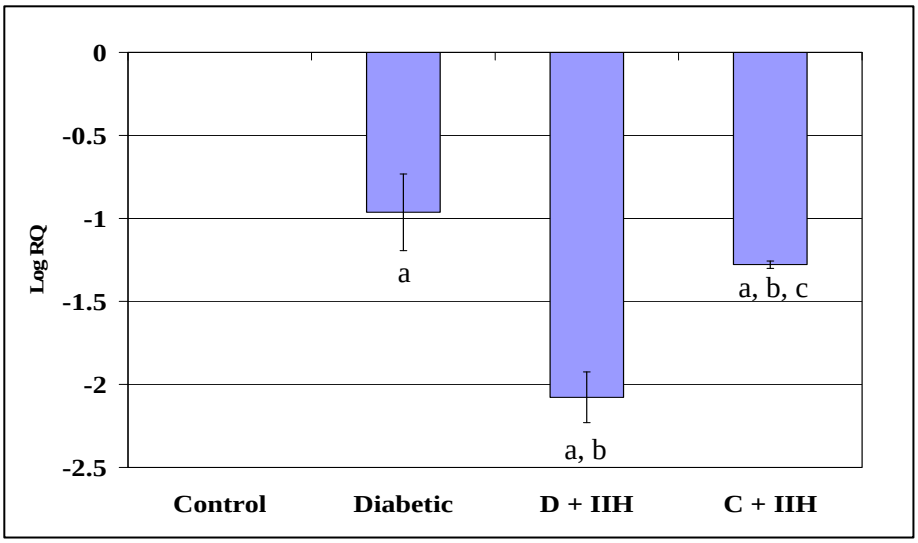


Table-79

Real Time PCR amplification of muscarinic M3 receptor mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.96 ± 0.23 [a]
D + IIH	-2.07 ± 0.15 [a, b]
C + IIH	-1.27 ± 0.02 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-80

Real Time PCR amplification of α7 nicotinic acetylcholine receptor mRNA in the corpus striatum of control and experimental rats

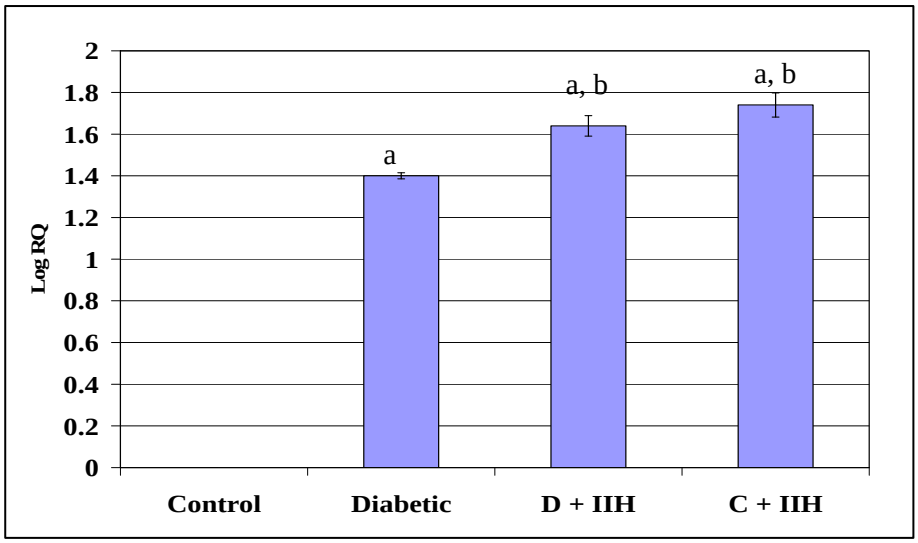


Table-80

Real Time PCR amplification of α7 nicotinic acetylcholine receptor in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	1.40 ± 0.01 [a]
D + IIH	1.62 ± 0.04 [a, b]
C + IIH	1.70 ± 0.05 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic. IIH - Insulin Induced Hypoglycemia

Figure-81

Real Time PCR amplification of GAD mRNA in the corpus striatum of control and experimental rats

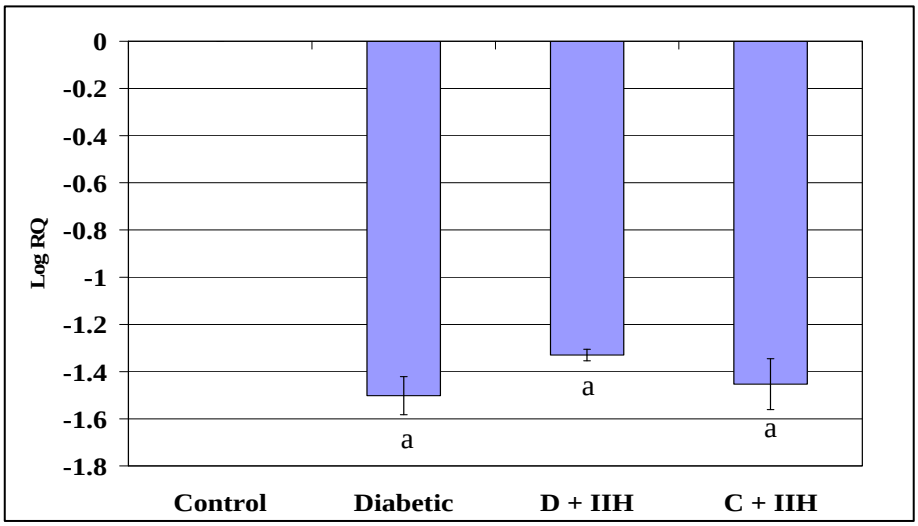


Table-81

Real Time PCR amplification of GAD mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.50 ± 0.08 [a]
D + IIH	-1.32 ± 0.02 [a]
C + IIH	-1.45 ± 0.10 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control. IIH - Insulin Induced Hypoglycemia

Figure-82

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the corpus striatum of control and experimental rats

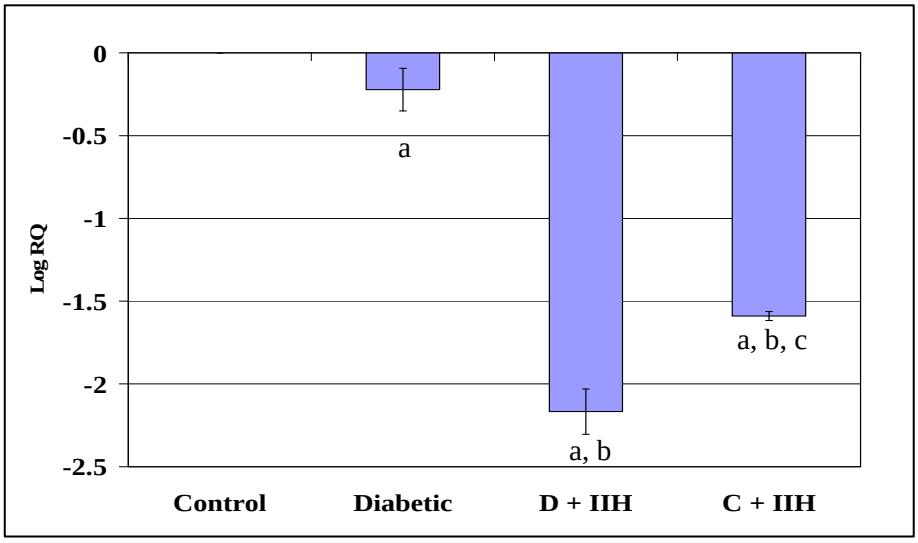


Table-82

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.22 ± 0.13 [a]
D + IIH	-2.16 ± 0.02 [a, b]
C + IIH	-1.58 ± 0.03 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-83

Real Time PCR amplification of $GABA_B$ mRNA in the corpus striatum of control and experimental rats

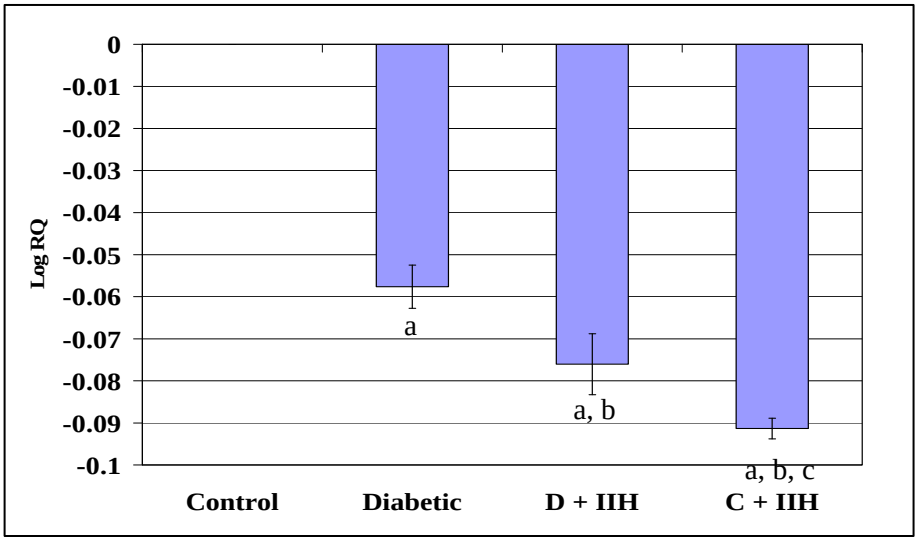


Table-83

Real Time PCR amplification of $GABA_B$ mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.05 ± 0.005 [a]
D + IIH	-0.07 ± 0.007 [a, b]
C + IIH	-0.09 ± 0.002 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-84

Real Time PCR amplification of GLUT3 mRNA in the corpus striatum of control and experimental rats

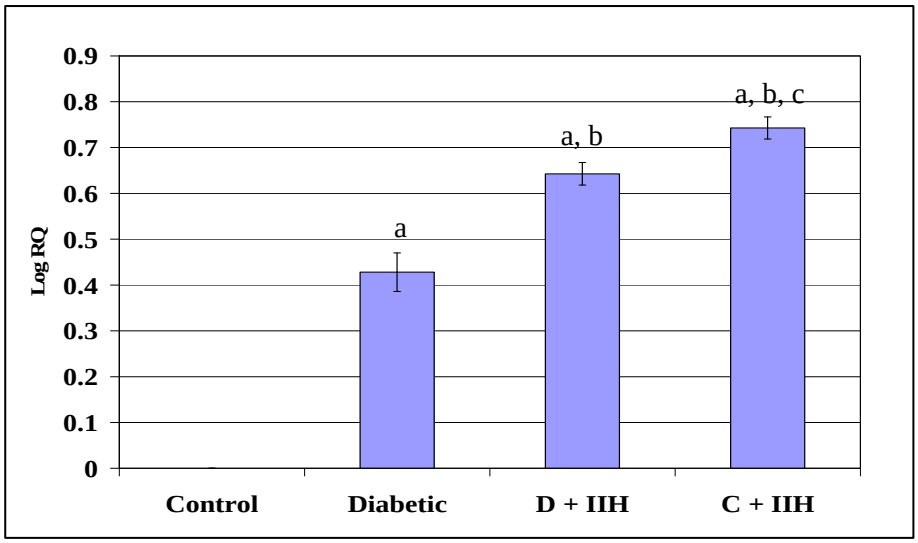


Table-84

Real Time PCR amplification of GLUT3 mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.43 ± 0.05 [a]
D + IIH	-0.64 ± 0.02 [a, b]
C + IIH	-0.73 ± 0.02 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-85

Real Time PCR amplification of insulin receptor mRNA in the corpus striatum of control and experimental rats

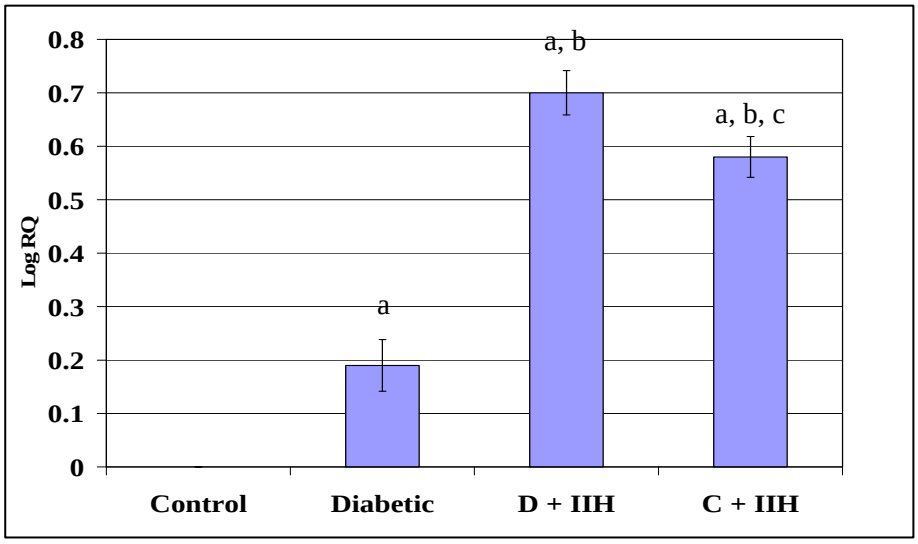


Table-85

Real Time PCR amplification of insulin receptor mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.19 ± 0.03^{a}
D + IIH	$0.70 \pm 0.03^{a, b}$
C + IIH	$0.58 \pm 0.03^{a, b, c}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia.

Figure-86

Real Time PCR amplification of SOD mRNA in the corpus striatum of control and experimental rats

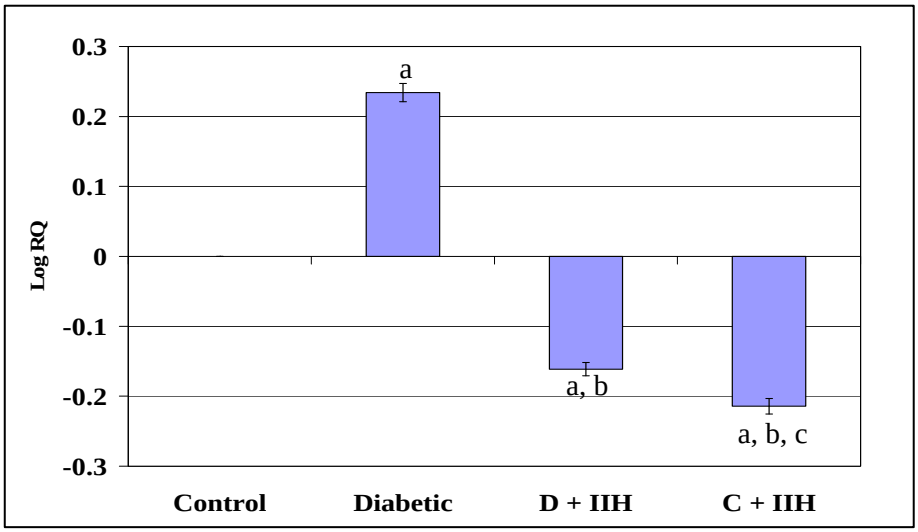


Table-86

Real Time PCR amplification of SOD mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.23 ± 0.01 [a]
D + IIH	-0.16 ± 0.01 [a, b]
C + IIH	-0.21 ± 0.01 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-87

Real Time PCR amplification of Bax mRNA in the corpus striatum of control and experimental rats

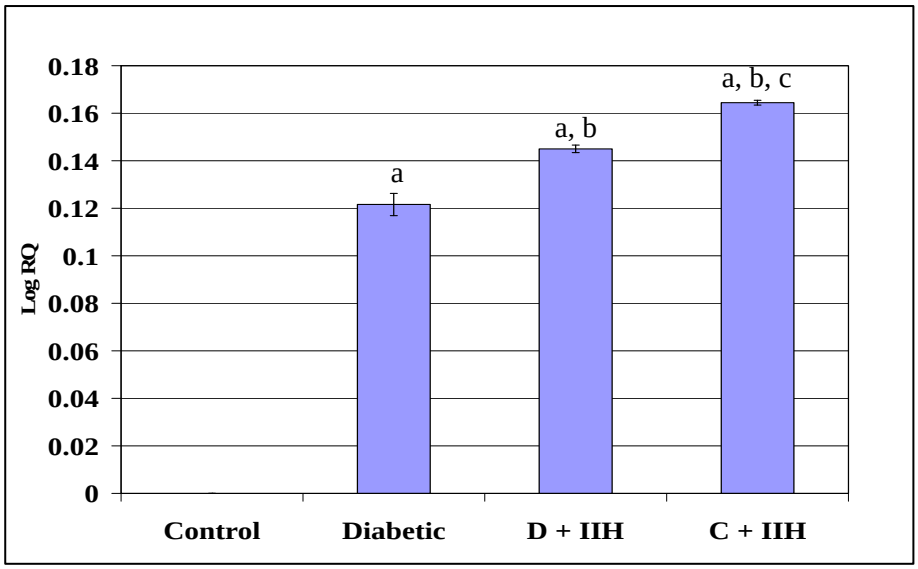


Table-87

Real Time PCR amplification of bax mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.12 ± 0.004 [a]
D + IIH	0.14 ± 0.001 [a, b]
C + IIH	0.16 ± 0.001 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-88

Real Time PCR amplification of phospholipase C mRNA in the corpus striatum of control and experimental rats

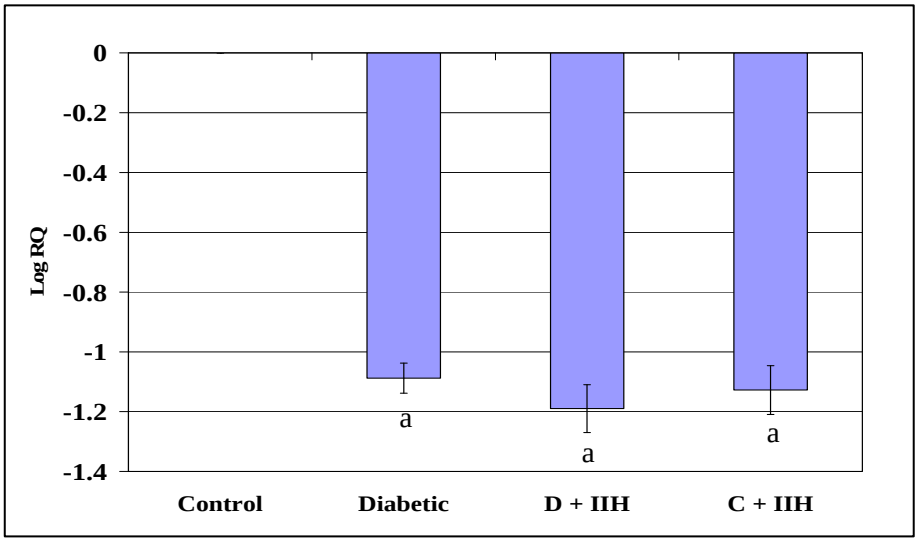


Table-88

Real Time PCR amplification of phospholipase C mRNA in the corpus striatum of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.08 ± 0.03 [a]
D + IIH	-1.18 ± 0.04 [a]
C + IIH	-1.18 ± 0.04 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control. IIH - Insulin Induced Hypoglycemia

Figure-89

Real Time PCR amplification of CREB mRNA in the corpus striatum of control and experimental rats

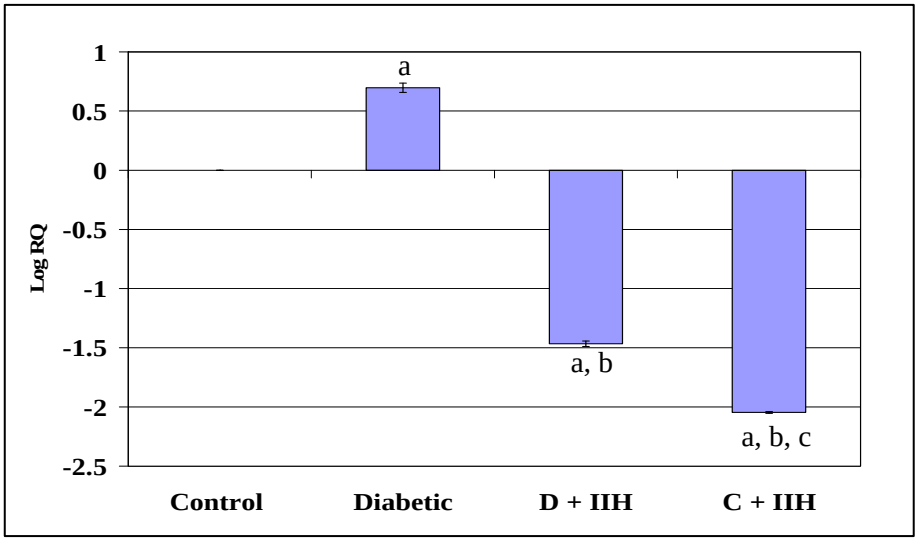


Table-89

Real Time PCR amplification of CREB mRNA in the corpus striatum of control and experimental rats

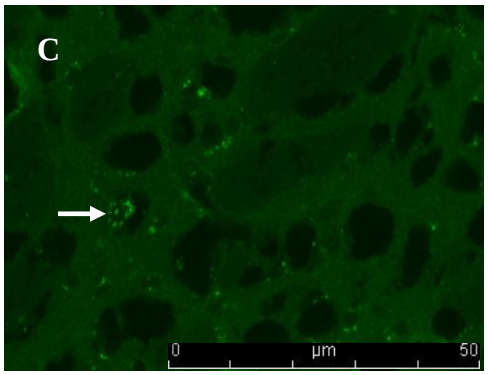
Experimental groups	Log RQ
Control	0
Diabetic	0.69 ± 0.03 [a]
D + IIH	-1.40 ± 0.02 [a, b]
C + IIH	-2.04 ± 0.02 [a, b, c]

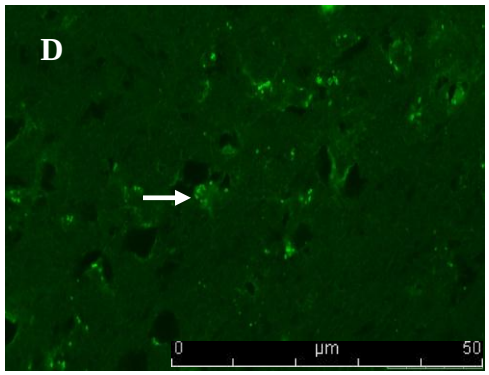
Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH . IIH - Insulin Induced Hypoglycemia

Figure--90

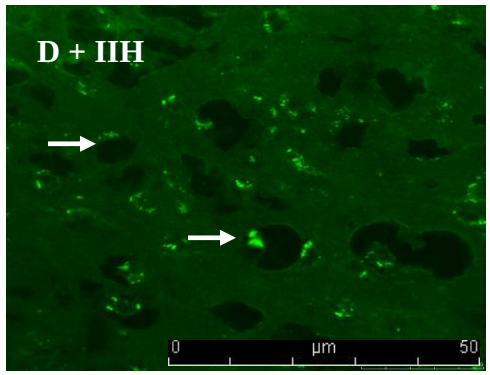
Muscarinic M1 receptor expression in the corpus striatum of control and experimental rats

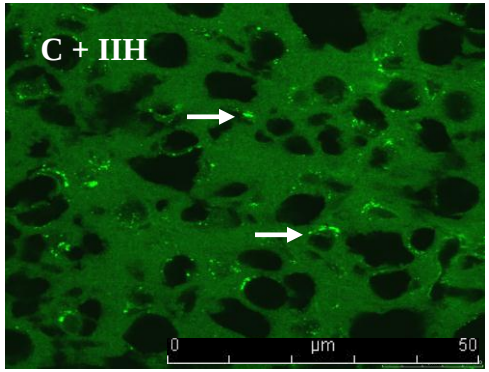
Table-90





Condition	Mean Pixel Value
Control	17.3 ± 3.3
Diabetic	36.4 ± 4.1 [a]
D + IIH	48.8 ± 4.8 [a, b]
C + IIH	56.1 ± 5.5 [a, b]

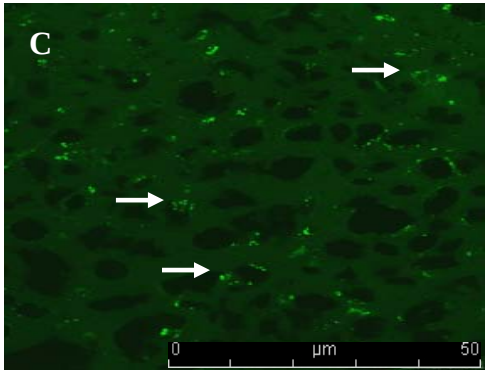


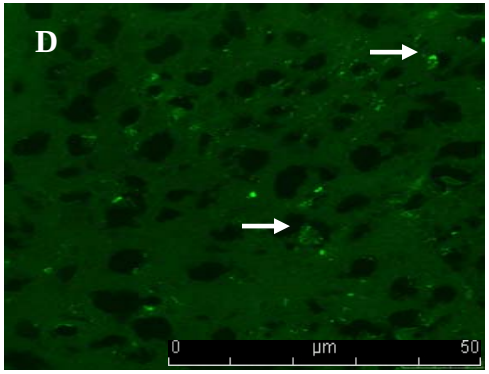


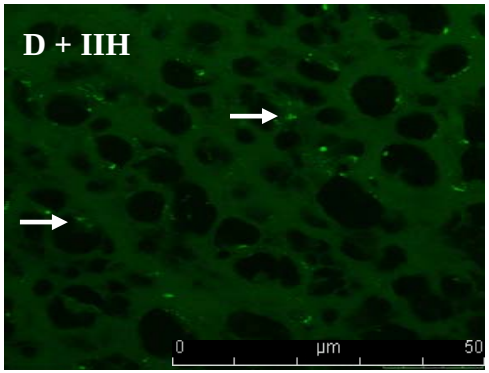
Confocal image of muscarinic M1 receptors in the corpus striatum of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M1 receptors. Scale bar = 50 µm. **a** when compared to control, **b** when compared to diabetic, **c** when compared to D + IIH.

Figure--91

Muscarinic M3 receptor expression in the corpus striatum of control and experimental rats







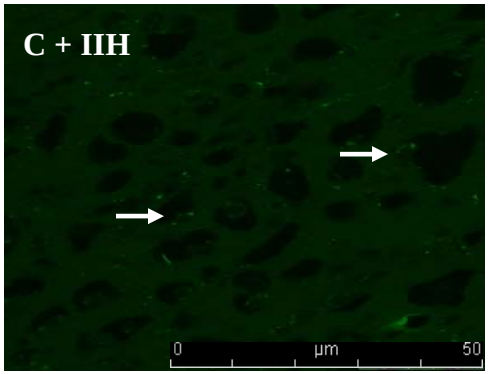


Table-91

Condition	Mean Pixel Value
Control	68.6 ± 3.3
Diabetic	46.2 ± 4.1 [a]
D + IIH	34.6 ± 4.8 [a, b]
C + IIH	26.5 ± 5.6 [a, b, c]

Confocal image of muscarinic M3 receptors in the corpus striatum of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M3 receptors. Scale bar = 50 µm. **a** $p<0.001$ when compared to control, **b** $p<0.001$when compared to diabetic, **c** $p<0.01$ when compared to D + IIH.

Figure--92

α7 nicotinic receptor expression in the corpus striatum of control and experimental rats

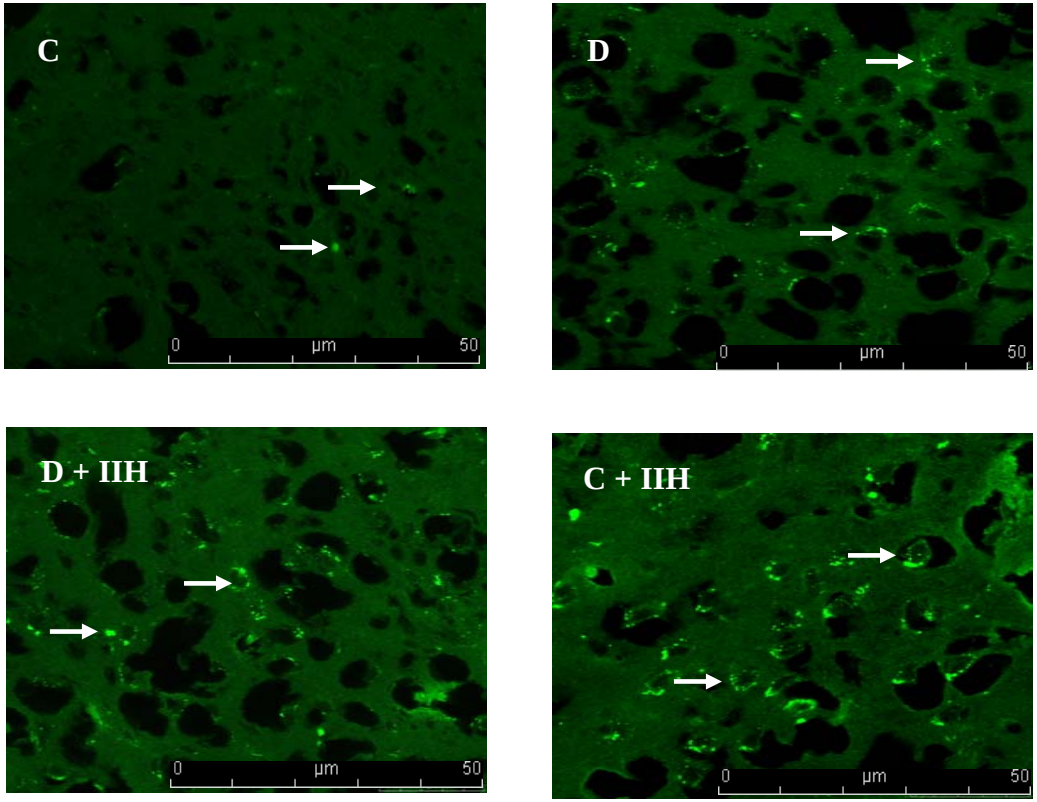


Table-92

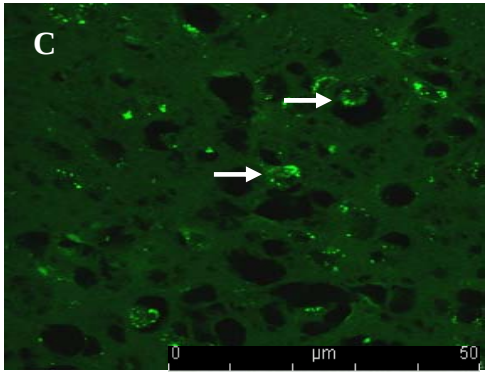
Condition	Mean Pixel Value
Control	18.6 ± 3.5
Diabetic	35.4 ± 6.2 [a]
D + IIH	49.5 ± 5.8 [a, b]
C + IIH	61.3 ± 2.9 [a, b, c]

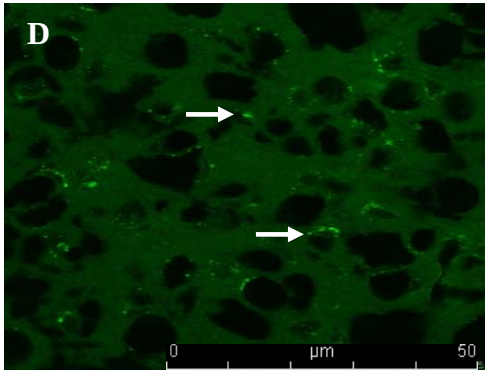
Confocal image of α7 nACh receptors in the corpus striatum of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent α7 nACh receptor specific primary antibody and FITC as secondary antibody. (→) in white shows α7 nACh receptors. Scale bar = 50 µm. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic, [c] $p<0.001$ when compared to D + IIH.

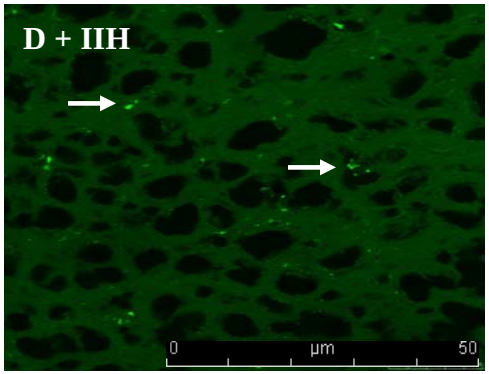
Figure--93

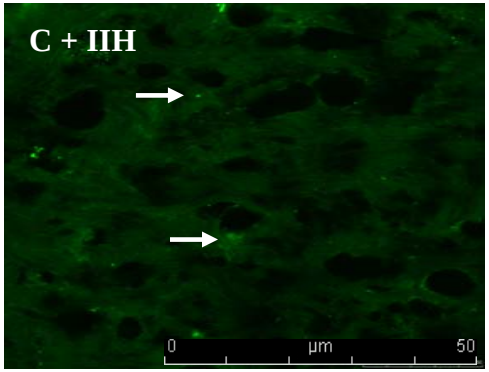
$GABA_{A\alpha1}$ receptor expression in the corpus striatum of control and experimental rats

Table-93









Condition	Mean Pixel Value
Control	68.6 ± 5.3
Diabetic	58.3 ± 4.6 [a]
D + IIH	46.7 ± 5.8 [a, b]
C + IIH	32.1 ± 4.9 [a, b]

Confocal image of $GABA_{A\alpha1}$ receptors in the corpus striatum of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent $GABA_{A\alpha1}$ receptor specific primary antibody and FITC as secondary antibody. (→) in white shows $GABA_{A\alpha1}$ receptors. Scale bar = 50 µm. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH.

Figure-94

Scatchard analysis of [^{3}H]QNB binding against total muscarinic receptor antagonist, atropine in the hippocampus of control and experimental rats

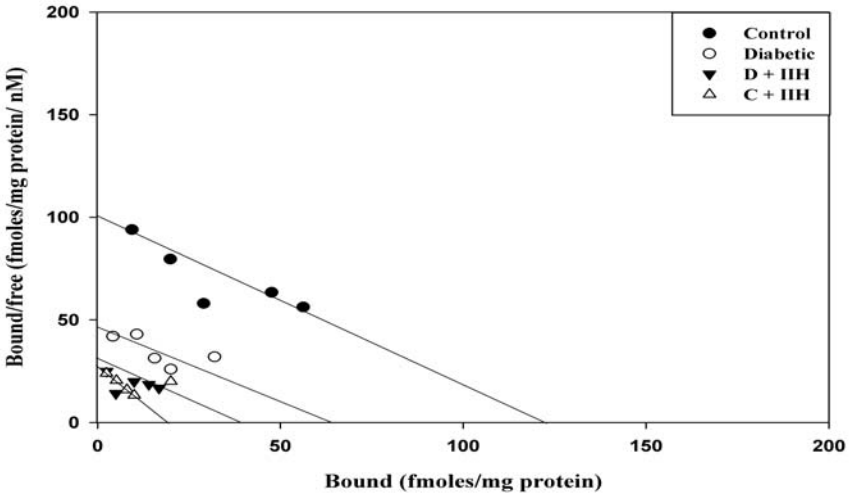


Table-94

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	122 ± 10.0	1.22 ± 0.08
Diabetic	64 ± 7.0 [a]	1.30 ± 0.07
D + IIH	39 ± 9.0 [a, b]	1.27 ± 1.00
C + IIH	18 ± 7.0 [a, b, c]	0.60 ± 0.02 [a, b, c]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH . IIH- Insulin Induced Hypoglycemia

Figure-95

Scatchard analysis of [^{3}H]QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the hippocampus of control and experimental rats

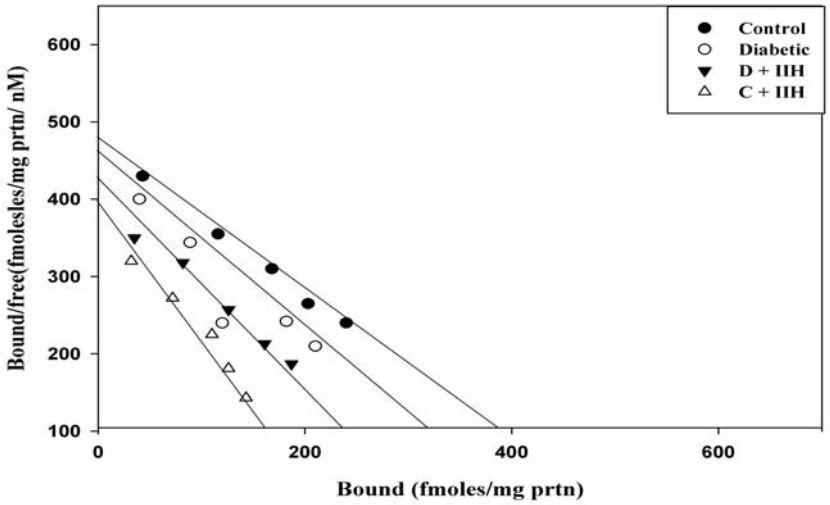


Table-95

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	386 ± 9.0	0.90 ± 0.02
Diabetic	319 ± 8.0 [a]	0.60 ± 0.03 [a]
D + IIH	236 ± 14.4 [a, b]	0.55 ± 0.05 [a, d]
C + IIH	160 ± 12.0 [a, b, c]	0.40 ± 0.02 [a, d]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.001$, **d** $p<0.01$ when compared to diabetic, **c** $p<0.01$when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-96

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the hippocampus of control and experimental rats

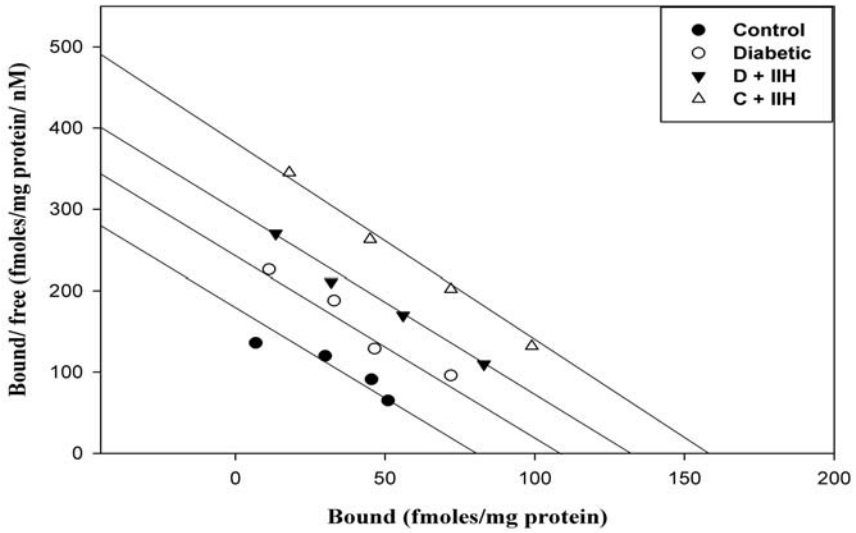


Table-96

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	80 ±4.3	0.28 ± 0.02
Diabetic	110 ± 8.0 [a]	0.32 ± 0.03
D + IIH	132 ± 14.4 [a, b]	0.33 ± 0.05
C + IIH	160 ± 12.0 [a, b, c]	0.32 ± 0.02

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-97

Scatchard analysis of [^{3}H] GABA binding against GABA in the hippocampus of control and experimental rats

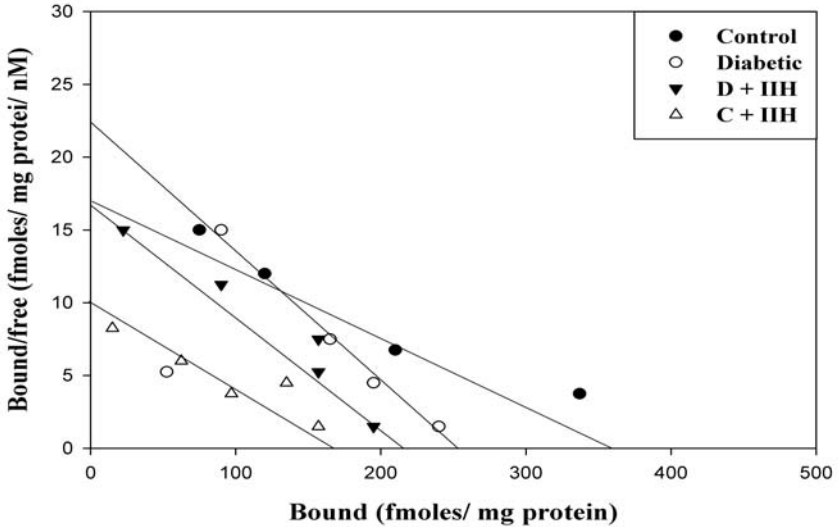


Table-97

Scatchard analysis of [^{3}H] GABA binding against GABA in the hippocampus of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	361 ± 5.3	22.0 ± 0.18
Diabetic	253 ± 7.2 [a]	11.5 ± 0.17 [d]
D + IIH	216 ± 9.3 [a, b]	16.0 ± 1.00 [d, e]
C + IIH	166 ± 11.4 [a, b, c]	16.6 ± 0.12 [d, e]

Values are Mean ± S.E.M. of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$, **d** $p<0.01$ when compared to control, **b** $p<0.001$, **e** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-98

Real Time PCR amplification of AChE mRNA in the hippocampus of control and experimental rats

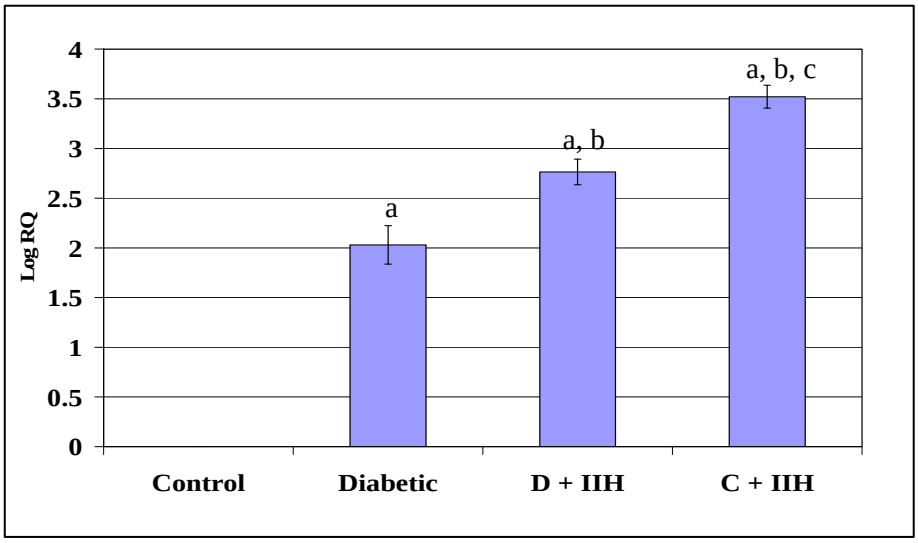


Table-98

Real Time PCR amplification of AChE mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	2.03 ± 0.19 [a]
D + IIH	2.76 ± 0.12 [a, b]
C + IIH	3.52 ± 0.12 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-99

Real Time PCR amplification of ChAT mRNA in the hippocampus of control and experimental rats

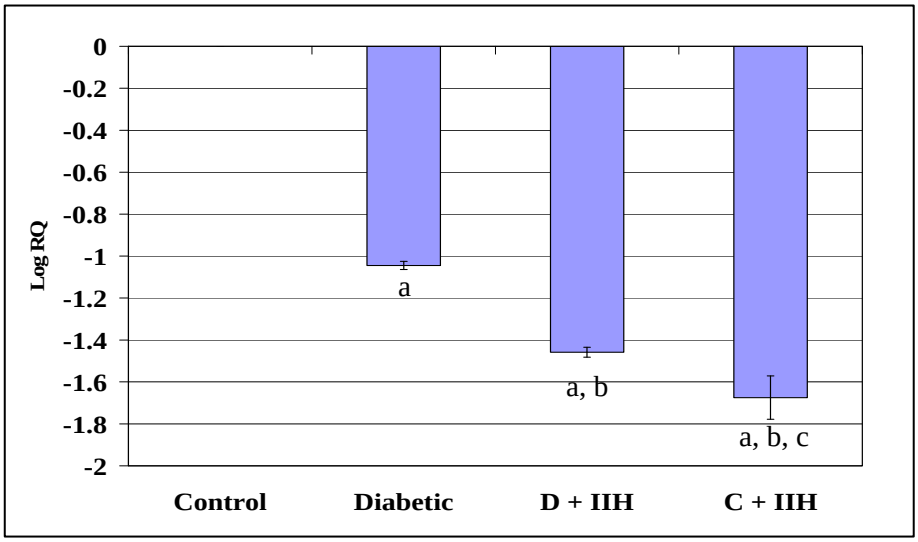


Table-99

Real Time PCR amplification of ChAT mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.04 ± 0.02 [a]
D + IIH	-1.45 ± 0.02 [a, b]
C + IIH	-1.65 ± 0.10 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-100

Real Time PCR amplification of muscarinic M1 receptor mRNA in the hippocampus of control and experimental rats

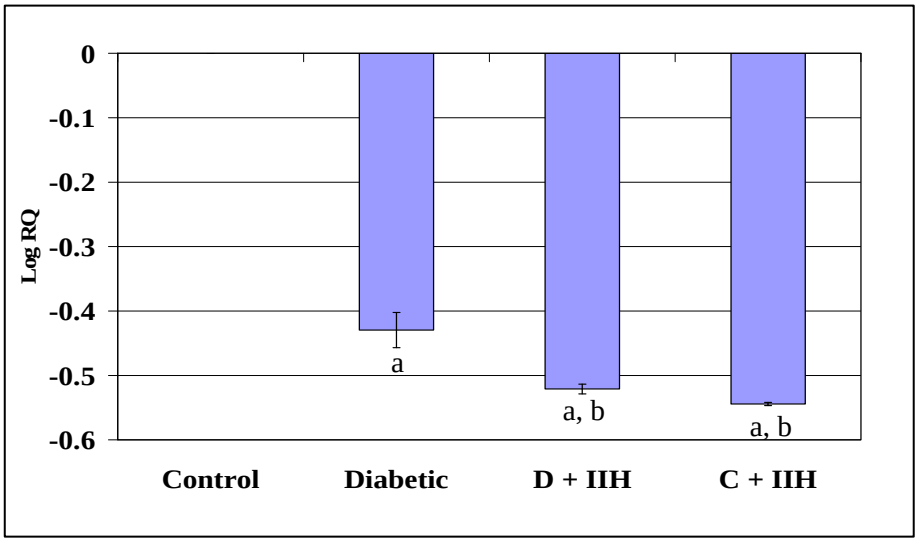


Table- 100

Real Time PCR amplification of muscarinic M1 receptor mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	- 0.42 ± 0.020 [a]
D + IIH	- 0.52 ± 0.010 [a, b]
C + IIH	- 0.54 ± 0.002 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **[a]** $p<0.001$ when compared with control, **[b]** $p<0.001$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-101

Real Time PCR amplification of muscarinic M3 receptor mRNA in the hippocampus of control and experimental rats

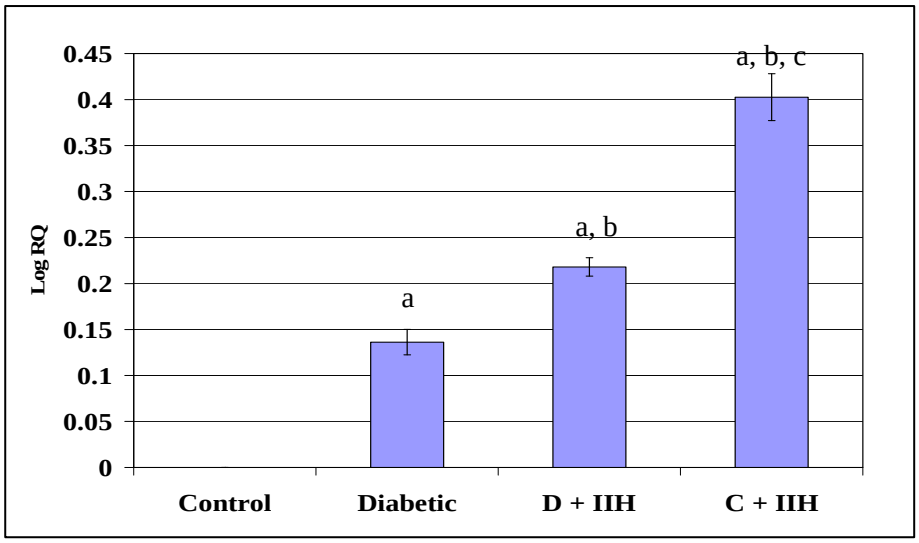


Table- 101

Real Time PCR amplification of muscarinic M3 receptor mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.14 ± 0.01^{a}
D + IIH	$0.21 \pm 0.01^{a, b}$
C + IIH	$0.40 \pm 0.02^{a, b, c}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic, **c** $p<0.001$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-102

Real Time PCR amplification of α7 nicotinic acetylcholine receptor mRNA in the hippocampus of control and experimental rats

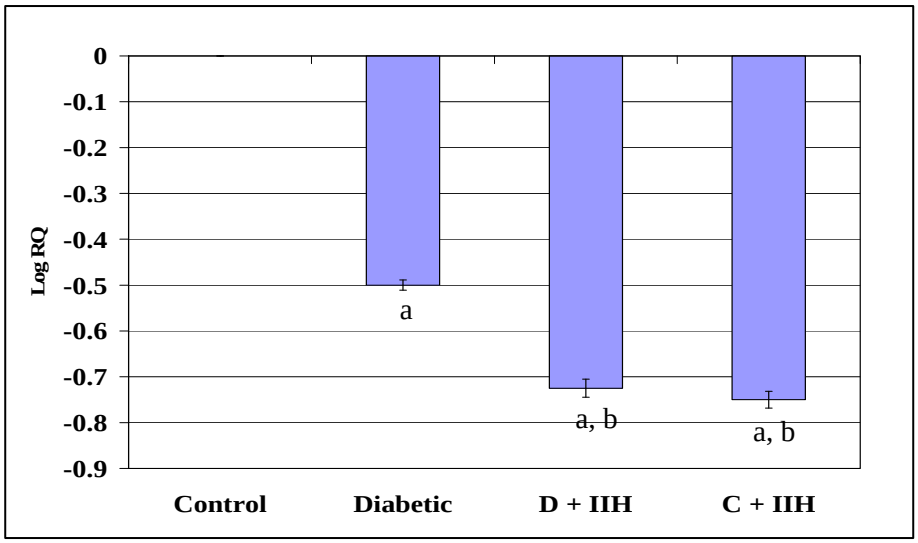


Table-102

Real Time PCR amplification of α7 nicotinic acetylcholine receptor mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.50 ± 0.01 [a]
D + IIH	-072 ± 0.03 [a, b]
C + IIH	-0.74 ± 0.02 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **[a]** $p<0.001$ when compared with control, **[b]** $p<0.001$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-103

Real Time PCR amplification of GAD mRNA in the hippocampus of control and experimental rats

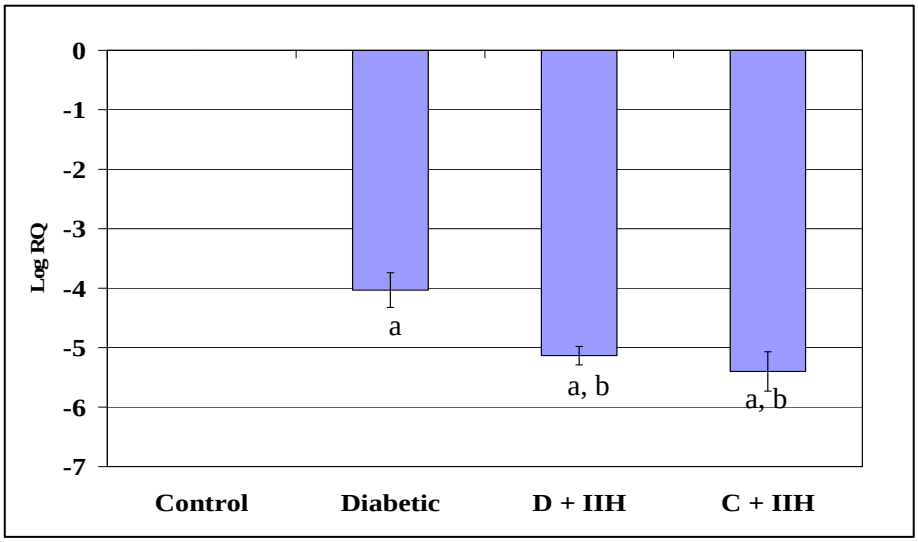


Table-103

Real Time PCR amplification of GAD mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-4.0 ± 0.20 [a]
D + IIH	-5.1 ± 0.15 [a, b]
C + IIH	-5.3 ± 0.20 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic. IIH - Insulin Induced Hypoglycemia

Figure-104

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the hippocampus of control and experimental rats

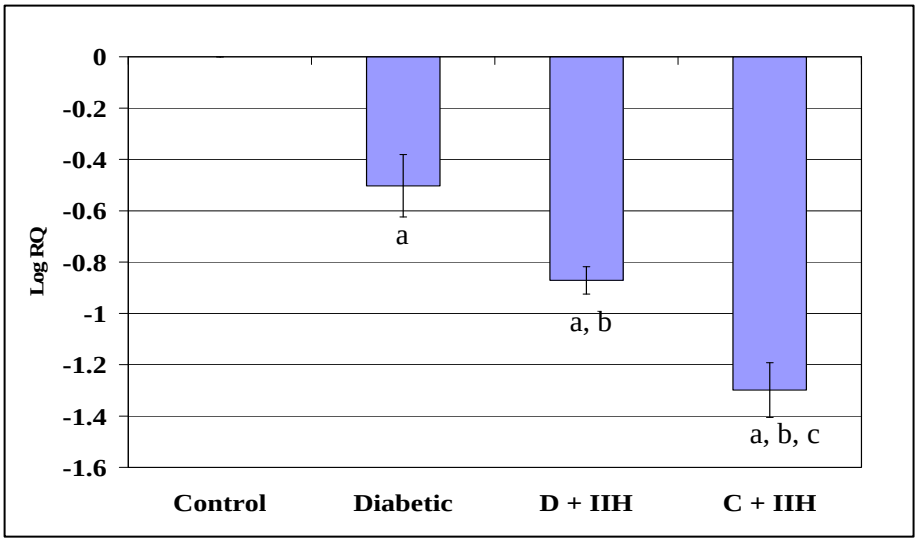


Table-104

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.05 ± 0.12 [a]
D + IIH	-0.87 ± 0.05 [a, b]
C + IIH	-1.30 ± 0.10 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-105

Real Time PCR amplification of $GABA_B$ mRNA in the hippocampus of control and experimental rats

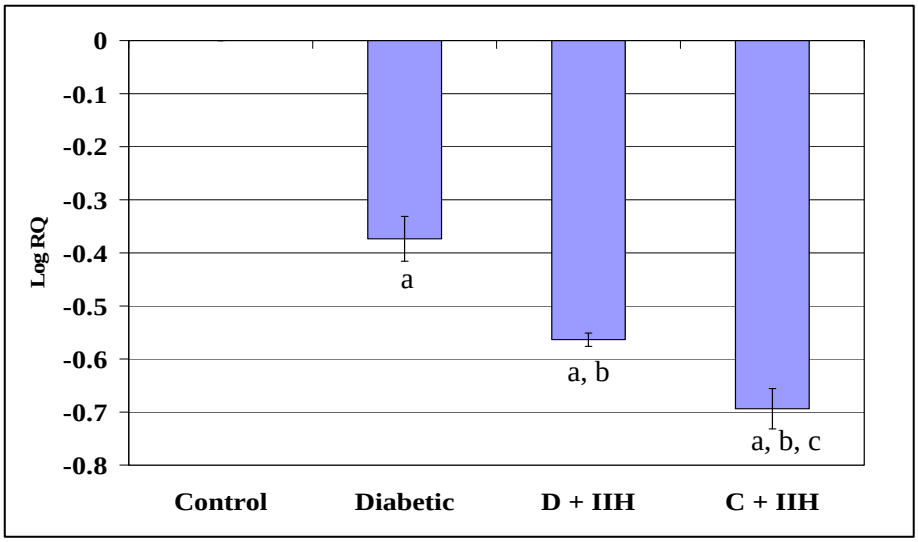


Table-105

Real Time PCR amplification of $GABA_B$ mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.37 ± 0.04 [a]
D + IIH	-0.43 ± 0.01 [a, b]
C + IIH	-0.69 ± 0.03 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-106

Real Time PCR amplification of GLUT3 mRNA in the hippocampus of control and experimental rats

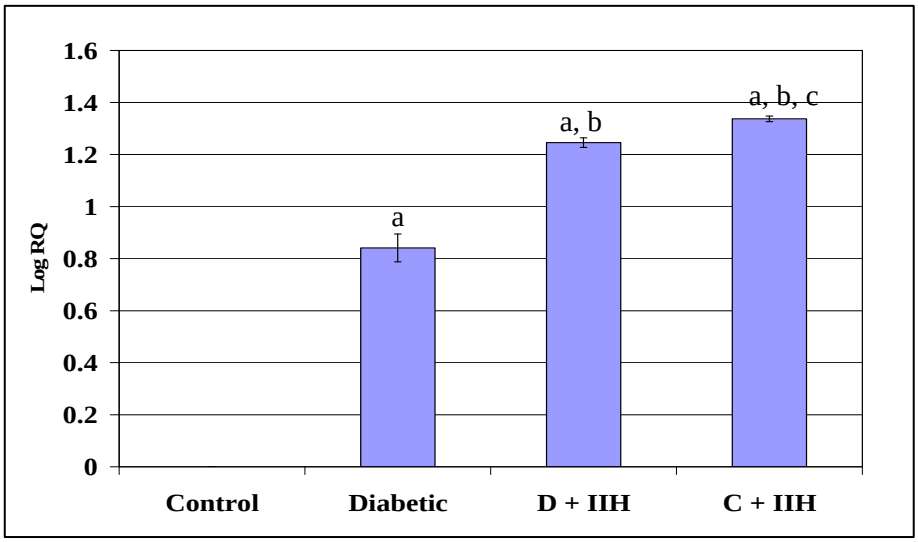


Table-106

Real Time PCR amplification of GLUT3 mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.84 ± 0.05 [a]
D + IIH	1.24 ± 0.02 [a, b]
C + IIH	1.33 ± 0.01 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-107

Real Time PCR amplification of insulin receptor mRNA in the hippocampus of control and experimental rats

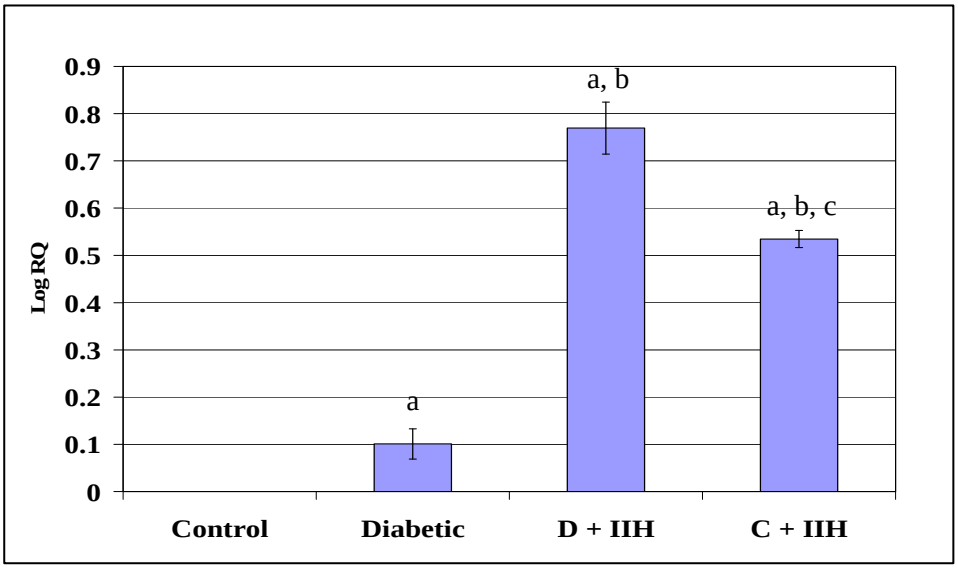


Table-107

Real Time PCR amplification of insulin receptor mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0. 10 ± 0.02 [a]
D + IIH	0.78 ± 0.05 [a, b]
C + IIH	0.55 ± 0.01 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-108

Real Time PCR amplification of SOD mRNA in the hippocampus of control and experimental rats

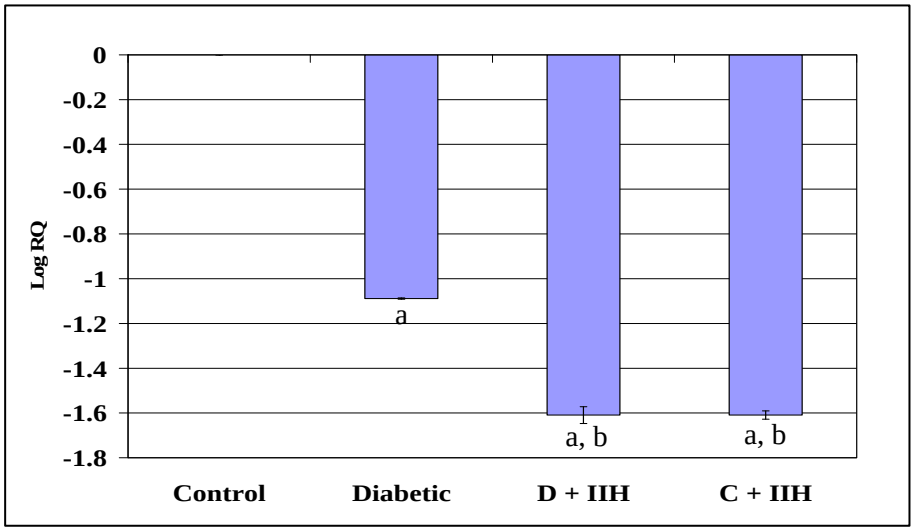


Table-108

Real Time PCR amplification of SOD mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-1.08 ± 0.01 [a]
D + IIH	-1.60 ± 0.03 [a, b]
C + IIH	-1.60 ± 0.02 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic. IIH - Insulin Induced Hypoglycemia

Figure-109

Real Time PCR amplification of Bax mRNA in the hippocampus of control and experimental rats

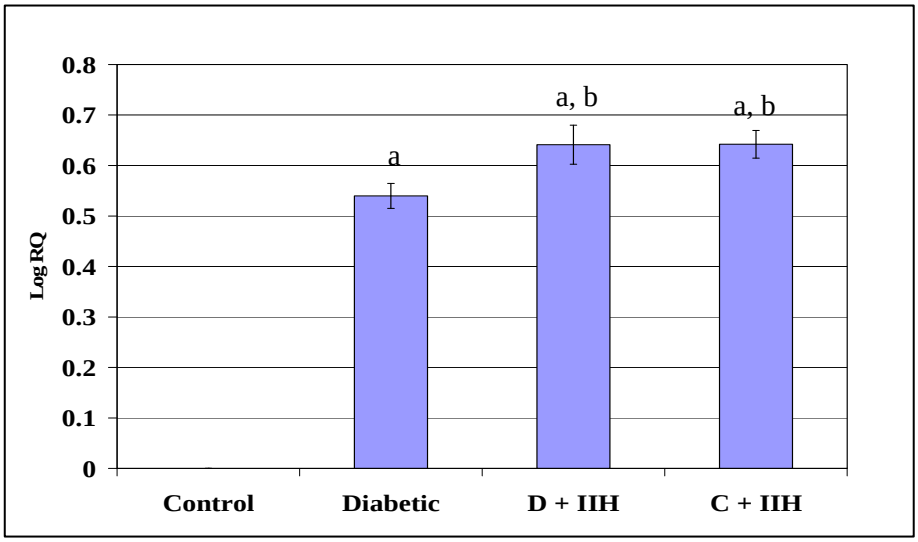


Table-109

Real Time PCR amplification of Bax mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.52 ± 0.02 [a]
D + IIH	0.64 ± 0.03 [a, b]
C + IIH	0.64 ± 0.02 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic. IIH - Insulin Induced Hypoglycemia

Figure-110

Real Time PCR amplification of phospholipase C mRNA in the hippocampus of control and experimental rats

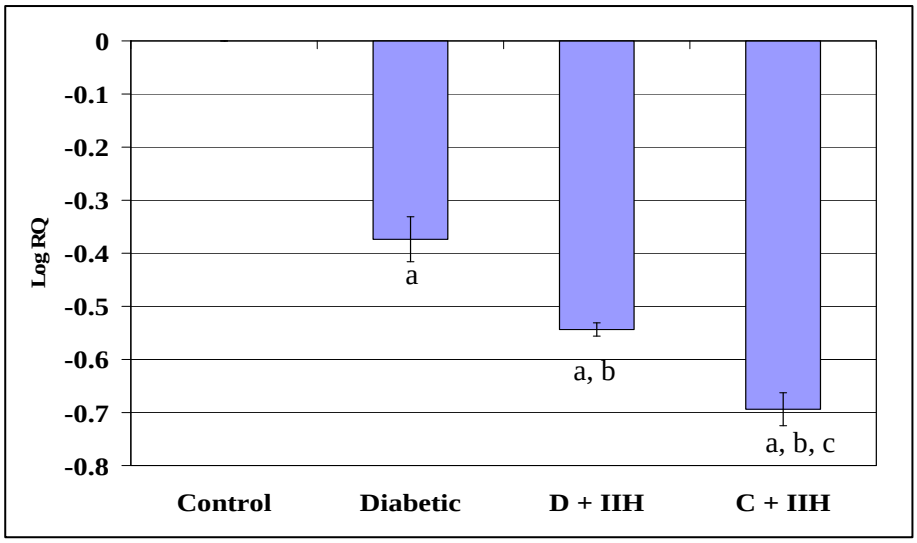


Table-110

Real Time PCR amplification of phospholipase C mRNA in the hippocampus of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.36 ± 0.02 [a]
D + IIH	-0.55 ± 0.03 [a, b]
C + IIH	-0.69 ± 0.02 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic, **c** $p<0.001$ when compared to D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-111

Real Time PCR amplification of CREB mRNA in the hippocampus of control and experimental rats

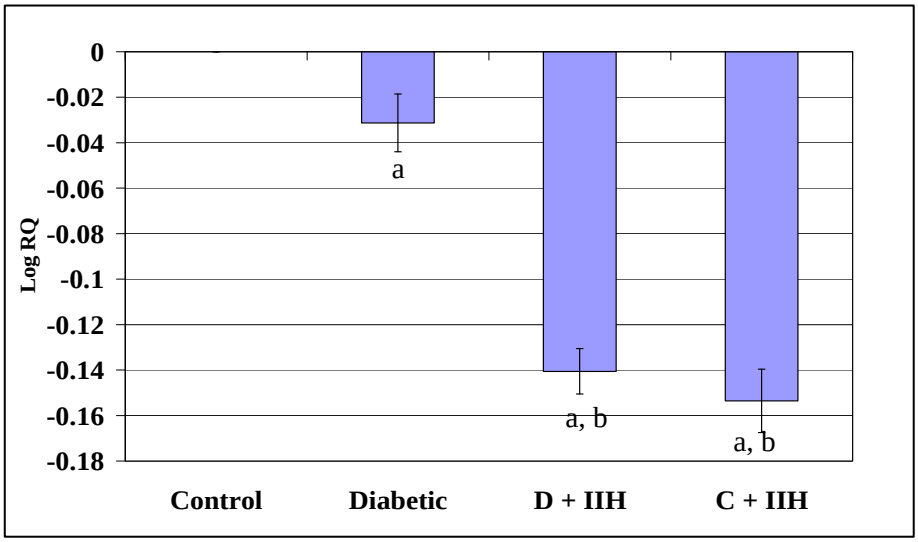


Table-111

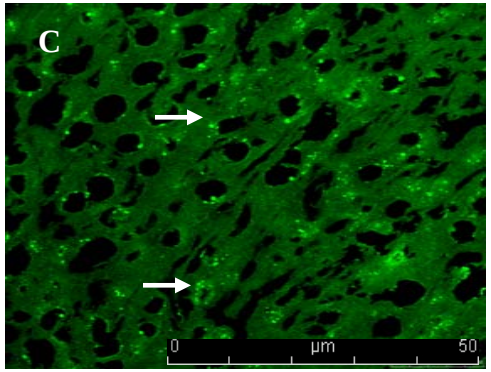
Real Time PCR amplification of CREB mRNA in the hippocampus of control and experimental rats

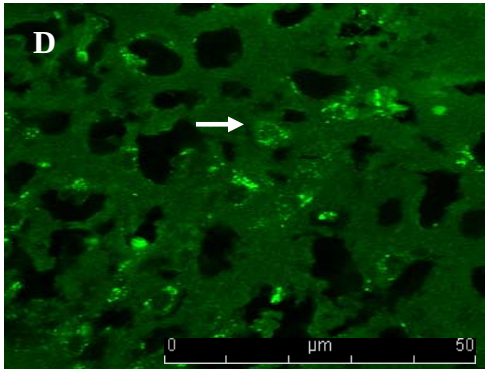
Experimental groups	Log RQ
Control	0
Diabetic	-0.03 ± 0.01[a]
D + IIH	-0.14 ± 0.01[a, b]
C + IIH	-0.15 ± 0.01[a, b]

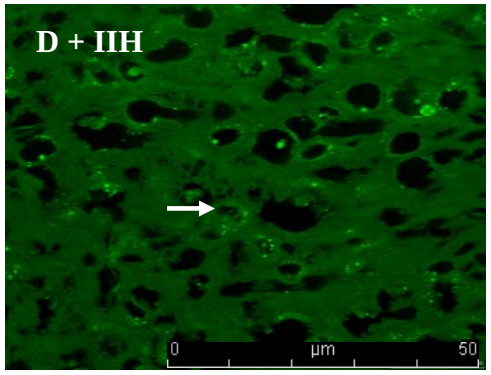
Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared to diabetic. IIH - Insulin Induced Hypoglycemia

Figure--112

Muscarinic M1 receptor expression in the hippocampus of control and experimental rats







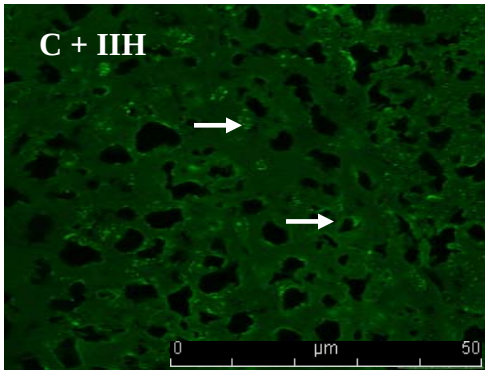


Table-112

Condition	Mean Pixel Value
Control	57.6 ± 3.5
Diabetic	32.5 ± 3.4 [a]
D + IIH	23.3 ± 2.8 [a, b]
C + IIH	17.6 ± 2.1 [a, b, c]

Confocal image of muscarinic M1 receptors in the hippocampus of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M1 receptors. Scale bar = 50 µm. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic, [c] $p<0.001$ when compared to D + IIH.

Figure—113
Muscarinic M3 receptor expression in the hippocampus of control and experimental rats

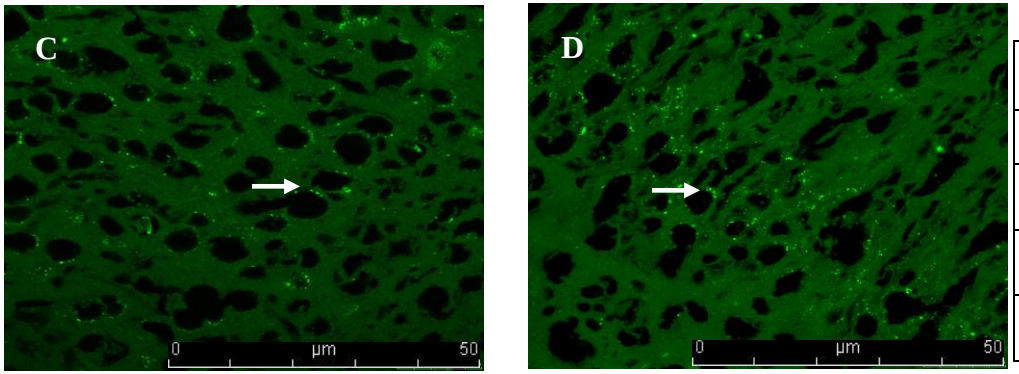
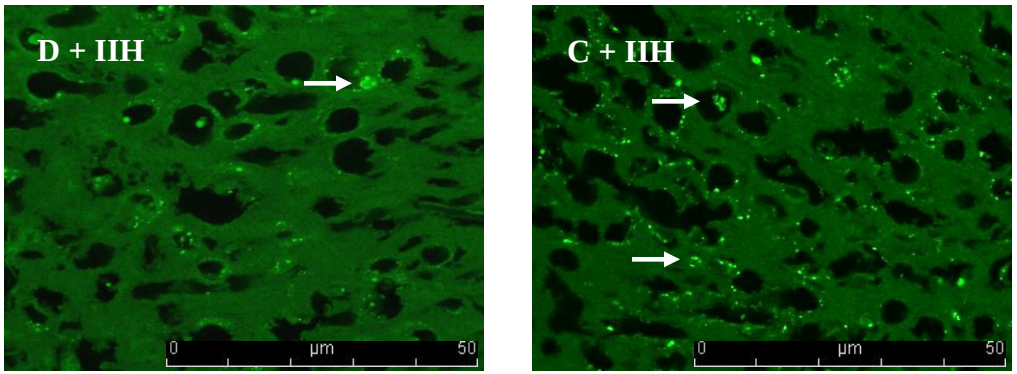


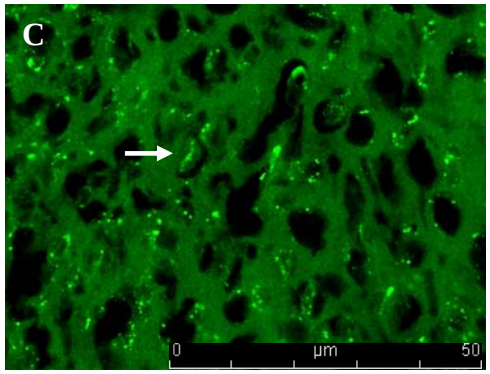
Table-113

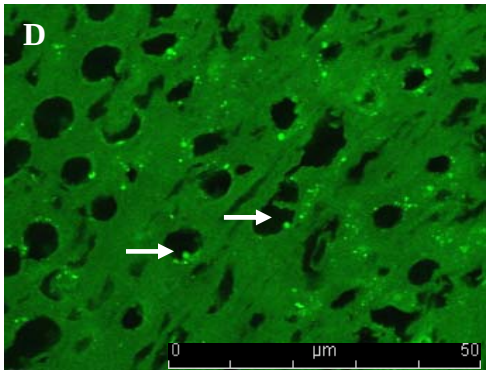
Condition	Mean Pixel Value
Control	16.8 ± 3.3
Diabetic	35.7 ± 4.1 [a]
D + IIH	48.1 ± 4.8 [a, b]
C + IIH	58.3 ± 2.9 [a, b, c]

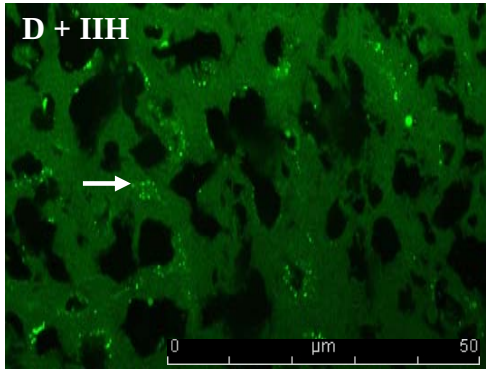


Confocal image of muscarinic M3 receptors in the hippocampus of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M3 receptors. Scale bar = 50 µm. [a] $p<0.01$ when compared to control, [b] $p<0.01$ when compared to diabetic, [c] $p<0.01$ when compared to D + IIH.

Figure—114
α7 nicotinic receptor expression in the hippocampus of control and experimental rats







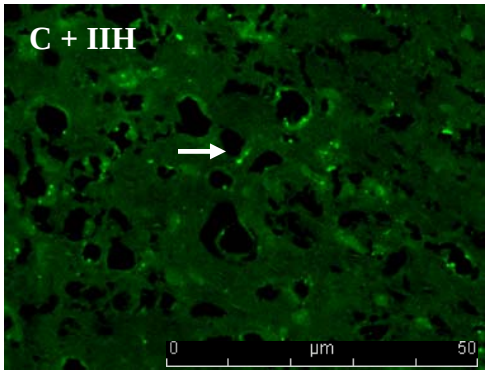


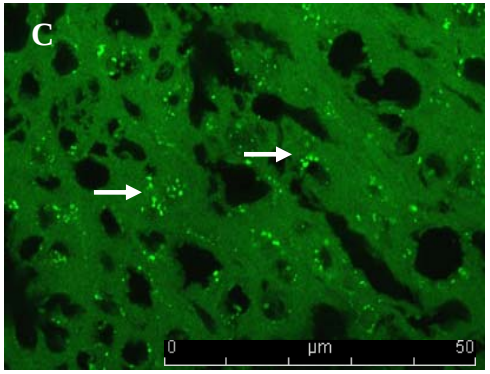
Table-114

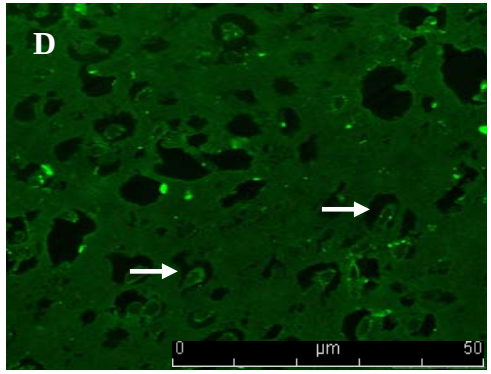
Condition	Mean Pixel Value
Control	58.3 ± 3.3
Diabetic	42.7 ± 4.1 [a]
D + IIH	30.2 ± 4.8 [a, b]
C + IIH	15.4 ± 2.9 [a, b, c]

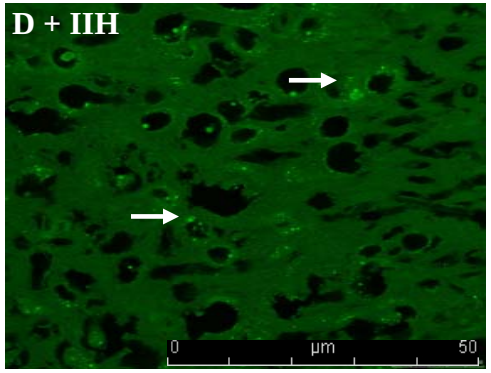
Confocal image of α7 nACh receptors in the hippocampus of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent α7 nACh receptor specific primary antibody and FITC as secondary antibody. (→) in white shows α7 nACh receptors. Scale bar = 50 µm. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic, [c] $p<0.01$ when compared to D + IIH.

Figure--115

$GABA_{A\alpha1}$ receptor expression in the hippocampus of control and experimental rats







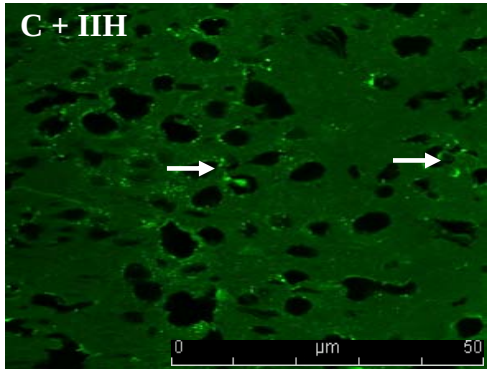


Table-115

Condition	Mean Pixel Value
Control	61.2 ± 3.3
Diabetic	40.4 ± 4.1 [a]
D + IIH	28.5 ± 4.8 [a, b]
C + IIH	22.6 ± 5.9 [a, b]

Confocal image of $GABA_{A\alpha1}$ receptors in the cerebellum of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent $GABA_{A\alpha1}$ receptor specific primary antibody and FITC as secondary antibody. (→) in white shows $GABA_{A\alpha1}$ receptors. Scale bar = 50 µm. **a** $p<0.001$ when compared to control, **b** $p<0.001$ when compared to diabetic.

Figure-116

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the pancreas of control and experimental rats

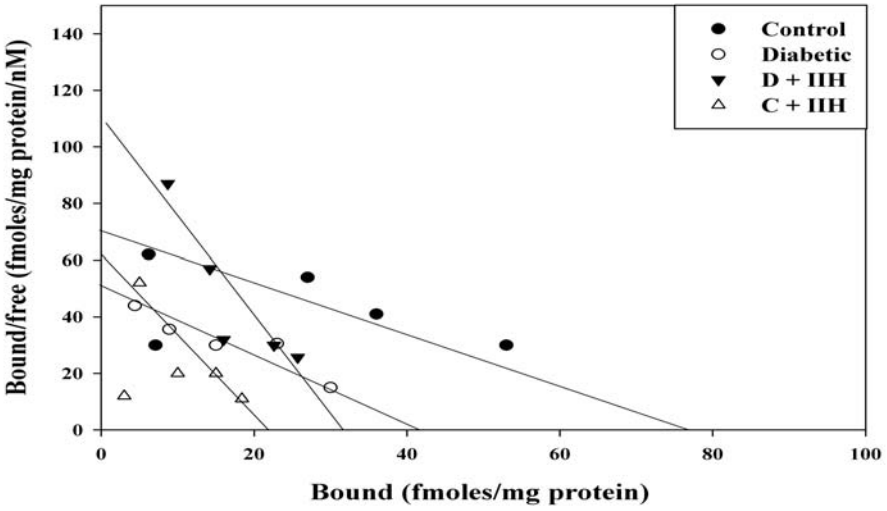


Table-116

Scatchard analysis of [^{3}H] QNB binding against total muscarinic receptor antagonist, atropine in the pancreas of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	76 ± 2.4	1.08 ± 0.02
Diabetic	41 ± 1.8 [a]	0.82 ± 0.05 [a]
D + IIH	31 ± 1.4 [a, b]	0.28 ± 0.03 [a, b]
C + IIH	21 ± 2.8 [a, b, c]	0.35 ± 0.04 [a, b]

Values are Mean ± S.E.M. of 3-5 separate experiments. Each group consists of 6-8 rats. **a** $p<0.01$ when compared to control, **b** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-117

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the pancreas of control and experimental rats

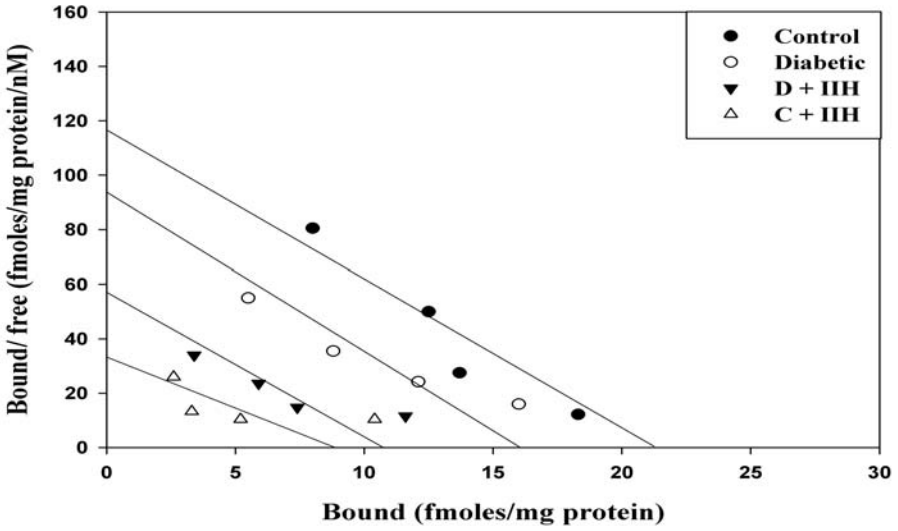


Table-117

Scatchard analysis of [^{3}H] QNB binding against muscarinic M1 receptor antagonist, pirenzepine in the pancreas of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	21.3 ± 2.4	0.18 ± 0.02
Diabetic	16.0 ± 1.8 [a]	0.17 ± 0.05
D + IIH	10.6 ± 1.4 [a, b]	0.18 ± 0.03
C + IIH	8.7 ± 2.8 [a, b]	0.26 ± 0.04 [a, b]

Values are Mean ± S.E.M. of 3-5 separate experiments. Each group consists of 6-8 rats. **a** $p<0.01$ when compared to control, **b** $p<0.01$ when compared to diabetic. IIH- Insulin Induced Hypoglycemia

Figure-118

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the pancreas of control and experimental rats

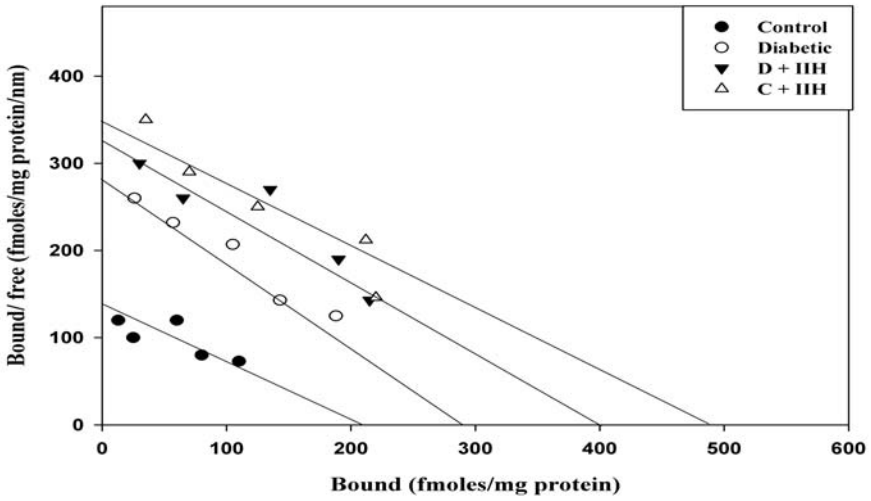


Table-118

Scatchard analysis of [^{3}H] DAMP binding against muscarinic M3 receptor antagonist, 4-DAMP mustard in the pancreas of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	121.3 ± 2.4	0.34 ± 0.06
Diabetic	86.9 ± 1.8 [a]	0.28 ± 0.05 [d]
D + IIH	68.8 ± 1.4 [a, b]	0. 25 ± 0.08 [d]
C + IIH	47.5 ± 2.8 [a, b, c]	0.20 ± 0.05 [d]

Values are Mean ± S.E.M. of 3-5 separate experiments. Each group consists of 6-8 rats. **a** $p<0.01$, **d** $p<0.05$ when compared to control, **b** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-119

Scatchard analysis of [^{3}H] GABA binding against GABA in the pancreas of control and experimental rats

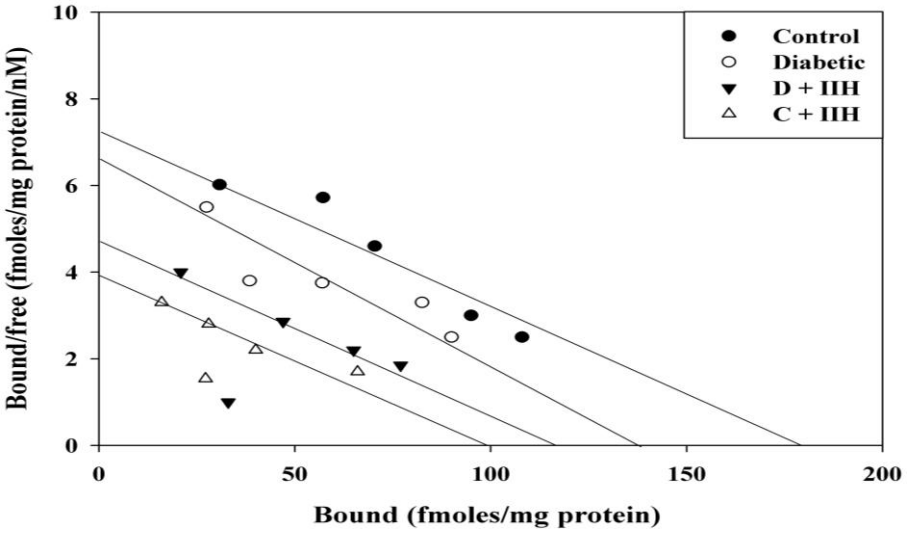


Table-119

Scatchard analysis of [^{3}H] GABA binding against GABA in the pancreas of control and experimental rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	136 ± 5.8	1.83 ± 0.02
Diabetic	120 ± 3.2 [a]	1.90 ± 0.05
D + IIH	95 ± 4.4 [a, b]	2.05 ± 0.03
C + IIH	84 ± 2.8 [a, b, c]	2.40 ± 0.04 [a, b]

Values are Mean ± S.E.M. of 3-5 separate experiments. Each group consists of 6-8 rats. **a** $p<0.01$ when compared to control, **b** $p<0.01$ when compared to diabetic, **c** $p<0.01$ when compared to D + IIH. IIH- Insulin Induced Hypoglycemia

Figure-120

Real Time PCR amplification of AChE mRNA in the pancreas of control and experimental rats

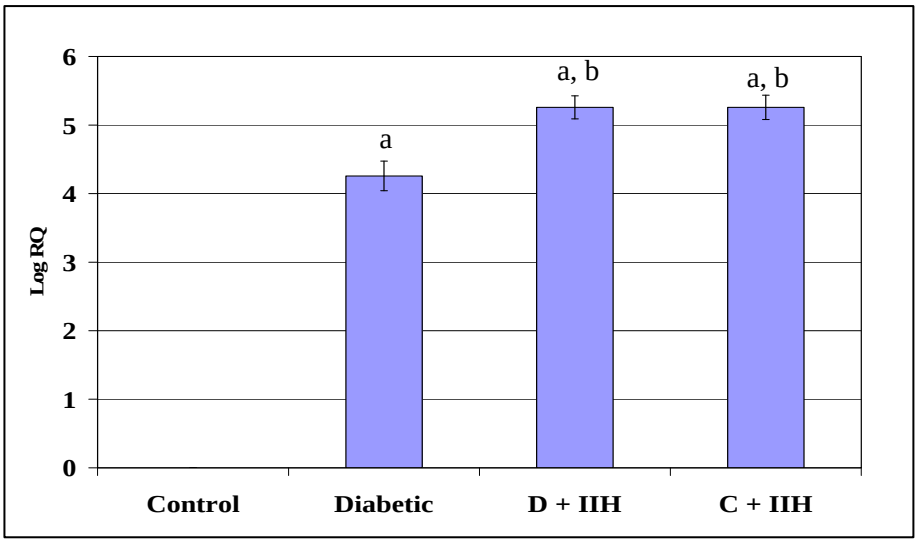


Table-120

Real Time PCR amplification of AChE mRNA in the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	4.25 ± 0.21 [a]
D + IIH	5.20 ± 0.16 [a, b]
C + IIH	5.35 ± 0.17 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-121

Real Time PCR amplification of ChAT mRNA in the pancreas of control and experimental rats

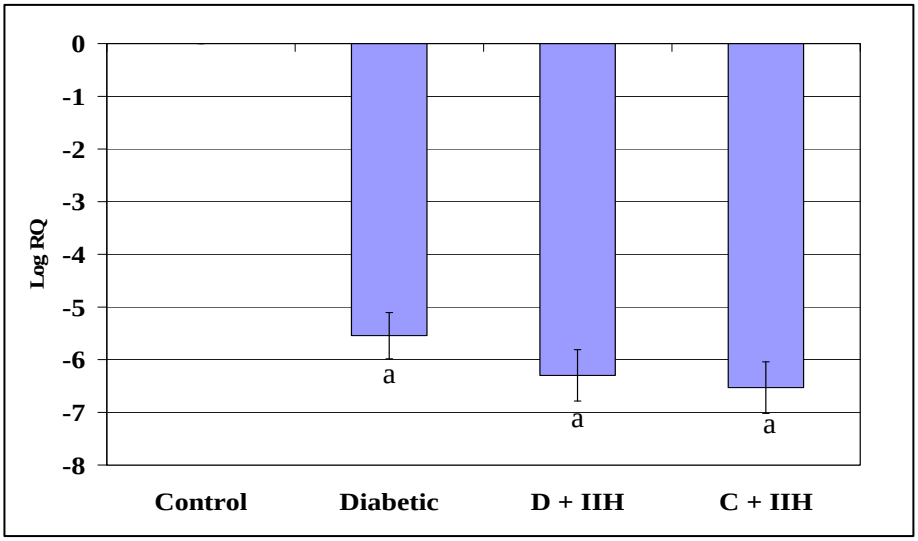


Table-121

Real Time PCR amplification of ChAT mRNA in the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-5.5 ± 0.43 [a]
D + IIH	-6.3 ± 0.48 [a]
C + IIH	-6.5 ± 0.48 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control. IIH - Insulin Induced Hypoglycemia

Figure-122

Real Time PCR amplification of muscarinic M1 receptor mRNA in the pancreas of control and experimental rats

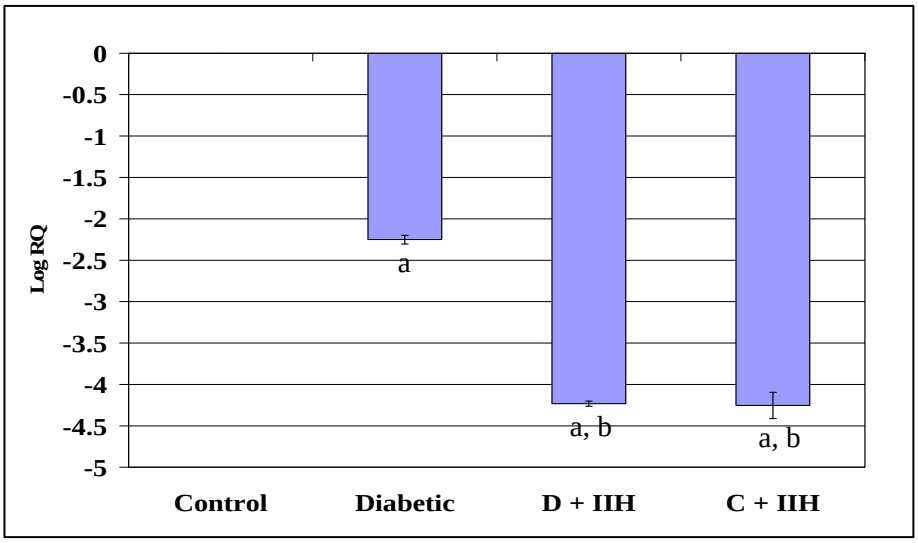


Table-122

Real Time PCR amplification of muscarinic M1 receptor mRNA in the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-2.2 ± 0.03 [a]
D + IIH	-4.3 ± 0.03 [a, b]
C + IIH	-4.4 ± 0.05 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-123

Real Time PCR amplification of muscarinic M3 receptor mRNA in the pancreas of control and experimental rats

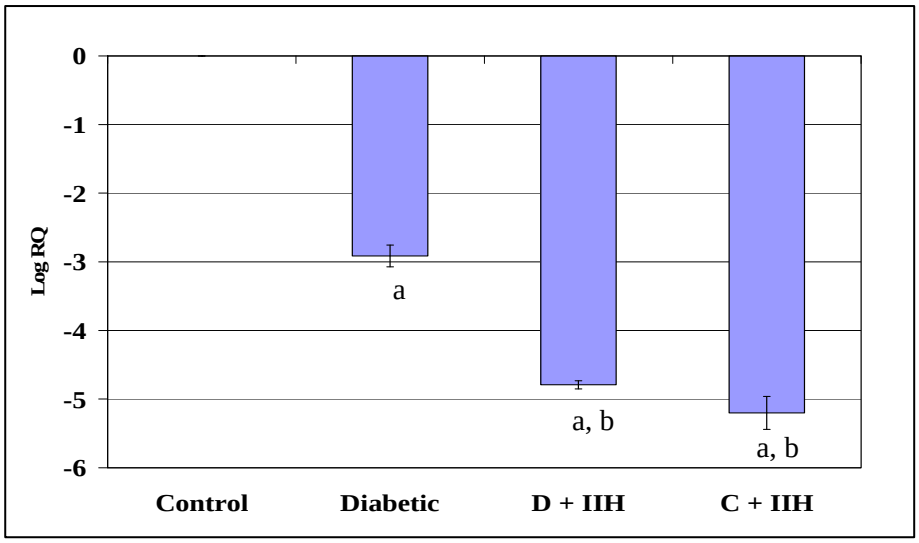


Table-123

Real Time PCR amplification of muscarinic M3 receptor mRNA in the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-2.9 ± 0.07 [a]
D + IIH	-4.8 ± 0.05 [a, b]
C + IIH	-4.2 ± 0.07 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **[a]** $p<0.001$ when compared with control, **[b]** $p<0.001$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-124

Real Time PCR amplification of GAD mRNA in the pancreas of control and experimental rats

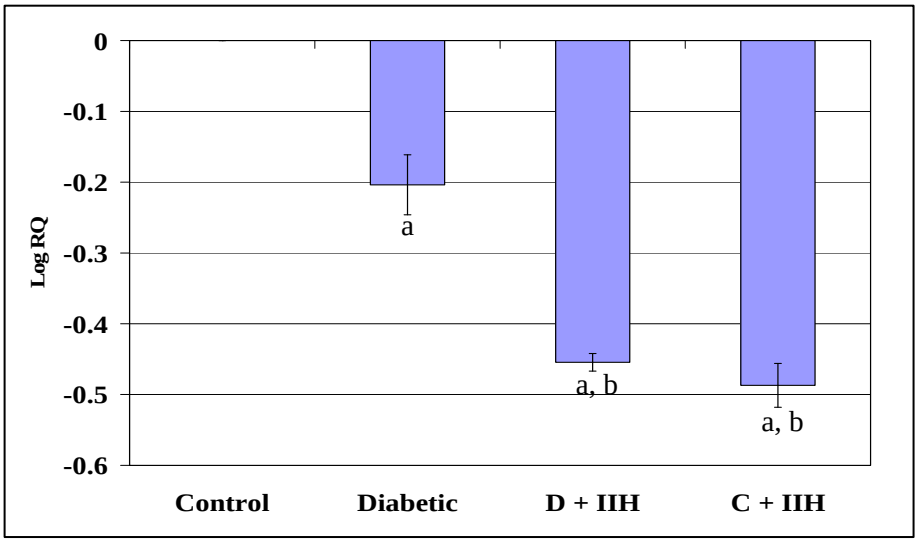


Figure-124

Real Time PCR amplification of GAD mRNA in the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.20 ± 0.04 [a]
D + IIH	-0.45 ± 0.01 [a, b]
C + IIH	-0.49 ± 0.03 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control. IIH - Insulin Induced Hypoglycemia

Figure-125

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the pancreas of control and experimental rats

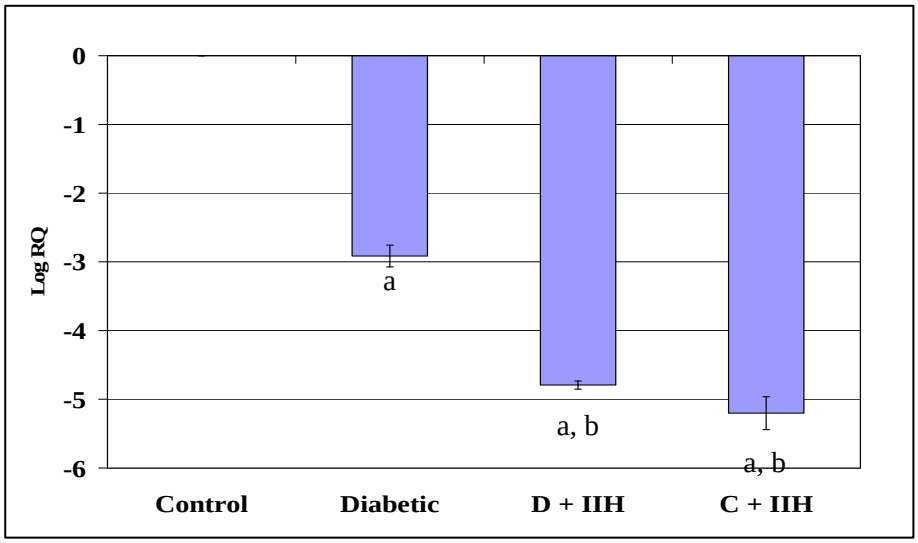


Table-125

Real Time PCR amplification of $GABA_{A\alpha1}$ mRNA in the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-2.92 ± 0.15 [a]
D + IIH	-4.79 ± 0.05 [a, b]
C + IIH	-5.20 ± 0.23 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic. IIH - Insulin Induced Hypoglycemia

Figure-126

Real Time PCR amplification of $GABA_B$ mRNA in the pancreas of control and experimental rats

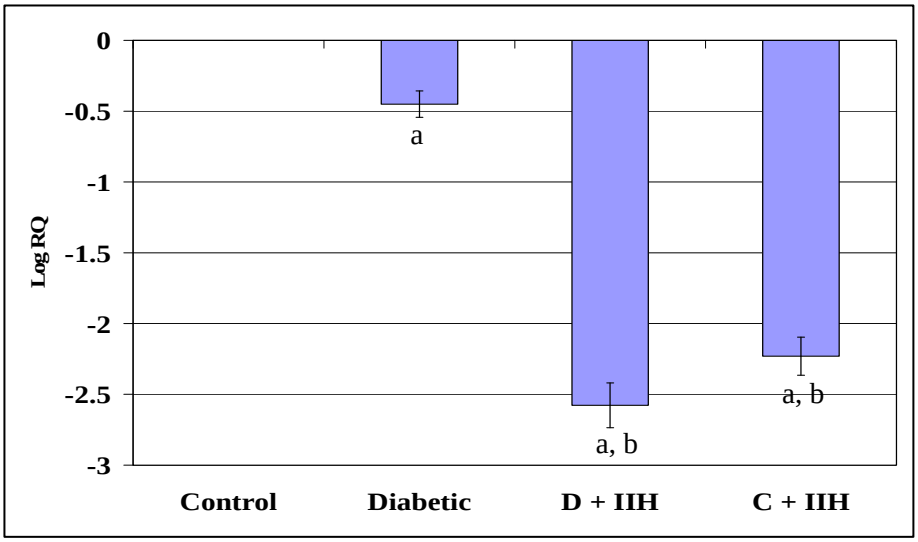


Table-126

Real Time PCR amplification of $GABA_B$ mRNA in the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.45 ± 0.09 [a]
D + IIH	-2.50 ± 0.15 [a, b]
C + IIH	-2.23 ± 0.13 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **[a]** $p<0.001$ when compared with control, **[b]** $p<0.001$ when compared with diabetic . IIH - Insulin Induced Hypoglycemia

Figure-127

Real Time PCR amplification of insulin receptor mRNA in the pancreas of control and experimental rats

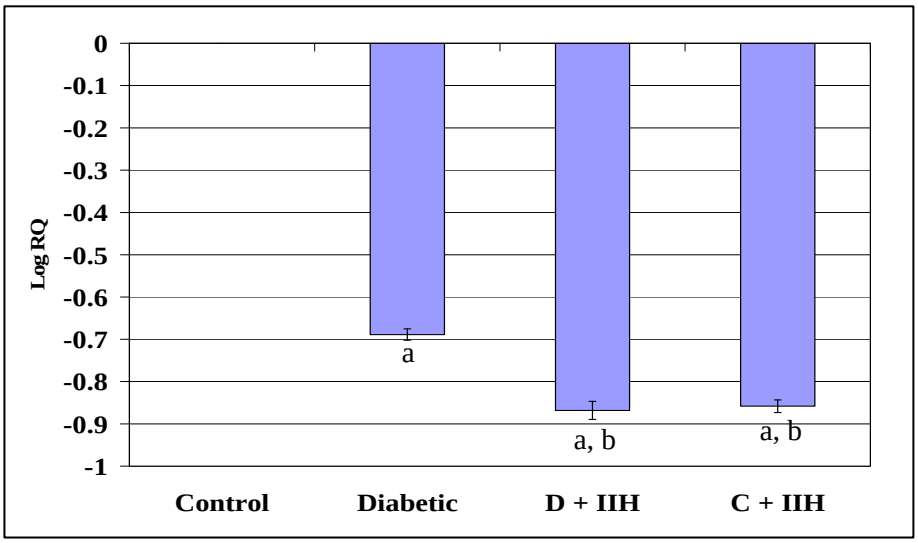


Table-127

Real Time PCR amplification of insulin receptor mRNA in the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.69 ± 0.01 [a]
D + IIH	-0.87 ± 0.02 [a, b]
C + IIH	-0.86 ± 0.01 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **[a]** $p<0.001$ when compared with control, **[b]** $p<0.001$ when compared with diabetic . IIH - Insulin Induced Hypoglycemia

Figure-128

Real Time PCR amplification of SOD mRNA in the pancreas of control and experimental rats

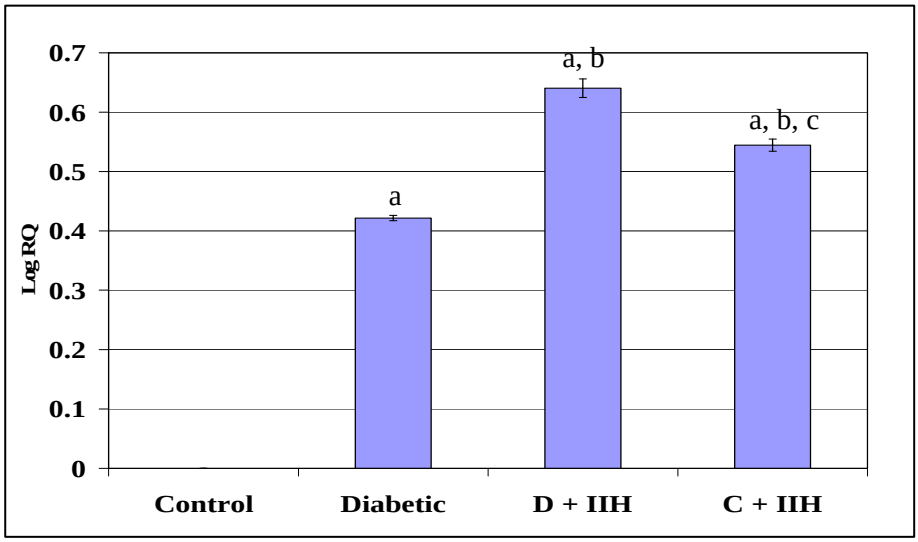


Table-128

Real Time PCR amplification of SOD mRNA in the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.42 ± 0.04 [a]
D + IIH	0.64 ± 0.03 [a, b]
C + IIH	0.55 ± 0.01 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic, **c** $p<0.01$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-129

Real Time PCR amplification of Bax mRNA in the pancreas of control and experimental rats

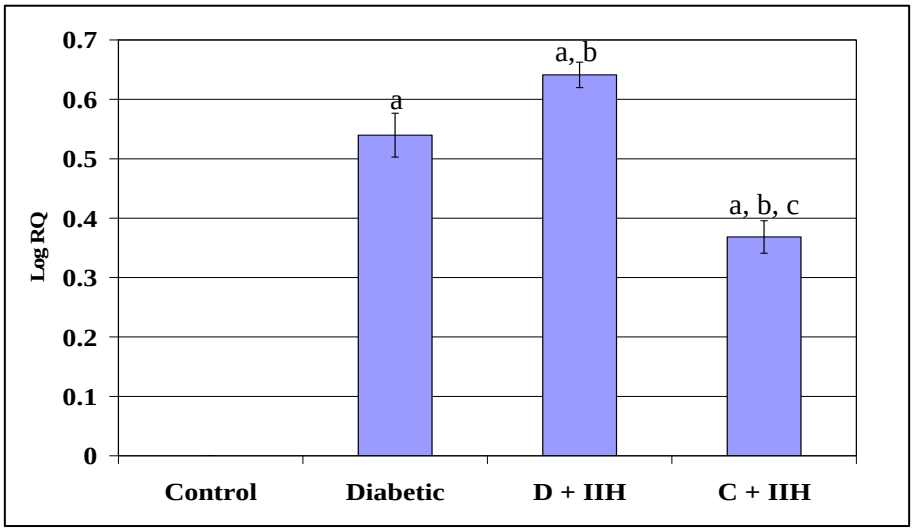


Table-129

Real Time PCR amplification of Bax mRNA in the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	0.53 ± 0.04 [a]
D + IIH	0.64 ± 0.03 [a, b]
C + IIH	0.36 ± 0.03 [a, b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control rats, **b** $p<0.001$ when compared with diabetic rats, **c** $p<0.01$ when compared with D + IIH. IIH - Insulin Induced Hypoglycemia

Figure-130

Real Time PCR amplification of phospholipase C mRNA in the pancreas of control and experimental rats

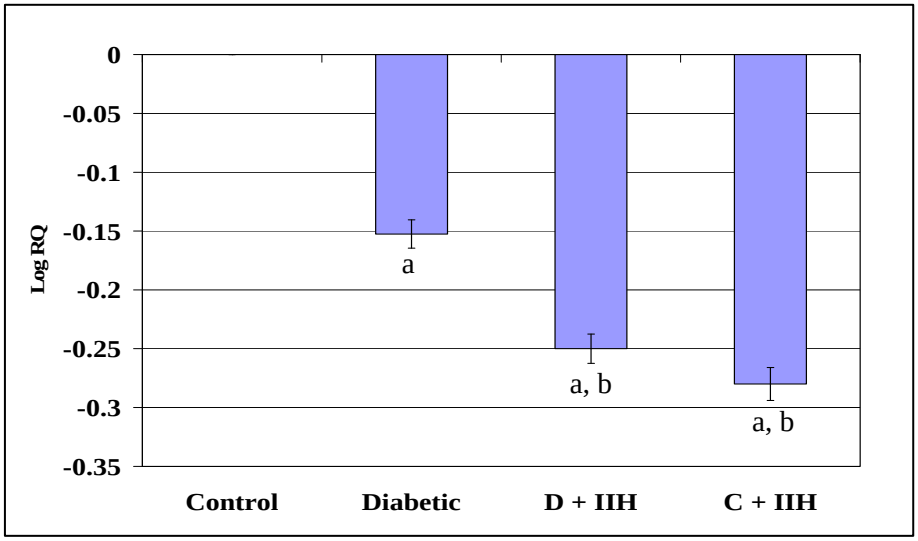


Table-130

Real Time PCR amplification of phospholipase C mRNA in the pancreas of control and experimental rats

Experimental groups	Log RQ
Control	0
Diabetic	-0.15 ± 0.01 [a]
D + IIH	-0.25 ± 0.01 [a, b]
C + IIH	-0.27 ± 0.01 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. **a** $p<0.001$ when compared with control, **b** $p<0.001$ when compared with diabetic . IIH - Insulin Induced Hypoglycemia.

Figure-131
Muscarinic M1 receptor expression in the pancreas of control and experimental rats

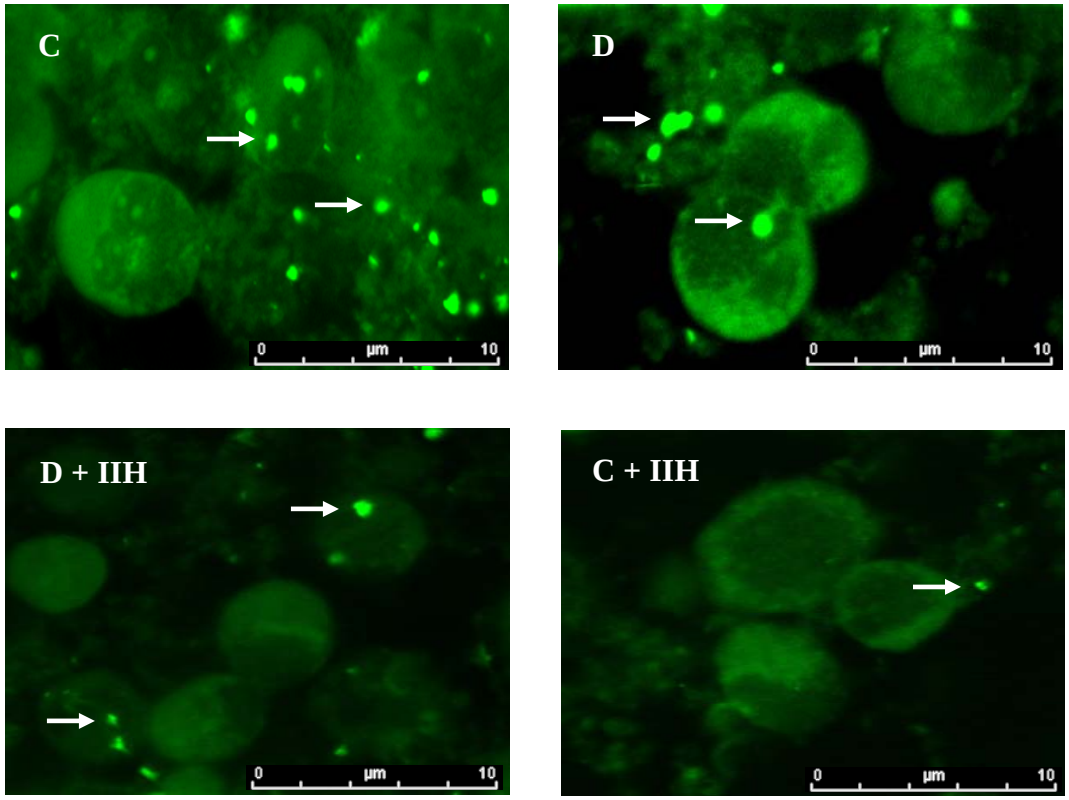
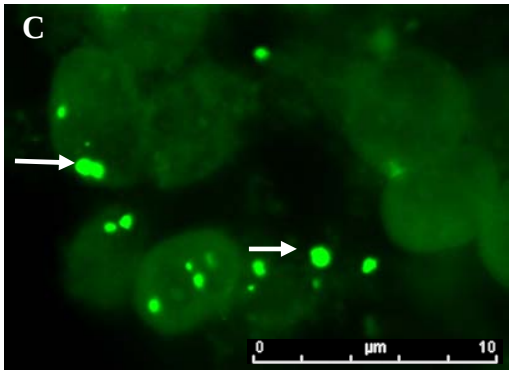


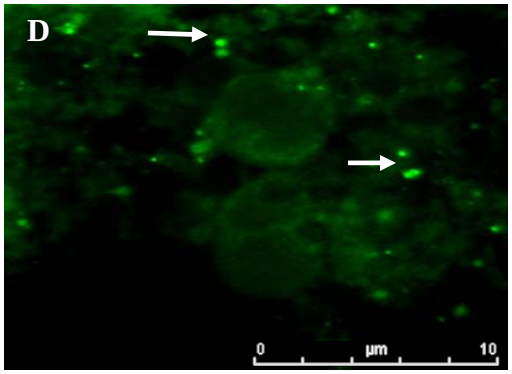
Table-131

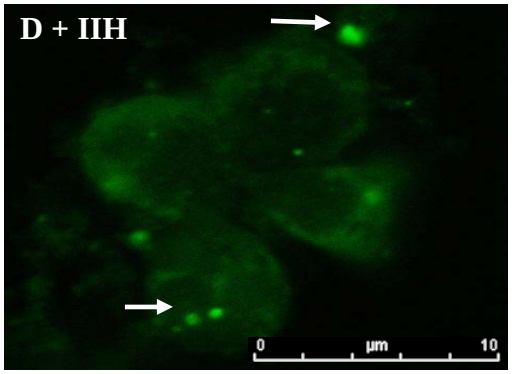
Condition	Mean Pixel Value
Control	48 ± 5.2
Diabetic	30 ± 6.1 [a]
D + IIH	18 ± 5.2 [a, b]
C + IIH	08 ± 4.5 [a, b, c]

Confocal image of muscarinic M1 receptors in the pancreas of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M1 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M1 receptors. Scale bar = 10 µm. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic, [c] $p<0.001$ when compared to D + IIH.

Figure-132
Muscarinic M3 receptor expression in the pancreas of control and experimental rats







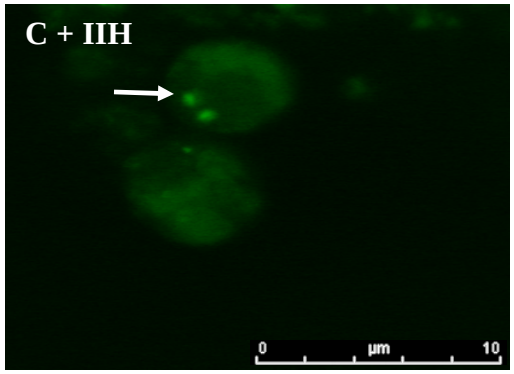


Table-132

Condition	Mean Pixel Value
Control	56 ± 3.3
Diabetic	32 ± 4.1 [a]
D + IIH	24 ± 4.8 [a, b]
C + IIH	19 ± 2.9 [a, b]

Confocal image of muscarinic M3 receptors in the pancreas of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent muscarinic M3 receptor specific primary antibody and FITC as secondary antibody. (→) in white shows muscarinic M3 receptors. Scale bar = 10 µm. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic.

Figure-133

$GABA_{A\alpha1}$ receptor expression in the pancreas of control and experimental rats

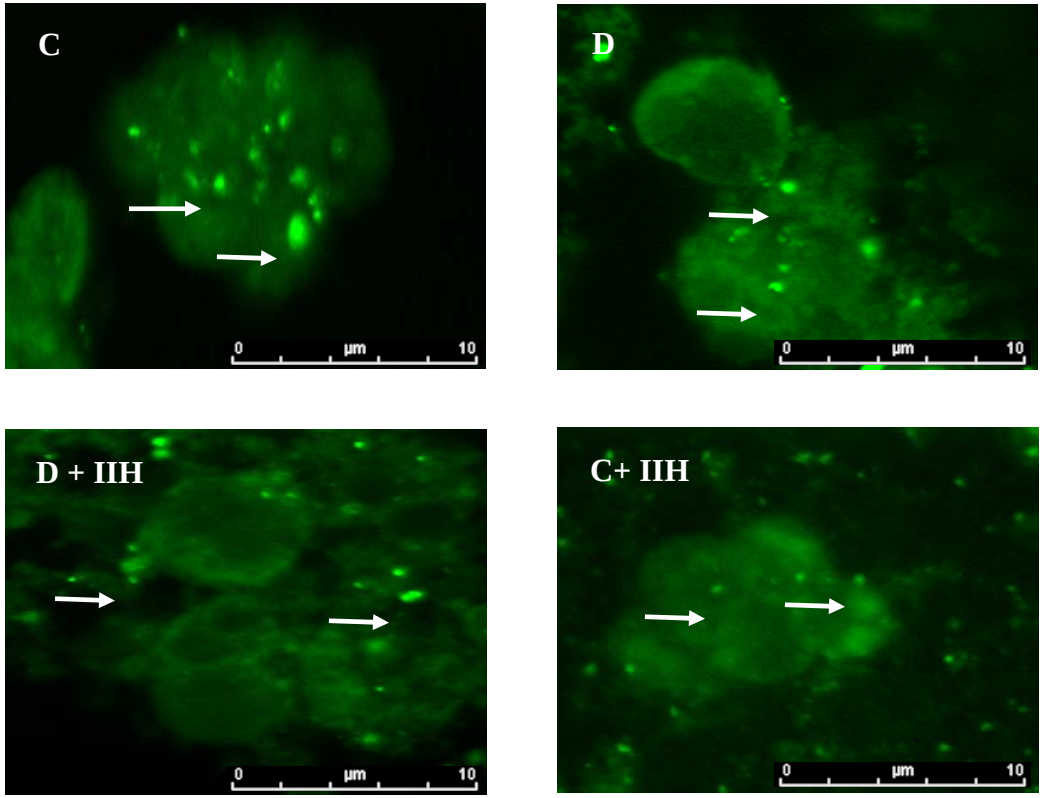


Table-133

Condition	Mean Pixel Value
Control	66.5 ± 5.3
Diabetic	35.1 ± 4.5 [a]
D + IIH	21.6 ± 5.7 [a, b]
C + IIH	10.8 ± 2.9 [a, b, c]

Confocal image of $GABA_{A\alpha1}$ receptors in the pancreas of control, Diabetic, D+IIH and C + IIH rats using immunofluorescent $GABA_{A\alpha1}$ receptor specific primary antibody and FITC as secondary antibody. (→) in white shows $GABA_{A\alpha1}$ receptors. Scale bar = 10 µm. [a] $p<0.001$ when compared to control, [b] $p<0.001$ when compared to diabetic., [c] $p<0.01$ when compared to D + IIH.