DOPAMINE RECEPTOR GENE EXPRESSION IN STREPTOZOTOCIN INDUCED DIABETIC RATS AND ITS ROLE IN INSULIN SECRETION

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CERTIFICATE

This is to certify that the thesis entitled "**DOPAMINE RECEPTOR GENE EXPRESSION IN STREPTOZOTOCIN INDUCED DIABETIC RATS AND ITS ROLE IN INSULIN SECRETION**" is a bonafide record of the research work carried out by **Mr. P.N.ESWAR SHANKAR** 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.

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ABBREVIATIONS USED IN THE TEXT

3-MT	3-methoxytyramine
5-HIAA	5-hydroxy indole acetic acid
5-HT	5-Hydroxytryptamine
7-OH-DPAT	7-Hydroxy-2(di-n-propylamino)-tetralin
AAD	Aromatic L-amino decarboxylase
Ach	Acetylcholine
AD	Aldehyde dehydrogenase
ADP	Adenosine diphosphate
APO	Apomorphine
ATP	Adenosine triphosphate
Bmax	Maximal binding
BRC	Bromocriptine
BS	Brain stem
cAMP	Cyclic adenosine monophosphate
CC	Cerebral cortex
CNS	Central nervous system
COMT	Catechol-*O*-methyltransferase.
CRF	Corticotropin releasing factor
CS	Corpus striatum
DA	Dopamine
DAT	Dopamine transporter protein
DBH	Dopamine β hydroxylase
DDC	DOPA decarboxylase
DOPAC	Dihydroxy phenyl acetic acid
EPI	Epinephrine
eSIN	Early sympathetic islet neuropathy
GABA	Gamma amino butyric acid
GOD	Glucose oxidase
GTP	Guanosine triphosphate

hDAT	human dopamine transporter protein
HPA	Hypothalamic- pitutary –axis
HPLC	High performance liquid chromatography
HVA	Homovanillic acid
HYPO	Hypothalamus
i.p.	Intraperitonial
IAPP	Islet amyloid polypeptide
I_{CRAN}	Ca^{2+} release activated non-selective cation current
IDDM	Insulin dependent diabetes mellitus
I-LHA	Intra lateral hypothalamic area
Kd	Dissociation constant
L-DOPA	L-3,4 dihydroxyphenylalanine
LH	Lateral hypothalamus
MAO-B	Monoamine oxidase B
MAPK	Mitogen activated protein kinase
Mu MLV	Murine moloney leukemia virus reverse transcriptase
MIF	Macrophage migration inhibitory factor
mRNA	Messenger ribonucleic acid
NAD	Nicotinamide adenine dinucleotide (oxidised)
NADH	Nicotinamide adenine dinucleotide (reduced)
NADP	Nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NE	Norepinephrine
NIDDM	Non insulin dependent diabetes mellitus
NPY	Neuropeptide Y
NTB	Non-tumor bearing
p	Level of significance
PEG	Polyethylene glycol
PFC	Prefrontal cortex
PHV	Parahypothalamic ventricular nucleus
PI 3-kinase	Phosphatidyl inositol (PI) 3-kinase
Pi	Inorganic phosphate

PKC	Protein kinase C
POD	Peroxidase
PTX	Pertussis toxin
RIA	Radioimmunoassay
RT-PCR	Reverse-transcriptase-polymerase chain reaction
S.E.M	Standard error of mean
SCN	Suprachiasmatic nuclei
SN	Substantia nigra
STZ	Streptozotocin
TB	Tumor bearing
VMH	Ventro medial hypothalamus
VTA/SNc.	Ventral tegmental area/ Substantia nigra compacta

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INTRODUCTION

Diabetes Mellitus is a metabolic disorder associated with insulin deficiency, which not only affects the carbohydrate metabolism but also is associated with various central and peripheral complications. Chronic hyperglycemia during diabetes mellitus is a major initiator of diabetic microvascular complications like retinopathy, neuropathy, nephropathy (Sheetz & King, 2002). Glucose processing uses a variety of diverse metabolic pathways. Chronic hyperglycemia can induce multiple cellular changes leading to metabolic disorders. The central nervous system (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 (Julius, *et al.*, 1989).

Neurotransmitters have been reported to show significant alterations during hyperglycemia resulting in altered functions causing neuronal degeneration (Kaur, *et al.*, 1999). Neurochemical and neuro-imaging evidences have been reported to show regionally selective sympathetic denervation in diabetic neuropathy (Goldstein, *et al.*, 2002). The changes in the brain monoamines during experimental diabetes have been reported. The serotonin content is doubled in the hypothalamus with no apparent alteration of its metabolite 5-hydroxy indole acetic acid (5-HIAA) levels, suggesting a reduced release. In the brain stem, serotonin and dopamine with the relative metabolites 5-HIAA and dihydroxyphenylacetic acid (DOPAC) are significantly reduced whereas noradrenaline is markedly increased (Chen, 1992). Insulin deficiency is the major factor involved as a trigger of the monoaminergic changes in the diabetic brain. Streptozotocin-induced diabetes produced marked alterations of monoamine concentrations in the brain regions of rats (Shimzu, 1991; Chen & Yang, 1991). The effects of streptozotocin (STZ)-induced diabetes on dopamine and serotonin release in striatum revealed that striatal dopamine release increased in acute diabetic state and this release depleted during the chronic state. The progression of diabetes is associated with an impaired ability of

the neurons in the CNS to release neurotransmitters resulting in behavioral changes (Broderick & Jacoby, 1989).

Hypothalamus epinephrine content increased in the suprachiasmatic nuclei but was decreased in the arcuate nucleus while dopamine decreased in the ventromedial nucleus during STZ induced diabetes (Linnoila, *et al.*, 1986). Experimental diabetes resulting in acute insulin deficiency (Onegova, *et al.*, 1980) causes a rapid onset of detectable alterations in epinephrine and dopamine activity in specific hypothalamic nuclei, which may contribute to the development of secondary neuroendocrine abnormalities known to occur in this condition. Insulin treatment partially normalised the altered neurotransmitter levels in streptozotocin induced diabetic rats.

Brain tissues absorb excessive glucose during hyperglycaemia due to abundance in the brain glucose I transporter. The brain interface for glucose transport is at the brain capillary endothelial cells which comprise the blood-brain barrier (BBB). Glucose transport across these barriers is mediated exclusively by the sodium-independent glucose transporter GLUT1. Changes in endothelial glucose transport and GLUT1 abundance in the barriers of the brain have profound consequences on glucose delivery to these tissues and major implications in the development of two major diabetic complications, namely insulin-induced hypoglycemia and diabetic retinopathy. The hyperglycaemic state is accompanied by increased metabolic activity in some specific brain regions and decreased metabolic activity in others. This regional variation is due to the distribution of glucose transporter sites within the brain. Hyperglycaemia is reported to be associated with hyperactivity of dopaminergic system.

Dopamine is a neurotransmitter that has been implicated in various central neuronal degenerative disorders like Parkinson's disease and behavioral diseases like Schizophrenia. Dopamine is synthesised from tyrosine, stored in vesicles in axon terminals and released when the neuron is depolarised. Dopamine interacts with specific membrane receptors to produce its effects. These effects are terminated by re-uptake of dopamine into the presynaptic neuron by a dopamine transporter or by metabolic inactivation by monoamine oxidase B (MAO-B) or catechol-0-methyltransferase

(COMT). Dopamine plays an important role both centrally and peripherally. The recent identification of five dopamine receptor subtypes provides a basis for understanding dopamine's central and peripheral actions. Dopamine receptors are classified into two major groups: DA D_1 like and DA D_2 like. Dopamine D_1 like receptors consists of DA D_1 and DA D_5 receptors. Dopamine D_2 like receptors consists of DA D_2, DA D_3 and DA D_4 receptors. Stimulation of the DA D_1 receptor gives rise to increased production of cAMP. Dopamine D_2 receptors inhibit cAMP production, but activate the inositol phosphate second messenger system. Impairment of central dopamine neurotransmission causes muscle rigidity, hormonal regulation, thought disorder and cocaine addiction. Peripheral dopamine receptors mediate changes in blood flow, glomerular filtration rate, sodium excretion and catecholamine release.

Dopamine itself has a regulatory effect on the synthesis of post-synaptic receptors. Dopamine D_2 receptors decreases when there is an increased dopaminergic transmission, while a decrease in the transmission has an opposite effect. Schizophrenia causes an increased dopamine D_2 receptor synthesis due to dopaminergic blockade by neuroleptics. In Parkinson's disease dopamine deficiency causes an increase in DA D_2 receptors.

Diabetes causes an increase in striatal dopamine with a decrease in its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Lim & Lee, 1995; Saller, 1984; Kwok & Juorio, 1986). The central vagal connection with dopaminergic innervation is reported to reach the pancreatic islets through the parahypothalamic ventricular (PHV) nucleus while adrenergic and serotonergic innervations reach the pancreas through the brain stem (Smith and Davis, 1983, Lowey *et al.*, 1994). Substantia nigra is an important autonomic area involved in controlling islet growth and development. It plays a role in modulating the outflow of both sympathetic and parasympathetic signals which ultimately reach the islets (Smith and Davis, 1983). In obese (ob/ob) diabetic rats activation of dopamine receptors reduce glucose, insulin resistance, obesity and certain lipids associated with cardiovascular risk. A series of studies conducted in ob/ob mice demonstrated that dopamine agonist therapy concurrently reduced elevated levels of chemical messengers in hypothalamus like

corticotropin releasing factor (CRF) and neuropeptide Y (NPY). Dopamine agonist combination treatment normalized the increased circadian organisation of the suprachiasmatic nuclei (SCN) to normal in obese animals. Blood glucose is regulated by the ventromedial hypothalamus (VMH) and changes in SCN temporal organization during diabetes are believed to influence the VMH affecting its function (Cincotta, *et al.*, 1998; 1999). Dopamine agonist treatment was also associated with changes in VMH activity known to improve hyperglycemia (Cincotta, *et al.*, 1998).

The central dopaminergic postsynaptic receptor supersensivity due to decreased dopamine release is reported to decrease locomotor activity in STZ-induced diabetic rats (Kobayashi, *et al.*, 1990). The metabolic abnormalities during diabetes in the striatum cause alterations in dopaminergic neurons by decreasing their firing rate (Saller, 1984; Saller, & Chiodo, 1980). Streptozotocin induced diabetic rats are reported to be associated with decreased ambulatory activity (Shimomura, *et al.*, 1990). Striatal DA D_1 receptors affinity decreased while there was an increase in number of DA D_2 receptors during diabetes (Trulson & Himmel, 1983; Salkovic & Lackovic, 1992).

Streptozotocin induced diabetes in rats is reported to cause peripheral neuropathy. The autonomic and enteric innervation of the gut were denervated and hyperinnervated in the small intestine of diabetic animals (Di Giulio, *et al.*, 1989; Gorio, *et al.*, 1989). It was previously reported that the cholinergic parasympathetic innervation of the intestine was markedly reduced and noradrenergic sympathetic axons hyperinnervate the duodenum of diabetic rats. Also, noradrenaline levels were reported to be high in the duodenum of diabetic rats (Gorio, *et al.*, 1989). The intrinsic serotoninergic innervation was not affected in the gut (Di Giulio, *et al.*, 1989).

Dopamine exerts its inhibitory effect through the DA D_1 receptors in the pancreatic exocrine secretion of conscious rats (Miyasaka, *et al.*, 1998). Pancreatic β-cell secretory granules have the ability to store substantial amounts of calcium, dopamine and serotonin. Dopamine plays an important role in the modulation of the glucose-induced insulin secretion. L-3, 4-dihydroxyphenylalanine (L-DOPA) is rapidly converted to dopamine in islet β-cells. Dopamine accumulation in pancreatic islets is accompanied by

an increase in MAO activity which has an inhibitory effect on glucose-stimulated insulin response (Ahren & Lundquist, 1985). It is suggested that increased hydrogen peroxide production, following increased MAO activity augments the inhibitory effect of dopamine accumulation on insulin release.

The reports so far stated did not attempt to emphasis the functional correlation of dopaminergic receptors during diabetes. In the present study a detailed investigation on the alterations of dopamine and its receptors in the brain and pancreatic islets of streptozotocin induced diabetic rats were carried out. Our studies confirmed the stimulatory role of dopamine through dopamine D_2 receptors in glucose induced insulin secretion.

OBJECTIVES OF THE PRESENT STUDY

1. To study the histological changes in the brain regions - corpus striatum, cerebral cortex and hypothalamus using periodic acid stain.

2. To study the changes in the pancreas of control, diabetic and insulin treated diabetic rats using hematoxylin and eosin stain.

3. To quantify the striatal dopamine and homovanillic acid in streptozotocin induced diabetic rats at different time intervals using High Performance Liquid Chromatography.

4. To assess the change in corpus striatal dopamine and homovanillic acid in rats injected with different doses of streptozotocin using High Performance Liquid Chromatography.

5. To study the changes in dopamine and homovanillic acid content in the brain regions – corpus striatum cerebral cortex, brain stem, hypothalamus -pancreas, adrenals and plasma of control, diabetic and insulin treated diabetic rats using High Performance Liquid Chromatography.

6. To study the dopamine and dopamine D_2 receptor changes in corpus striatum, cerebral cortex, brain stem, hypothalamus and pancreatic islets of control, diabetic and insulin treated diabetic rats.

7. To study the role of dopamine and dopamine D_2 receptors on glucose induced insulin secretion *in- vitro* using rat pancreatic islets.

8. To study the dopamine D_2 receptor gene expression in the brain regions- corpus striatum, cerebral cortex, brain stem and hypothalamus of control, diabetic and insulin treated diabetic rats.

REVIEW OF LITERATURE

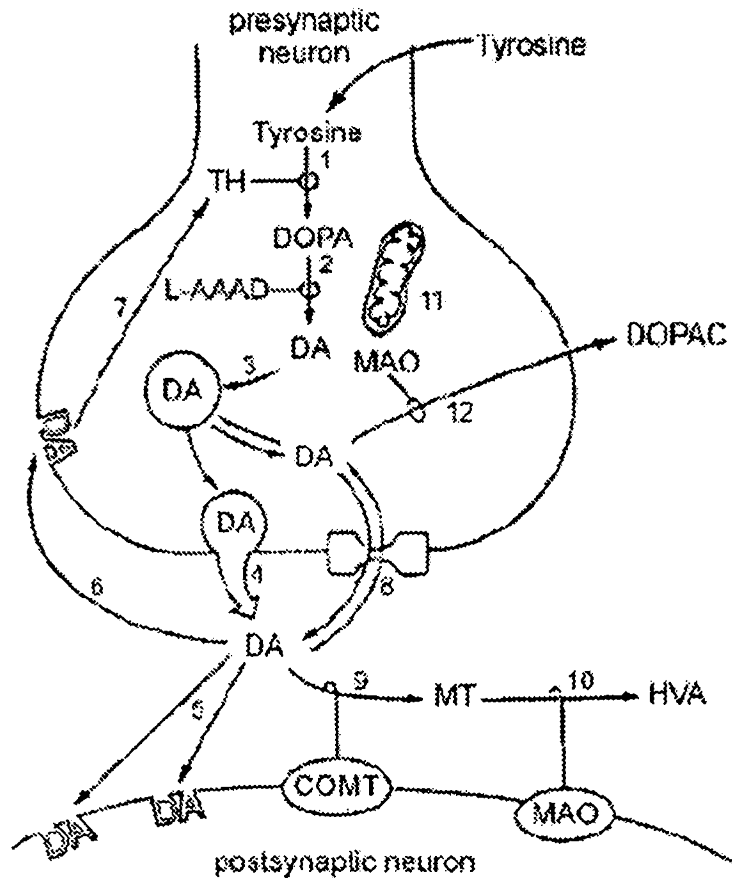
Dopamine, a neurotransmitter in the central nervous system

Dopamine (DA) is a major neurotransmitter within the mammalian central and peripheral nervous system (CNS). Changes in central dopamine neurotransmission are implicated in processes as diverse as muscle rigidity, hormonal regulation, thought disorder and cocaine addiction. Peripheral dopamine mediate changes in blood flow, glomerular filtration rate, sodium excretion and catecholamine release.

Dopamine containing neurons arise mainly from DA cell bodies in the substantia nigra and ventral tegmental area in mid-brain region (Tarazi, *et al.*, 2001; 1996; Creese, *et. al.*, 1997; Royh, *et. al.*, 1991; Carlsson, 1993; Lookingland, *et al.*, 1995). Dopaminergic system is organized into four major subsystems (i) the *nigrostriatal* system involving neurons projecting from the substantia nigra pars compacta to the caudate-putamen of the basal ganglia. This is the major DA system in the brain as it accounts for about 70% of the total DA in the brain, and its degeneration makes a major contribution to the pathophysiology of Parkinson's disease; (ii) the *mesolimbic* system that originates in the midbrain tegmentum and projects to the nucleus accumbens septi and lateral septal nuclei of the basal forebrain as well as the amygdala, hippocampus, and the entorhinal cortex, all of which are considered components of the limbic system and so are of particular interest for the patho-physiology of idiopathic psychiatric disorders; (iii) the *mesocortical* system, which also arises from neuronal cell bodies in the tegmentum which project their axons to the cerebral cortex, particularly the medial prefrontal regions; (iv) the *tuberinfundibular* pathway, which is a neuroendocrinological pathway arising from the arcuate and other nuclei of the hypothalamus and ending in the median eminence of the inferior hypothalamus. DA released in this system exerts regulatory effects in the anterior pituitary and inhibits the release of prolactin. DA is involved in the control of both motor and emotional behavior. Despite the large number of crucial functions it performs, this chemical messenger is found in a relatively small number of brain cells. In

Figure: 1

NEURONAL BIOCHEMICAL PROCESSES INVOLVING DOPAMINE



(1) Conversion of tyrosine to DOPA by tyrosine hydroxylase.
(2) Conversion of DOPA to DA by aromatic L- amino acid decarboxylase.
(3) Pooling of DA in a vesicle.
(4) Exocytosis of a vesicle and DA-release into the synaptic cleft.
(5) Activation of postsynaptic DA-receptors.
(6) Activation of DA-autoreceptors.
(7) Inhibition of tyrosine hydroxylase.
(8) Reuptake of DA by the DA-transporter.
(9) Metabolism of DA: conversion to 3-methoxytyramine by COMT.
(10) Oxidation of MT to homovanillic acid by MAO.
(11) Mitochondrion.
(12) Mitochondrial oxidation of DA to DOPAC by MAO.

fact, while there are a total of 10 billion cells in the cerebral cortex alone, there are only one million dopaminergic cells in the entire brain.

Biosynthesis of dopamine

Dopamine is synthesized from the amino acid L-tyrosine (Fig: 1). L-tyrosine is hydroxylated by the enzyme tyrosine hydroxylase (TH) to give L-3, 4-dihydroxyphenylalanine (L-DOPA) which is the rate limiting step. L-DOPA is subsequently decarboxylated to dopamine by the enzyme aromatic L-amino acid decarboxylase. Therefore, it is not possible to enhance the levels of DA by providing L-tyrosine. The activity of tyrosine hydroxylase is regulated by several endogenous mechanisms. For example, the enzyme is activated by increased neuronal impulse flow, but is inactivated either by DA itself as an end-product inhibitor, or by activation of presynaptic DA receptors. On the other hand, the enzyme aromatic L-amino acid decarboxylase converts L-DOPA to DA instantaneously. Therefore, providing L-DOPA creates a possibility to enhance the formation of DA (Fig: 1).

Dopamine reuptake and metabolism

Dopamine exerts its functions mediated through various receptors and these actions are terminated to prevent continuous stimulation of the receptors. This inactivation is brought about by reuptake mechanisms and metabolism of DA. Reuptake of DA is accomplished by a high affinity carrier present in the membrane, the dopamine transporter (DAT) (Fig: 1). The dopamine transporter recycles extracellular DA by actively pumping it back into the nerve terminal. The dopamine content which is about 70 to 80 % in the striatal synaptic cleft is inactivated by this process. Drugs, such as cocaine, are able to block the action of the dopamine transporter, thereby sustaining the presence of dopamine in the synaptic cleft and its action on dopamine receptors. Part of the dopamine is inactivated by conversion to inactive compounds by metabolic enzymes, which are present both intra- and extraneuronally. Monoamine oxidase (MAO), aldehyde dehydrogenase (AD) and catechol-*O*-methyltransferase (COMT) are responsible for the metabolism of DA. Dopamine after reuptake may intraneuronally be deaminated by MAO to give dihydroxyphenyl acetaldehyde, which subsequently is converted to 3, 4-

Figure: 2

Dopamine D_1 Receptor

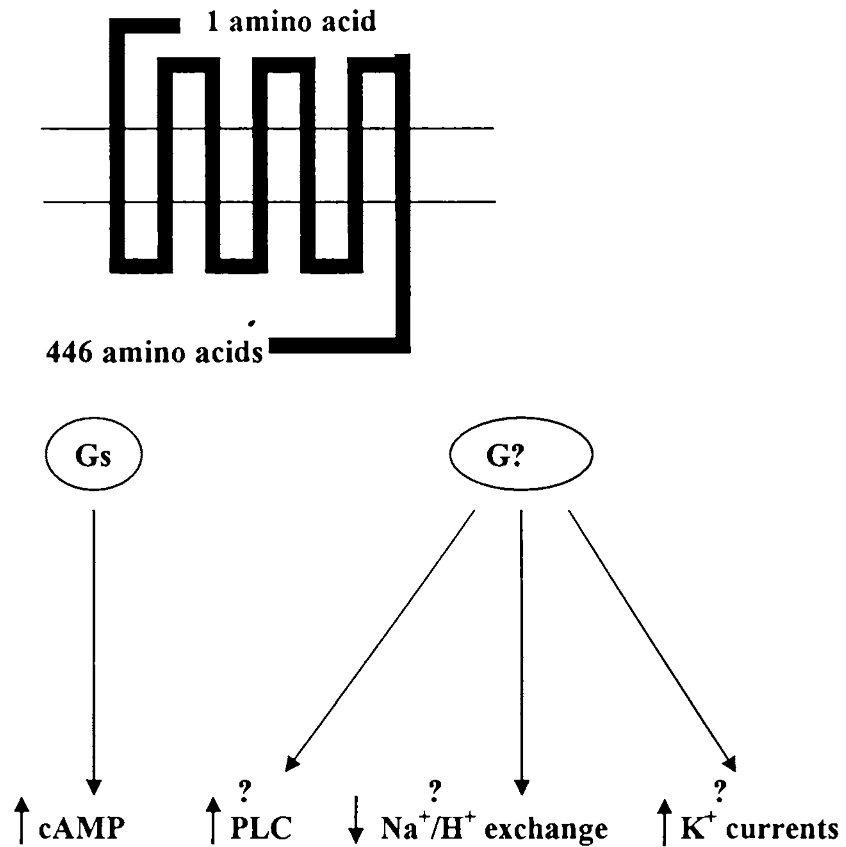


Fig:2 Signalling pathways of D_1 like receptors.

Gs: Stimulatory G proteins α subunits.
G? Unknown or novel G protein α subunits with which the receptor may interact.
cAMP: cyclic Adenosine monophosphate
PLC: Phospholipase C
K^+ Pottassium ions
Na^+ Sodium ions
H^+ Hydrogen ions

dihydroxyphenylacetic acid (DOPAC) by AD. DOPAC is then methylated by COMT to give homovanillic acid (HVA). Extraneuronally, DA is metabolized by an alternative route in which it is first *O*-methylated to 3-methoxytyramine (3-MT) through the action of COMT and subsequently oxidized by MAO and AD to HVA.

Dopamine receptors

Dopamine mediates its actions via membrane receptor proteins. DA receptors are found on postsynaptic neurons in brain regions that are DA-enriched. In addition, they reside presynaptically on DA neuronal cell bodies and dendrites in the midbrain as well as on their terminals in the forebrain. Dopamine receptors belong to a family of large peptides that are coupled to G-proteins which are modified by attached carbohydrate, lipid-ester or phosphate groups. The topologies of the five dopamine receptors are predicted to be the same as all the other G-protein-coupled receptors (Fig: 2 & 3). They are characterized by having seven hydrophobic transmembrane-spanning regions. The third intracytoplasmic loop is functionally critical and interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects (Tarazi, *et al.*, 1996; Creese, *et. al.*, 1997; Royh, *et. al.*, 1991; Carlsson, 1993). In their putative transmembrane domains, the DA D_1 and D_5 receptors are 79% identical to each other, while they are only 40–45% identical to the DA D_2, D_3, and D_4 receptors. Conversely, the DA D_2, D_3, and D_4 receptors are between 75% and 51% identical to each other. They contain seven putative membrane-spanning helices which would form a narrow dihedral hydrophobic cleft surrounded by three extracellular and three intracellular loops. The receptor polypeptides are probably further anchored to the membranes through palmitoylation of a conserved Cys residue found in their carboxy tails, 347 in DA D_1, the C-terminus in DA D_2 like receptors. The dopamine receptors are glycosylated in their N-terminal domains. Dopamine D_1 like subtypes have potential glycosylation sites in their first extra cytoplasmic loop.

Dopamine receptors are divided into two families on the presence or absence of ability of DA to stimulate adenylyl cyclase and produce the second-messenger molecule cyclic-AMP (cAMP) (Calne, 1979; Schwartz, *et al* 1992; Civelli, *et al*, 1993; O'Dowd,

Figure: 3
Dopamine D_2 receptor

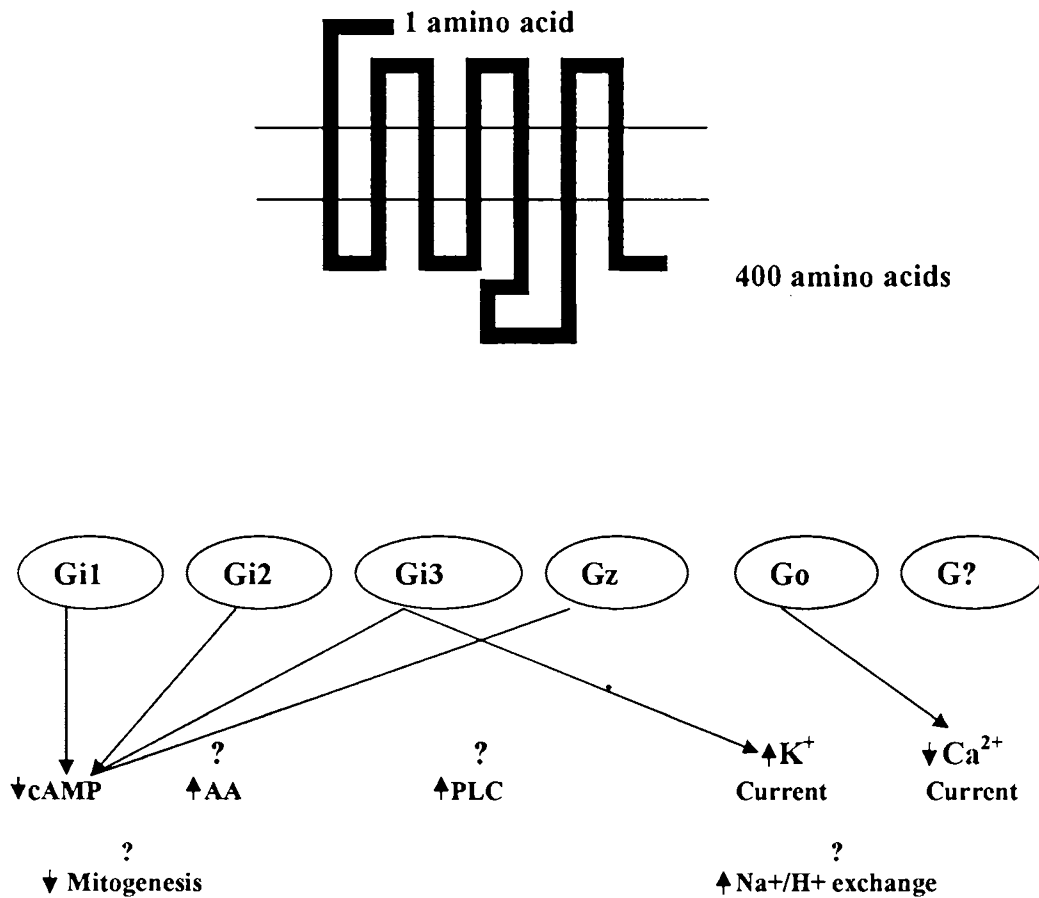


Figure: 3 Signalling pathways of dopamine D_2 receptor.

Gi: Inhibitory G protein α subunits;
Gz, Go, G? Unknown or novel G protein α subunits with which the receptor may interact.
cAMP: cyclic Adenosine monophosphate
AA: Amino Acid
PLC Phospholipase C
K^+ Pottassium ions
Ca^{2+} Calcium ions
Na^+ Sodium ions
H^+ Hydrogen ions

1993; Jackson, *et al.* 1994; Ogawa, 1995; Strange, 1996). This classification is based on similarities in structure, pharmacology, function and distribution. Dopamine D_1 like receptors are characterized initially as mediating the stimulation of cAMP production (Fig: 2). Dopamine D_2 like receptors inhibit the production of cAMP (Fig: 3) This pharmacological characterization is based on the ability of some DA agents to block adenylyl cyclase activity to inhibit the release of prolactin *in vivo* and *in vitro* in a cAMP-independent fashion (Seeman, 1980). Applications of recent technical advances in molecular genetics have greatly facilitated the isolation and characterization of novel DA receptors, DA D_3, D_4 and D_5, with different anatomical localization from traditional DA D_1 or DA D_2 receptors. Based upon their pharmacological profiles, including their effects on different signal transduction cascades, these receptors are currently divided into two families: the DA D_1like family which includes dopamine D_1 and D_5 receptors. The DA D_2 like family includes dopamine D_2, D_3 and D_4 receptors (Grandy, *et al.*, 1993; Shen, *et al.*, 1993; Schwartz, *et al.*, 1995). The genomic organizations of the DA receptors demonstrate that they are derived from the divergence of two gene families that mainly differ in the absence or the presence of introns in their coding sequences. Dopamine D_1 like receptors genes do not contain introns in their coding regions, a characteristic shared with most G protein-coupled receptors. The genes encoding the dopamine D_2 like receptors are interrupted by introns (Marc, *et al.*, 1998). Furthermore, most of the introns in the DA D_2-like receptor genes are located in similar positions.

Dopamine D_1-like family

The dopamine D_1 receptor is the most abundant DA receptor in the central nervous system. The DA D_1-like receptors are characterized by a short third loop as in many receptors coupled to Gs protein (Civelli, *et al.*, 1993; Gingrich & Canon, *et al.*, 1993; O'Dowd, 1993). The dopamine D_1like receptors have short third intracellular loops and long carboxy terminal tails. The DA D_1 like receptors are classified into dopamine D_1 and D_5. In the DA D_1 and D_5 receptor third intracellular loop and the carboxy terminus are similar in size but divergent in their sequence. In contrast, the small cytoplasmic loops 1 and 2 are highly conserved so that any difference in the biology of these receptors can be probably related to the third cytoplasmic loop and the

carboxy terminal tail (Civelli, *et al.*, 1993, Gingrich & Canon, *et al.*, 1993; O'Dowd, 1993). The external loop between transmembrane domain (TM) TM4 and TM5 is considerably different in the two receptor subtypes, being shorter (27 amino acids) in the D_1 receptor than in the D_5 receptor (41 amino acids). The amino acid sequence of this loop is divergent in the dopamine D_5 receptor (Marc, *et al.*, 1998).

Dopamine D_1 receptor

Dopamine D_1 receptors are found at high levels in the typical dopamine regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercles. Dopamine D_1 receptor seems to mediate important actions of dopamine to control movement, cognitive function and cardiovascular function. The dopamine D_1 receptor gene, which lacks any introns, encodes a protein that extends for 446 amino acids (Caron, *et al.*, 1991). In humans dopamine D_1 receptor gene has been localized to chromosome 5 (Kennedy, *et al.*, 1990). The dopamine D_1 receptors show characteristic ability to stimulate adenylyl cyclase and generate inositol 1, 4, 5-trisphosphate (IP_3) and diacylglycerol via the activation of phospholipase C (Sibley, *et al.*, 1990; Monsma, *et al.*, 1990). Dopamine D_1 receptors are highly expressed in basal ganglia followed by cerebral cortex, hypothalamus and thalamus. Dopamine D_1 receptors mRNA is colocalized in striatal neurons of the basal ganglia with mRNA for dopamine receptor phosphor protein (DARPP-32; KD) which is a dopamine and cyclic-AMP-regulated phosphoprotein. Dopamine Receptor Phosphor Protein contributes to the actions of D_1 receptor (Hemmings & Greengard, 1986; Greengard, *et al.*, 1987). The dopamine D_1 receptors in the brain are linked to episodic memory, emotion, and cognition.

Dopamine D_5 receptors

The dopamine D_5 receptor gene is intronless and encodes a protein that extends for 477 amino acids (George, *et al.*, 1991). This protein has an overall 50% homology with DA D_1 receptor and 80% if only the seven transmembrane segments are considered. The gene encoding the human dopamine D_5 protein is located at the short arm of chromosome 4, the same region where the Huntington disease gene has been located (Gusella, 1989). Two dopamine D_5 receptor pseudogenes having 154 amino acids have

been identified with 90% homology (Gusella, 1989). These pseudogenes, however, contain stop codons in their coding regions that prevent them from expressing functional receptors. The functions of these pseudogenes, which appear so far to be specific to humans, are not yet known (Allen, *et al.*, 1991).

Dopamine D_5 receptor mRNA expression is unique and limited to the hippocampus and parafascicular nucleus of the thalamus (Civelli, *et al.*, 1992). It is involved in the thalamic processing of painful stimuli (Basbaum, *et al.*, 1979). Dopamine D_5 receptors, like DA D_1 receptors, appear to interact with G-proteins and can stimulate adenylyl cyclase, with relatively high affinity for DA and DA D_1-selective agonists (George, *et al.*, 1991).

Dopamine D_2 like family

Dopamine D_2 like receptors belong to the G-protein coupled receptors and has 400 amino acid residues. Dopamine D_2-like receptors are characterized by a long extracellular amino terminus which has several glycosylation sites and a shorter carboxy terminal tail with putative phosphorylation sites. The function of sugar moieties is unclear (Marc, *et al.*, 1998; Sibley, 1999). It is generally believed that the membrane enclosed part of the amino-acid chain of G-protein coupled receptors is folded into seven α-helices. The transmembrane helices consist primarily of hydrophobic amino-acid residues. The unique feature of DA D_2 like receptors family is that they posses a bigger third cytoplasmic (intracellular) loop in common, which is thought to be the site where the G-protein couples (Marc, *et al.*, 1998). Between the different dopamine receptors, the third loop also displays the greatest variability in amino-acid sequence. This may have consequences for their respective second messenger systems. The DA D_2-like receptors are coupled to Gi-protein and inhibit the formation of cyclic AMP. The DA D_2 receptors tertiary structure is stabilized by two cysteine disulphide bridges.

Dopamine D_2 receptors

The DA D_2 receptor gene encodes a protein that extends for 415 amino acids. Similar to other G-protein coupled receptors, the DA D_2 receptor has seven

transmembrane segments, but in contrast to DA D_1-like receptors, the third cytoplasmic domain is long and the carboxy terminus is short. Unlike the DA D_1-like receptor genes, the DA D_2 receptor gene contains seven introns that are spliced out during mRNA transcription (Fischer, *et al.*, 1989). The gene encoding this receptor was found to reside on q22-q23 of human chromosome 11 (Makam, *et al.*, 1989). The dopamine D_2 receptor was the first receptor to be cloned (Chrisre, *et al.*, 1988). The dopamine D_2 receptors are involved in several signal transduction cascades, including inhibition of cAMP production (Vallar & Meldolesi, 1989), inhibition of phosphoinositide turnover (Epelbaum J, *et al.*, 1986), activation of potassium channels and potentiation of arachidonic acid release (Axelrod, *et al.*, 1991). The dopamine D_2 receptors are highly expressed in basal ganglia, nucleus accumbens septi and ventral tegmental area (Schwartz, *et al.*, 1991).

The dopamine D_2 receptor exists as two alternatively spliced isoforms differing in the insertion of a stretch of 29 amino acids in the third intracellular loop and are designated as dopamine D_{2S} and dopamine D_{2L} (Seeburg, *et al.*,1989; Marc, *et al.*, 1998). Because this loop seems to play a central role in receptor coupling, the existence of a splicing mechanism at this level could imply functional diversity. However, in spite of the efforts of several groups, no obvious differences have emerged so far between the two DA D_2 receptor isoforms. The two isoforms derived from the same gene by alternative RNA splicing which occurs during the maturation of the DA D_2 receptor pre-mRNA (Schwartz, *et al.*, 1989). Dopamine D_2 receptor isoforms (DA D_{2L} and DA D_{2S}) vary within each species by the presence or absence of a 29-amino acid sequence in the third cytoplasmic domain of the DA D_2 receptor peptide chain. Both variants share the same distribution pattern; with the shorter form less abundantly transcribed in addition they appear to differ in their mode of regulation (Marc, *et al.*, 1998). Pharmacologically, both isoforms exhibit nearly similar profiles in terms of their affinities to different DA D_2-selective agents, and inhibit adenylyl cyclase activity. However, these isoforms display an opposite regulatory effect (Sibley, *et al.*, 1994). These isoforms have the same pharmacological profile, even though a marginal difference in the affinity of some substituted response to dopamine treatment is reported: Dopamine induces the up-regulation of DA D_{2L} isoform of dopamine D_2 receptors (Mohell, *et al.*, 1993; Castro,

&Strange, 1993). When expressed in host cell lines, both isoforms inhibited adenylyl cyclase (Marc, *et al.*, 1998; Sibley, 1999). However, the DA D_{2S} receptor isoform displayed higher affinity than the DA D_{2L} in this effect (Seeburg, *et al.*, 1989, Marc, *et al.*, 1998). The isoforms of DA D_2 mediate a phosphatidylinositol-linked mobilization of intracellular calcium in mouse Ltk [-] fibroblasts. Protein kinase C (PKC), however, differentially modulates dopamine D_{2S} and D_{2L}-activated transmembrane signalling in this system with a selective inhibitory effect on the dopamine D_{2S}-mediated response.

Dopamine D_3 receptors

Dopamine D_3 receptor gene contains five introns and encodes a 446 amino acid protein (Schwartz, *et al.*, 1990). The gene encoding this receptor resides on chromosome 3 (Giros, *et al.*, 1990). The DA D_3 receptors bear close structural and pharmacological similarities to the DA D_2 receptors. Dopamine D_3 mRNA occurs in longer and shorter spliced forms generated from the same gene (Schwartz, *et al.*, 1991). Distribution of dopamine D_3 receptor mRNA are distributed and expressed mainly in subcortical limbic regions including islands of Calleja, nucleus accumbens septi and olfactory tubercle, with low levels of expression in the basal ganglia (Marc, *et al.*, 1998). D_3 receptor mRNA has also been found in neurons of the cerebellum, which may regulate eye-movements (Lévesque, *et al.*, 1992). The status of the DA D_3 molecular entity as a functional receptor remains uncertain since it neither couples to G-proteins nor consistently transduces an effector mechanism (Schwartz, *et al.*, 1990; Sokoloff, *et al.*, 1992; Marc, *et al.*, 1998). However, the structural similarity with DA D_2 receptor raises the possibility that DA D_3 receptor may also inhibit adenylyl cyclase activity in its normal cellular setting. More recent studies reported that DA D_3 receptors might mediate positive regulatory influences of DA on production of the peptide neurotensin (Lévesque, *et al.*, 1995; Marc, *et al.*, 1998).

Dopamine D_4 receptors

Dopamine D_4 receptor gene contains four introns and encodes a 387 amino acid protein (Van Tol *et al.*, 1991). The overall homology of the DA D_4 receptor to the DA D_2 and D_3 receptors is about 41% and 39% respectively, but this homology increases to

56% for both receptors when only the transmembrane spanning segments are considered. The gene encoding the human DA D_4 protein is located at the tip of the short arm of chromosome 11 (Civelli, *et al.*, 1992; Marc, *et al.*, 1998). Dopamine D_4 receptor gene has been localized in brain regions like hippocampus and frontal cortex using specific histoprobes (Civelli, *et al.*, 1994). The stimulation of DA D_4 receptor inhibits adenylyl cyclase activity and release arachidonic acid in brain neurons (Huff, *et al.*, 1994, Marc, *et al.*, 1998). In humans, dopamine D_4 receptor occurs in several genomic polymorphic variants that contain two to eleven repeats of a 48 base pair segment that is expressed in the third cytoplasmic domain (Van Tol, *et al.*, 1992; Marc, *et al.*, 1998). These are called the dopamine D_4 alleles which are represented as DA $D_{4.2}$, $D_{4.4}$ and $D_{4.7}$. These may contribute to the pathophysiology of certain neuropsychiatric disorders (Jackson & Westlind, 1994).

Effect of glucose on brain dopamine and its receptors

Dopamine has two distinct pathways that connect the striatum to the basal ganglia output nuclei - a direct pathway originating from neurons bearing dopamine DA D_1 receptors and an indirect pathway originating from neurons expressing DA D_2 receptors. Intrastriatal injection of selective DA D_1, DA D_2 or general dopamine agonists, in freely-moving rats reduced glucose utilization (Orzi, *et al.*, 2001). Glucose modulates substantia nigra (SN) dopamine (DA) neuronal activity and its release by acting on an ATP-sensitive potassium channel (K_{ATP}) (Levin, 2000). Changing SN glucose levels is reported to affect activities of (K_{ATP}) channel and dopamine neurons. Glucose modulates the motor activity involved in food intake. In experimental rats food deprivation cause a decrease in the activity of striatal dopamine transporters (DAT) (Figlewicz, *et al.*, 1998). Dopamine D_1 receptor binding significantly increased in the accumbens and DA D_2 binding decreased in the dorsal striatum as a result of excessive intake of sugar because palatable food stimulates the neural system (Hoebel, *et al.*, 2001). Dopamine antagonists are reported to effectively modulate brain energy metabolism and release of dopamine thus effecting cerebral glucose utilisation (Piercey *et al.*, 1999). Stimulation or blockade of DA D_3 receptors in cerebral cortex alters local glucose utilsation producing a unique pattern suggestive of potential antipsychotic activity (Levant, *et al.*, 1998).

Insulin and Dopamine

Insulin is involved in glucose disposal into skeletal muscles, inhibition of hepatic glucose production and inhibition of lipolysis in adipocytes. Intracerebroventricular infusion of insulin results in an increase in mRNA levels for the DA reuptake transporter (Figlewicz, *et al.*, 1998). Dopamine analogues are reported to inhibit glucose-stimulated insulin release from the endocrine pancreas (Fischer, *et al.*, 1984). Islets cells have been shown to contain the putative enzymes that synthesis dopamine like tyrosine hydroxylase and di-hydroxy phenylalanine decarboxylase (Gagliardino & Borelia, 2001). Dopamine and increased glucose stimulus reduced the insulin release from the pancreatic islets with no change in calcium efflux (Carpinelli, *et al.*, 1994). Acute L-DOPA-induced dopamine accumulation in pancreatic islets is reported to cause an inhibitory effect on glucose-stimulated insulin response resulting in an increased MAO activity (Lundquist, *et al.*, 1991; Lundquist, 1985).

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. In diabetic individuals the regulation of glucose levels by insulin is defective, either due to defective insulin production which is called as Insulin Dependent Diabetes Mellitus (IDDM) or due to insulin resistance that is termed as Non Insulin Dependent Diabetes Mellitus (NIDDM).

Diabetes mellitus has been reported to cause degenerative changes in neurons of the central nervous system (Bhattacharya & Saraswathi, 1991; Garris, 1990; Lackovic, *et al*, 1990). The concentration of 5-HT, DA 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.*, 1990). 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. Strepozotocin -induced diabetes and acute deficiency of insulin is reported to result in increased concentrations of EPI in the supra chiasmatic nucleus. 5-hydroxy tryptamine content in the brain is reported to be decreased during diabetes to be decreased (Jackson & Paulose, 1999; Chu, *et al.*, 1986; Sumiyoshi, *et al.*, 1997; Thorre, *et al.*, 1997). Garris (1995) had 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, *et al.*, 1994).

Norepinephrine, DA and 5-HIAA are reported to be increased in the heart and adrenal gland in STZ rats. In the heart the initial changes in short-term diabetes included an increase in NE concentration but did not persist in the longer term diabetic animals. In the adrenal gland there was an initial reduction followed by a steady increase in the concentration of NE and EPI (Sheen, *et al.*, 2001).

Dopamine and its receptor alterations during diabetes

Dopamine is implicated in diabetes. Hyperglycemia in rats is reported to decrease dopaminergic activity in the striata suggesting the up-regulation of dopamine receptors possibly due to the decreased dopamine metabolism (Ho, *et al.*, 1994). In experimental diabetes and insulin deficiency there is a rapid onset of detectable alterations in hypothalamic DA activity leading to secondary neuroendocrine abnormalities. Lim, *et al.*, (1995) have described an increase in the striatal dopamine and decrease in its metabolites dihydroxyphenylacetic acid and homovanillic acid. Tyrosine hydroxylase is reported to be depleted in nigrostriatal neurons in the genetically diabetic rat causing

marked reduction mesolimbic dopamine system. Insulin treatment could not restore the decaresed DA to controlled conditions, impairing the dopamine biosynthesis (Kamei & Saitoh, 1994). Dopamine uptake affinity and velocity in synaptosomes is decreased significantly during diabetes. The dopamine content was increased in cerebral cortex and hypothalamus of diabetic rats (Chen & Yang, 1991; Ohtani, *et al.*, 1997; Tassava, *et al.*, 1992; Shimizu, 1991). Diabetes is reported to cause increased dopamine release with altered turnover ratio of dopamine metabolites from the mesolimbic systems. This resulted in the enhanced spontaneous locomotor activity which is suggested to be due to the up regulation of δ-opioid receptor-mediated functions (Kamei, *et al.*, 1994). The decrease in striatal dopamine transporter mRNA in experimental diabetes is suggested to a possible cause for the disturbance in dopamine metabolism (Figlewicz, *et al.*, 1996). The dopamine turnover ratio in the limbic forebrain and midbrain in diabetic mice were significantly greater than those in non-diabetic mice (Kamei & Saitoh, 1996). Yawning behaviour in streptozotocin induced diabetes was significantly lowered when compared with their age-matched normal controls as a result of altered dopamine metabolism and decreased turnover to its metabolites (Heaton & Varrin, 1993).

Dopamine receptors are reported to be increased in diabetes causing significant alterations in central dopaminergic system (Lozovsky, *et al.*, 1981). Dopamine D_2 receptor density has been reported to be increased in the striatum of diabetic rats (Lozovsky, *et al.*, 1981; Trulson & Hummel, 1983; Serri *et al.*, 1985). Intracerebroventricular application of alloxan and streptozotocin in rat striatum is reported to have caused an alteration in dopamine receptors and increased dopamine content which had a similar effect to peripheral, diabetogenic administration of these drugs (Salkovic, *et al.*, 1995). The affinity of striatal DA D_1 receptors was significantly increased without changes in the number of binding sites, while the binding of dopamine D_2 receptors was significantly increased without affecting its affinity in the diabetic rats (Ho, *et al.*, 1994). Dopamine D_1 receptors are reported to decrease in hyporesponsiveness (Kamei, *et al.*, 1998). The increase in the central dopaminergic postsynaptic receptors has been related to decrease the locomotor and ambulatory activity in STZ-induced diabetic rats (Kobayashi, *et al.*, 1990; Shimomura, *et al.*, 1990).

Diabetes mellitus causes a condition called as neurocytoglucopenia where the increased glucose results in an increased sympathetic outflow into the liver, pancreas, adrenal medulla, adipose tissue and the circulation. This causes an increased hepatic glucose production, inhibition of insulin secretion and free fatty acid mobilization from the adipose tissue (Oliveira, *et al.*, 1998). Participation of dopaminergic tone in the control of insulin secretion and hyperglycemia has been given little focus. These studies recently shown that dopamine agonists play an important role in lowering the elevated shift in the sympathetic tone as a result of increased glucose levels and stimulate the parasympathetic tone which increases the insulin response (Oliveira, *et al.*, 1998).

Factors affecting insulin regulation from pancreatic β-cells

D-Glucose is the major physiological stimulus for insulin secretion. The mechanism of glucose induced insulin release is not completely understood. Phosphorylation of glucose to glucose-6-phosphate serves as the rate limiting step in glucose oxidation (Schuit, 1996). Glucokinase acts as a glucose sensor during this process. Glucokinase is also linked to the phosphate potential, [ATP]/([ADP] [Pi]) (Sweet *et al.*, 1996). An increased ATP/ADP ratio is believed to close K^+-ATP channel at the plasma membrane, resulting in decreased K^+ efflux and subsequent depolarisation of the β-cell (Dunne, 1991). Depolarisation activates voltage-dependent Ca^{2+} channels, causing an influx of extracellular Ca^{2+} (Liu, *et al.*, 1996). Although intracellular Ca^{2+} activates protein kinases such as Ca^{2+} and calmodulin dependent protein kinase (Breen & Aschcroft, 1997), it remains unclear how increase in intracellular Ca^{2+} leads to insulin release. Intracellular Ca^{2+}stores appear to regulate a novel plasma membrane current [Ca^{2+} release activated non-selective cation current, I_{CRAN}], whose activity may control glucose activated secretion. Lesions in these pathways lead to the pathogenesis of diabetes mellitus (Dukes, *et al.*, 1997). Glucose induced insulin secretion is also partly dependent upon the activation of typical isoforms of protein kinase C (PKC) within the β-cell (Harris, *et al.*, 1996). It is suggested that PKC may be tonically active and effective in the maintenance of the phosphorylated state of the voltage-gated L-type Ca^{2+} channel, enabling an appropriate function of this channel in the insulin secretory process (Arkhammar *et al.*, 1994).

channel, enabling an appropriate function of this channel in the insulin secretory process (Arkhammar *et al.*, 1994).

Amino acids

Amino acids also act as potent stimulators of insulin release. L-Tryptophan which is the precursor of 5-HT can act as a stimulator of insulin release (Bird, *et al.*, 1980). L-Arginine also causes insulin release from pancreatic β-cells. Several *in vitro* studies have suggested the production of nitric oxide from islets. Nitric oxide system may have a negative regulation on the L-arginine induced secretion of insulin and glucagon in mice.

Fatty acids

Short chain fatty acids and their derivatives are highly active stimulators of insulin release in sheep (Horino, *et al.*, 1968). A novel ester of succinic acid, 1, 2, 3-tri-(methyl-succinyl) glycerol ester displayed stimulation of insulin release and biosynthetic activity in pancreatic islets of Goto-Kakizaki rats (Laghmich, *et al.*, 1997). A monomethyl ester of succinic acid along with D-glucose is required to maintain the β-cell response to D-glucose (Fernandez, *et al.*, 1996).

Substrates derived from nutrients

Pyruvate, citrate, ATP, NADH and NADPH are derived from nutrients that are involved in the intake or local islet stimulation (Lisa, *et al.*, 1994; Tahani, 1979; Iain, *et al.*, 1994). Adenosine diphosphate acts as an intracellular regulator of insulin secretion. Mg^{+}-ADP is required for the stimulation of K^{+}-ATP channels in intact β-cells. There are other intracellular factors such as arachidonate, guanine nucleotides, small monomeric GTP-binding proteins such as rab 3A (Regazzi, *et al.*, 1996) and the heterotrimeric GTP-binding protein $G_{\alpha i}$ are involved in regulating glucose induced insulin release (Konrad, *et al.*, 1995). GTP analogues are also important regulators of insulin secretion (Lucia, *et al.*, 1987). Glucose induced insulin secretion is accompanied by an increase in the islet content of cAMP (Rabinovitch, *et al.*, 1976).

Glucagon

Glucagon is the hormone secreted by pancreatic α-cells. It has been shown that glucagon has a striking stimulation of insulin release in the absence of glucose (Sevi & Lillia, 1966). The presence of specific glucagon receptors on isolated rat pancreatic β-cells and subpopulation of α- and δ-cells shows the relevance of glucagon on regulation of insulin secretion (Kiefer, 1996). Intra-islet glucagon appears to be a paracrine regulator of cAMP *in vitro* (Schuit, 1996). Glucagon stimulates insulin release by elevating cAMP. The cAMP through activation of protein kinase A, increases Ca^{2+} influx through voltage dependent L-type Ca^{2+} channels, thereby elevating $[Ca^{2+}]i$ and accelerating exocytosis (Carina, *et al.*, 1993). Protein phosphorylation by Ca^{2+}/Calmodulin and cAMP dependent protein kinase play a positive role in insulin granule movement which results in potentiation of insulin release from the pancreatic β-cell (Hisatomi, *et al.*, 1996).

Somatostatin

Somatostatin is secreted by the pancreatic δ-cells of the islets of Langerhans. Somatostatin inhibits insulin release (Ahren, *et al.*, 1981). Its action is dependent on the activation of G-proteins but not associated with the inhibition of the voltage dependent Ca^{2+} currents or adenylyl cyclase activity (Renstrom, *et al.*, 1996).

Epinephrine and norepinephrine

Epinephrine and norepinephrine are secreted by the adrenal medulla. Norepinephrine is the principal neurotransmitter of sympathetic nervous system. Epinephrine and norepinephrine inhibit insulin secretion, both *in vivo* and *in vitro* (Renstrom, *et al.*, 1996; Porte, 1967). Epinephrine and norepinephrine exerts opposite effects on peripheral glucose disposal and glucose stimulated insulin secretion (Avogaro, *et al.*, 1996).

Pancreastatin

Pancreastatin is known to be produced in islet β-cells and inhibits insulin secretion. Pancreastatin is a modulator of the early changes in insulin secretion after increase of glucose concentration within the physiological range (Ahren, *et al.*, 1996). Pancreastatin is reported to increase Ca^{2+} in insulin secreting RINm5F cells, independent of extracellular calcium (Sanchez, *et al.*, 1992).

Amylin

Amylin is a 37 amino acid peptide hormone co-secreted with insulin from pancreatic β-cells. Amylin appears to control plasma glucose via several mechanisms that reduce the rate of glucose appearance in the plasma. Amylin is absolutely or relatively deficient in type-I diabetes and in insulin requiring type-II diabetes (Young, 1997). Islet amyloid polypeptide (IAPP) or amylin inhibits insulin secretion via an autocrine effect within pancreatic islets. Amylin fibril formation in the pancreas may cause islet cell dysfunction and cell death in Type-II Diabetes mellitus (Alfredo, *et al.*, 1994).

Adrenomedullin

Adrenomedullin is a novel hypotensive adrenal polypeptide isolated from a human pheochromocytoma and is structurally related to calcitonin gene related peptide and islet amyloid polypeptide. It has been suggested that besides being an adrenal hypotensive peptide, adrenomedullin may be a gut hormone with potential insulinotropic function (Mulder, *et al.*, 1996).

Galanin

Galanin is a 29 amino acid neuropeptide localised in the intrinsic nervous system of the entire gastrointestinal tract and the pancreas of man and several animal species (Scheurink, *et al.*, 1992). Galanin inhibits insulin release (Ahren, *et al.*, 1991), probably

via activation of G-proteins by the mediation of activated galanin receptors. However, galanin receptors are not as effective as α_2-adrenergic receptors in activating G-proteins (Renstrom, *et al.*, 1996).

Macrophage migration inhibitory factor

Macrophage migration inhibitory factor (MIF) is as cytokines secreted by T lymphocytes. It wasfound recently to be both a pituitary hormone and a mediator released by immune cells in response to glucocorticoid stimulation. It has been demonstrated that insulin secreting β-cells of the islets of Langerhans expresses MIF and its production is regulated by glucose in a time and concentration dependent manner. MIF and insulin co-localise within the secretory granules of the pancreatic β-cells and once released, MIF appears to regulate insulin release in an autocrine fashion. MIF is therefore a glucose dependent islet cell product that regulates insulin secretion in a positive manner and may play an important role in carbohydrate metabolism (Waeber, *et al.*, 1997).

Other agents

Coenzyme Q_{10} improved insulin release and it may also have a blood glucose lowering effect (Conget, *et al.*, 1996). Inositol hexa bisphosphate stimulates non Ca^{-} mediated and purine-Ca^{2+} mediated exocytosis of insulin by activation of protein kinase C (Efanov, *et al.*, 1997). Insulin secretion and release in rats and hamsters are also reported to be controlled by small GTP-ases of the rab 3A family expressed in insulin secreting cell lines (Regazzi, *et al.*, 1996).

ROLE OF NEUROTRANSMITTERS IN INSULIN REGULATION

Epinephrine and Norepinephrine

Epinephrine and norepinephrine has an antagonistic effect on insulin secretion and glucose uptake (Renstrom, *et al.*, 1996; Porte, 1967). They also inhibit insulin - stimulated glycogenesis through inactivation of glycogen synthase and activation of

phosphorylase with consequent accumulation of glucose-6-.phosphate. In addition, it has been reported that epinephrine enhances glycolysis through an increased activation of phosphofructokinase.

The adrenergic receptors are seven-pass transmembrane receptors that are coupled to G-proteins. Adrenergic receptors are mainly classified into α_1, α_2 and β-adrenergic receptors. α_1 adrenergic receptor has three subclasses- α_{1A}, α_{1B}, α_{1C} (Price, *et al.*, 1994) and α_2 has $\alpha_{2A/D}$, α_{2B} and α_{2C} (Hamamdzic, *et al.*, 1995). β-adrenergic receptors are subclassified into β_1, β_2 and β_3 (Dohlman, *et al.*, 1991). Epinephrine and NE bind to these receptors in a concentration dependent manner. Epinephrine and NE at low concentrations can bind and activate β-adrenergic receptors which in turn stimulate the insulin secretion from pancreatic islets and at high concentration they can bind to α_{2A} receptors and inhibit insulin secretion (Lacey *et al.*, 1993). Previous studies had shown that in diabetic condition α_{2A} receptors are more activated which brought out the insulin inhibition and in turn hyperglycaemia (Lacey, *et al.*, 1993). Rat islet cell membrane is equipped with α_{2A}-adrenoceptors (Filipponi, *et al.*, 1986) which are linked to adenylyl cyclase inhibiting insulin secretion. β_3 adrenoreceptors stimulation also results in enhanced insulin secretion (Alef, *et al.*, 1996).

Acetylcholine

Acetylcholine is the neurotransmitter of the parasympathetic system. Cholinergic receptors are classified as ionotropic nicotinic receptor and metabotropic muscarinic receptor. Acetylcholine increases insulin secretion through muscarinic receptors in pancreatic islet cells (Tassava, *et al.*, 1992; Greenberg & Pokol, 1994). Muscarinic receptors are classified as M_1, M_2, M_3, M_4 and M_5. They are G protein coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions that interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects. Expression studies have revealed the presence of M_1 and M_3 receptors in the pancreas. Acetylcholine is reported to be involved in the activation of glucose transport in the chromaffin cells. The cholinergic activation affecting this process is coupled with calmodulin and protein kinase C (Skar, *et al.*, 2002).

γ-Aminobutyric acid

Gamma aminobutyric acid (GABA) is the major inhibitory neurotransmitter in central nervous system. GABA is reported to be present in the endocrine pancreas at concentrations comparable with those found in central nervous system. The highest concentration of GABA within the pancreatic islet is confined to β-cells (Sorenson, *et al.*, 1991). 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, *et al.*, 1995). GABA which is present in the cytoplasm and in synaptic-like microvesicles is co-released with insulin from β-cells in response to glucose (Reetz, *et al.*, 1991). GABA inhibits islet α and δ-cell hormonal secretion in a paracrine manner. GABA release is decreased in diabetes resulting in the enhancement of glucagon secretion from α-cells leading to hyperglycaemia. GABA is involved in the maintenance of glucose homeostasis and inhibition of central $GABA_A$ receptors increasing the plasma glucose concentration (Lang, 1995). Thus, any impairment in the GABAergic mechanism in central nervous system and/or pancreatic islets is important in the pathogenesis of diabetes.

Serotonin

Brain serotonin content decreased during diabetes (Jackson & Paulose, 1999). This decrease is reported to be due to a decrease in uptake of tryptophan through the blood brain barrier (BBB) (Madras, *et al.*, 1974; Fernstrom & Wurtman, 1972; Fernstrom & Wurtman, 1971) and a decrease in rate of 5-HT synthesis (Carndall, *et al.*, 1981). The turnover rate of 5-HT to 5-HIAA in diabetic rats was also reported to be lower (Sandrini, *et al.*, 1997; Kwok & Juorio, 1987). A decrease in brain 5-HT will lead to an up regulation of $5\text{-}HT_{2A}$ receptors of cerebral cortex and brain stem which in turn can inhibit insulin secretion due to increased sympathetic activity (Jackson & Paulose, 1999).

Central Nervous System regulation of pancreatic insulin secretion

The pancreas has innervations of nerves from the central nervous system. Three types of nerve endings are reported with in the pancreas. They are the sympathetic, parasympathetic and peptidergic nerves. The neurotransmitters found in these nerves are catecholamines, serotonin, acetylcholine and vascoactive intestinal polypeptides and cholecystokinin respectively. The nerve fibres enter the pancreas in association with the vascular supply. Adrenergic fibres innervate vessels, acini and islets whereas cholinergic nerves are found in the islets alone (Miller, 1981). The peptidergic nerves are present in both the exocrine and endocrine tissues of this gland and there is considerable interspecies variability as to which part receives a greater proportion of these fibres (Bishop, *et al.*, 1980). The nerve terminals end approximately 20-30nm from the endocrine cells thus implying that neurotransmitters affect several cells by diffusing through the extracellular space (Miller, 1981). Acetylcholine infusion or *in vivo* stimulation of the vagus nerve increases insulin secretion from the pancreatic islets (Kaneto *et al.*, 1981). Vagotomy often has a little effect on the basal hormone secretion, but effect the release of hormones (Helman, *et al.*, 1982). Intact nerve supply to the pancreas is supposed to be necessary for the islet growth and development (Smith and Davis 1983).

The vagus and splanchnic nerves travel via the pancreas and supply autonomic signals (Helman, *et al.*, 1982). These nerves are related to the ventral hypothalamus which plays a pivotal role in the integration of neurohormonal functions (Oommura & Yoshimatsu, 1984). The ventro-medial hypothalamic nuclei are considered as the sympathetic centre and the stimulation of this area decreases insulin secretion (Helman, *et al.*, 1982). Lesions in the ventro-medial hypothalamus resulted in behaviour alterations and morphological changes in the pancreatic islets (Sclafani, 1981). Ventro-lateral hypothalamus is the parasympathetic centre, stimulation of which increases the circulating level of insulin (Helman, 1982). Lesions in the ventral lateral hypothalamus resulted in decreased body weight, food intake plasma levels and decrease in islet size (Powley & Opsahl, 1976).

The substantia nigra (SN) is one autonomic area in the central nervous system which plays an important role in controlling structure and activity of pancreatic islets. Lesions in the substantia nigra not only resulted in reduced size and number of islets cell populations but also decreased the content of insulin and glucagon in the pancreas (Smith and Davis 1983). Studies have focused on the existence of pathways between the SN and intermediolateral cells in the spinal cord and between the SN and hypothalamic paraventricular nucleus (Schmidt, *et al.*, 1982). The hypothalamic paraventricular nucleus has direct connections with the dorsal vagal complex (Wright, *et al.*, 1980; Swanson & Sawchenko, 1980). These reports underlined the role of SN in modulating the outflow of both sympathetic and parasympathetic signals that ultimately reach the pancreas.

The central vagal connection with dopaminergic innervation is reported to reach the pancreatic islets through the parahypothalamic ventricular (PHV) nucleus while adrenergic and serotonergic innervations reach the pancreas through the brain stem (Smith and Davis 1983; Lowey *et al.*, 1994).

Effect of dopamine on blood glucose levels

Dopamine and its agonists have been reported to affect the blood glucose levels. Increase in glucose level has been suggested to be due to sympathoadernal activation. Plasma glucose levels are reported to be under separate serotonergic and dopaminergic control exerted via 5-HT $_{1A}$ and DA D_3 receptors respectively (Hillegaart, *et al.*, 1996). Dopamine D_3 receptor agonist, 7-OH DPAT, injection caused an increase in blood glucose level and decreased plasma insulin content showing the involvement of this receptor in glucose homeostasis. Evidences show that DA D_2 receptor-mediated increase in plasma glucose is via sympathoadrenal activation (Saller & Kreamer, 1991). Dopamine analogues like lergotrile, pergolide, bromocriptine, d-amphetamine and apomorphine when injected has reported to cause hyperglycemia in rats (Fischer, *et al.*, 1984). In contrary obese diabetic rats treated with a combination of dopaminergic receptor agonists SKF/38393 and bromocriptine (BC) is reported to reduce hyperglycemia (Cincotta, *et al.*, 1999).

Pancreatic dopamine and insulin secretion

The role and the peripheral mechanism of action of central dopamine on basal pancreatic exocrine secretion in conscious rats revealed that central dopamine inhibited pancreatic exocrine secretion via DA D_1like receptors and that the inhibitory effect is mediated via sympathetic nerves, especially α-adrenoceptors (Miyasaka, *et al.*, 1998). Presence of dopamine is reported in peripheral tissues (Hakanson, *et al.*, 1989). Dihydroxy phenyl acetic acid decarboxylase (DDC), dopamine β hydroxylase (DBH) and aromatic L-amino decarboxylase (AAD) are present in endocrine cells of adult rats (Takayanagi, Watanabe, 1996; Gagliardino, *et al.* 1997; Yamada, *et al.*, 1999; Kampe, *et al.*, 1995). As dihydroxy phenyl acetic acid decarboxylase and DBH are enzymes specifically involved in catecholamine synthesis and insular cells are reported to possess the capacity to synthesise these amines. Thus, endogenously-synthesised islet catecholamines have been suggested to participate in paracrine regulation of insulin secretion. Secretory granules of pancreatic β-cells have the ability to store (Ahren & Lundquist, 1985) substantial amounts of calcium, dopamine and serotonin. L-3, 4-dihydroxyphenylalanine is rapidly converted in islet beta-cells to dopamine. Acute L-DOPA-induced dopamine accumulation in pancreatic islets is accompanied by rapid changes in MAO activity, concomitant with an inhibitory effect on glucose-stimulated insulin response (Ahren & Lundquist, 1985). It is reported that increased hydrogen peroxide production, following increased MAO activity, may possibly augment the inhibitory effect of dopamine accumulation on insulin release (Lundquist, *et al.*, 1991). Dopamine is reported to suppress the somatostatin secretion predominantly through activation of dopaminergic receptors, whereas it suppresses insulin release through an alpha adrenergic mechanism and stimulates glucagon release through a β-adrenergic mechanism (Malaisse, *et al.*, 1992). There has not been any detailed study on the distribution of dopamine receptor subtypes in the pancreatic islets or the pancreas except for these studies. Sympathetic α_1 receptors and dopamine D_1 are reported to be distributed on the beta cells while β_2 receptors are located on the D cells and dopamine D_2 receptors in the beta neurons (Imamura, *et al.*, 1990).

Dysfunction of pancreatic islets plays an important role in the etiology of diabetes as chronic hyperglycemia impairs islet function. It has been proposed that chronic hyperglycemia resulting from peripheral insulin resistance may impair secretogogue-induced insulin release. Dopamine agonists influence central circadian neuroendocrine activities regulating metabolism to reduce insulin secretion (Lang, *et al.*, 1998). Timed dopaminergic stimulation is reported to normalize the circardian rhythm of corticosterone release in obese insulin resistant animals (Lang, *et al.*, 1998). It has been reported that administration of dopamine receptor agonists, bromocriptine and/or SKF38393 in diabetic rats decreased insulin resistance, increased secretion of insulin from the islet cells and normalized the daily corticosterone rhythm. Dopamine receptor agonists are suggested to improve the decreased regulatory mechanisms in the hypothalamic-neuroendocrine system during diabetes and reduce β-cell toxicity.

Hyperglycemia causes functional deficits in the CNS aminergic neurons which are too subtle and take a longer time to manifest. Reports emphasized that treatment of gastric stasis in diabetic patients using dopamine blocker metoclopramide resultsed in increased frequency and severity of dopamine associated tardive dyskinesia. Also, diabetes caused a shift in the CNS resulting in an increased sympathetic tone that resulted in a decreased insulin secretion. Recently the presence of DA in the adrenal medulla is being stated to draw importance as it is necessary to control secretions of NE and EPI. Dopamine regulates pancreatic insulin secretion in a concentration dependent manner (Zern, *et al.*, 1980). But the molecular mechanisam is not well studied in detail. Further studies on the involvement of dopamine will lead to improved therapeutic strategies to treat diabetes.

MATERIALS AND METHODS

BIOCHEMICALS AND THEIR SOURCES

Biochemicals used in the present study were purchased from Sigma Chemical Co., USA. All other reagents were of analytical grade purchased locally. HPLC solvents were of HPLC grade obtained from SRL and MERCK, India.

Chemicals used for this study

Biochemicals:

(±)Norepinephrine, (±)epinephrine, normetanephrine, 5-hydroxytryptamine, dopamine, homovanillic acid, sodium octyl sulfonic acid, ethylene glycol bis (β-aminoethyl ether)-EGTA, ethylene diamine tetra acetic acid-EDTA, HEPES - [n' (2-hydroxy ethyl)] piperazine-n'-[2-ethanesulfonic acid], ascorbic acid, streptozotocin, pargyline, D-glucose, calcium chloride, butaclamol, (±) 7-hydroxy-2-(di-n-propylamino) tetralin hydrogen bromide, (7-OH-DPAT), (-) sulpiride, collagenase type XI and bovine serum albumin fraction V. (Sigma Chemical Co., St. Louis, MI, USA)

YM-09151-2: *cis–N-(1-benzyl-2-methylpyrrolidine-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide*] was a gift from YamanouchiPharmaceuticals Ltd, Tokyo, Japan, Bromocriptine was a gift from Dr.Shaji George, USA and Dr. Jacqueline Trouillas *Laboraitoire d'Histologie-Embryologie (J.T.,P.C., C.G.), Alexis Carrel, France.*

Radiochemicals

[^{3}H] Dopamine (Sp. activity- 45.1Ci/mmol), [^{3}H] Spiperone (Sp. activity 16.5Ci/mmol) and [^{3}H]YM-09151-2 (*cis–N-(1-benzyl-2-methylpyrrolidine-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide* Sp. activity - 85.0Ci/mmol) were purchased from NEN Life Sciences Products, Inc. Boston, USA.

Radioimmunoassay kit for insulin assay was purchased from Bhabha Atomic Research Centre, Mumbai, India.

Molecular biology chemicals

Reverse transcriptase enzyme MuMLV was purchased from Amersham Biosciences, USA, Taq polymerase, random hexameres, Human RNAse inhibitor, DNA

molecular weight markers, dNTPs were purchased from Bangalore Genei Pvt. Ltd. India. Tri-reagent kit was purchased from Sigma Chemical Co., USA. Dopamine D_2 receptor primers for PCR and β-actin primers for PCR were synthesised by Sigma Chemical Co., USA.

ANIMALS

Adult male Wistar rats of 200-240g body weight were purchased from Central Institute of Fisheries Technology, Cochin and 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*.

Induction of diabetes

Diabetes was induced by a single intrafemoral dose (65 mg/kg body weight) of STZ prepared in citrate buffer, pH 4.5 (Hohenegger & Rudas, 1971; Arison, *et al.*, 1967). Animals were divided into the following groups as i) Control [C] ii) Diabetic [D] iii) Insulin treated diabetic rats [D+I]. Each group consisted of 4-6 animals. The insulin treated diabetic group received a daily dose (1Unit/kg body weight) of Lente and Plain insulin (Boots India). The dose was increased daily according to the blood glucose level (Sasaki & Bunag, 1983).

Streptozotocin (65mg/kg body wt.) was injected to adult male Wistar rats and corpus striatal dopamine and HVA content were measured at different time periods (3 hrs, 12 hrs, 24 hrs and 48 hrs). In the dose dependent study different doses of streptozotocin (5, 10, 20, 40 & 65 mg/ kg body wt.) were injected to adult male Wistar rats and the striatal dopamine and HVA changes were quantified after 48 hrs. Control rats were injected with citrate buffer.

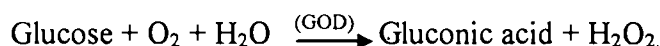
Tissue preparation

Rats were sacrificed by decapitation on the 14th day of the experiment. The cerebral cortex, corpus striatum, brain stem and hypothalamus were dissected out quickly over ice according to the procedure of Glowinski & Iversen, (1966). The tissues were stored at -70^0 C until assay.

The rats were sacrificed by decapitation in time dependent studies at intervals-3 hrs, 12 hrs, 24 hrs and 48 hrs and in dose dependent studies after 48 hrs of STZ injection. Brain was dissected out quickly over ice according to the procedure of Glowinski & Iversen, (1966) and the corpus striatum was used for both the experiments.

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:



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

The hydrogen peroxide formed in this reaction 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 505nm in (Milton Roy Genesys 5 Spectronic) spectrophotometer.

HISTOLOGY

Brain region- corpus striatum, cerebral cortex and hypothalamus from control, diabetic and insulin treated diabetic rats were used to make paraffin sections of 0.5µm for histological studies. These were stained using Nissl's periodic acid stain (PAS) and studied under light microscope for large accumulation of glycogen scattered in the brain tissues.

The pancreas from control, diabetic and insulin treated diabetic rats were used to make paraffin sections of 0.5µm. They were stained using hematoxylin-eosin stain and studied under light microscope for showing the degeneration in the pancreas.

QUANTIFICATION OF BRAIN MONOAMINES AND THEIR METABOLITES

The monoamines were assayed according to Paulose *et al.*, (1988). The tissues from brain regions were homogenised in 0.4N perchloric acid. The homogenate was centrifuged at 5000xg for 10 minutes at 4°C (Kubota refrigerated centrifuge) and the

clear supernatant was filtered through 0.45 μm HPLC grade filters and used for HPLC analysis.

Norepinephrine (NE), epinephrine (EPI), dopamine (DA) and Homovanillic acid (HVA) were determined in high performance liquid chromatography (HPLC) with electrochemical detector (HPLC-ECD) (Shimadzu, Japan) fitted with CLC-ODS reverse phase column of 5 μm particle size. The mobile phase consisted of 75 mM sodium dihydrogen orthophosphate, 1mM sodium octyl sulfonate, 50mM EDTA and 7% acetonitrile. The pH was adjusted to 3.25 with orthophosphoric acid, filtered through 0.45 μm filter (Millipore) and degassed. A Shimadzu (model 10 AS) pump was used to deliver the solvent at a rate of 1 ml/min. The catecholamines were identified by an amperometric detection using an electrochemical detector (Model 6A, Shimadzu, Japan) with a reduction potential of + 0.8 V, with the range set at 16 and a time constant of 1.5 seconds. Twenty microlitre aliquots of the acidified supernatant were injected into the system. The peaks were identified by relative retention times compared with external standards and quantitatively estimated using an integrator (Shimadzu, C-R6A - Chromatopac) interfaced with the detector. Data from different brain regions of the experimental and control rats were statistically analysed and tabulated.

In the case of pancreas and adrenals the tissues were homogenised in 0.1N perchloric acid. The homogenate was centrifuged at 5000xg for 10 minutes at 4°C (Kubota refrigerated centrifuge) and the clear supernatant was filtered through 0.45 μm HPLC grade filters and used for HPLC analysis. Data from pancreas and adrenals of the experimental and control rats were statistically analysed and tabulated.

Determination of Plasma Monoamines and their metabolites

Plasma monoamines were assayed as per Jackson *et al.*, (1997). 1.0 ml of plasma was diluted with 1.0 ml of distilled water. To this was added 50 μl of 5mM sodium bisulfite was added and mixed, followed by 25μl of 1M Tris buffer pH 8.6. Acid alumina (20mg) was then added and the contents were mixed well using a shaker. The supernatant was aspired out by means of a pasture pipette. The alumina was washed twice with 2.0 ml of 5mM sodium bisulfite. To the final pellet of alumina 0.2ml of 0.1 N perchloric acid was added and mixed in a shaker for 15 minutes. The supernatant was

filtered using a syringe top filter and used in the determination of monamines and its metabolites. Data from the plasma of the experimental and control rats were statistically analysed and tabulated

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 Spectrophotometer at 660nm.

DOPAMINE RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS IN THE BRAIN REGIONS OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS

Dopamine DA receptor binding studies using [^{3}H] Dopamine

Dopamine DA receptor assay was done using [^{3}H] DA as per Madras, *et al.*, (1988) and Hamblin & Creese, (1982). Brain tissues were homogenised in a polytron homogeniser with 10 volumes of cold 50mM Tris-HCl buffer, along with EDTA 1mM, ascorbic acid 0.01%, $MgCl_2$ 4mM, $CaCl_2$ 1.5 mM pH.7.4 and centrifuged at 38,000xg for 30min. at 4°C. The pellet was washed twice by rehomogenization and centrifuged twice at 38,000 g for 30min. at 4°C. This was resuspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.25nM-1.5nM of [^{3}H] DA in 50mM Tris-HCl buffer, along with EDTA 1mM ascorbic acid 0.01%, $MgCl_2$ 1mM, $CaCl_2$ 2 mM, NaCl 120mM, 5mM KCl pH.7.4 in a total incubation volume of 300μl containing 200-300 μg of proteins. Specific binding was determined using 100μM unlabelled butaclamol. Competition studies were carried out with 0.25nM [^{3}H] DA in each tube with unlabelled ligand concentrations varying from 10^{-9} - 10^{-4}M of DA.

Tubes were incubated at 25°C for 60 min. and filtered rapidly through GF/Bfilters (Whatman). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Corpus striatal dopamine D_2 receptor binding studies using [^{3}H] spiperone of control, diabetic and insulin treated diabetic rats.

Dopamine D_2 receptor binding assay in the striatum was done according to the modified procedure of Trulson and Himmel, (1983), Grigoridias and Seeman, (1985) and Paulose *et al.*, (1981). Three samples of striatum were pooled from each group. The tissues were weighed and homogenized in 10 volumes of ice cold 50mM Tris-HCl buffer, along with 1mM EDTA, 4mM $MgCl_2$, 1.5mM $CaCl_2$, 5mM KCl pH.7.4. The homogenate was centrifuged at 480g for 10 min and the supernatant was saved and the pellet was resuspended in 10 volumes of the buffer and centrifuged at 480g for 10 min. The final pellet was discarded and the supernatant were again centrifuged at 48,000xg for 30 minutes. The pellet was resuspended in 50 volumes of 50mM Tris HCl, pH 7.4 and recentrifuged at 48,000xg for 30 minutes. This was resuspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.01nM-1.5nM of [^{3}H] spiperone in 50mM Tris-HCl buffer, along with 1mM EDTA, 4mM $MgCl_2$, 1.5 mM $CaCl_2$ 5mM KCl with 12µM pargyline and 0.1% ascorbic acid in a total incubation volume of 300µl containing 150-200 µg proteins. For the study of dopamine D_2 receptors to preclude serotonergic component of the striatum 40nM ketanserine was used. Specific binding was determined using 1.0µM unlabelled butaclamol. Competition studies were carried out with 0.25nM [^{3}H]spiperone in each tube with unlabelled ligand concentrations varying from 10^{-12} - 10^{-4}M of spiperone.

Tubes were incubated at 25^{0}C for 60 min. and filtered rapidly through GF/B filters. The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Dopamine D_2 receptor binding studies using [^{3}H] YM-09151-2 in brain regions of control, diabetic and insulin treated diabetic rats.

Dopamine D_2 receptor binding assay was done according to the modified procedure of Unis, *et al.*, (1998) and Madras, *et al.*, (1988). The dissected brain tissues corpus striatum, hypothalamus, cerebral cortex tissues and brain stem were weighed and

homogenized in 10 volumes of ice cold 50mM Tris-HCl buffer, along with 1mM EDTA, 5mM $MgCl_2$, 1.5 mM $CaCl_2$, 120mM NaCl, 5mM KCl pH.7.4. The homogenate was centrifuged at 48,000xg for 30 min. The pellet was washed and recentrifuged with 50 volumes of the buffer at 48,000xg for 30 min. This was suspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.25nM-2.0nM of [^{3}H] YM-09151-2 in 50mM Tris-HCl buffer, along with 1mM EDTA, 5mM $MgCl_2$, 1.5 mM $CaCl_2$, 120mM NaCl, 5mM KCl with 10μM pargyline and 0.1% ascorbic acid in a total incubation volume of 300μl containing 200-300μg of protein. Specific binding was determined using 5.0 μM unlabelled sulpiride. Competition studies were carried out with 0.25nM [^{3}H] YM-09151-2 in each tube with unlabelled ligand concentrations varying from 10^{-12} - 10^{-4}M of YM-09151-2.

Tubes were incubated at 25^{0}C for 60 min. and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

DOPAMINE RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS IN THE PANCREATIC ISLETS OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS

Dopamine DA receptor binding studies using [^{3}H] Dopamine in the islets of control, diabetic and insulin treated rats

Pancreatic islets of control, diabetic and insulin treated diabetic rats were isolated by standard collagenase digestion procedure using aseptic techniques [Howell, 1968]. The islets were isolated in HEPES-buffered sodium free Hanks Balanced Salt Solution (HBSS) [Pipeleers, 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 $KHCO_3$, 10mM HEPES with 0.2% (w/v) BSA (Fraction V), equilibrated with 5% CO_2 and pH 7.3 at room temperature. Autoclaved triple distilled water was used in the preparation of the buffer.

Pancreatic islets isolated as per the above mentioned procedure was homogenised in a polytron homogeniser with 10 volumes of cold 50mM Tris-HCl buffer, along with EDTA 1mM ascorbic acid 0.01%, $MgCl_2$ 4mM, $CaCl_2$ 1.5 mM pH.7.4 and centrifuged at 38,000xg for 30min. at 4°C. The pellet was washed twice by rehomogenization and centrifuged twice at 38,000xg for 30min. at 4°C. This was resuspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.1nM-2.0 nM of [^{3}H] DA in 50mM Tris-HCl buffer, along with EDTA 1mM ascorbic acid 0.01%, $MgCl_2$ 1mM, $CaCl_2$ 2 mM, NaCl 120mM, 5mM KCl 10μM pargyline pH.7.4 in a total incubation volume of 300μ containing 50-75μg of proteins. Specific binding was determined using 100μM unlabelled butaclamol. Competition studies were carried out with 0.5nM [^{3}H] DA in each tube with unlabelled ligand concentrations varying from 10^{-9} - 10^{-4}M of DA.

Tubes were incubated at 25°C for 60 min. and filtered rapidly through GF/B filters. The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Dopamine D_2 receptor binding studies using [^{3}H] YM-09151-2 in the islets of control, diabetic and insulin treated diabetic rats.

The assay was done in a similar way as in brain regions. After isolation the islets were homogenized in 10 volumes of ice cold 50mM Tris-HCl buffer, along with 1mM EDTA, 5mM $MgCl_2$, 1.5 mM $CaCl_2$, 120mM NaCl, 5mM KCl pH.7.4. The homogenate was centrifuged at 48,000xg for 30 min. The pellet was washed and recentrifuged with 50 volumes of the buffer at 48,000xg for 30 min. This was suspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.1nM-2.0nM of [^{3}H] YM-09151-2 in 50mM Tris-HCl buffer, along with 1mM EDTA, 5mM $MgCl_2$, 1.5 mM $CaCl_2$, 120mM NaCl, 5mM KCl with 10μM pargyline and 0.1% ascorbic acid in a total incubation volume of 300μl containing 50-75 μg of protein. Specific binding was determined using 5.0 μM unlabelled sulpiride. Competition studies were carried out with

0.5nM [^{3}H] YM-09151-2 in each tube with unlabelled ligand concentrations varying from 10^{-12} - 10^{-4}M YM-09151-2.

ANALYSIS OF THE RECEPTOR BINDING DATA

Linear regression analysis for Scatchard plots

The data was 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.

Nonlinear regression analysis for displacement curve

The displacement data were analysed by nonlinear regression using Graphpad Prism software, GraphPad, Inc., USA. The concentration of the competing drug that competes for half the specific binding was defined as EC_{50}, which is same as IC_{50} (Unnerstall, 1990). The affinity of the receptor for the competing drug is designated as K_i and is defined as the concentration of the competing ligand that will bind to half the binding sites at equilibrium in the absence of radioligand or other competitors (Cheng & Prusoff, 1973).

[^{3}H] Dopamine uptake studies by pancreatic islets *in vitro*

Pancreatic islets of male Wistar rats were aseptically dissected out into a sterile petridish containing ice cold Hanks Balanced Salt Solution (HBSS) and isolated by standard collagenase digestion procedure (Howell, 1968). The islets were isolated in HEPES-buffered sodium free (Pipeleers, 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 $KHCO_3$, 10mM HEPES with 0.2% (w/v) BSA (Fraction V), equilibrated with 5% CO_2 and pH 7.3 at room temperature. Autoclaved triple distilled water was used in the preparation of the buffer. The pancreas was cut into small pieces and transferred to a

sterile glass vial containing 2ml collagenase type XI solution (1.5 mg/ml in HBSS), pH 7.4. The collagenase digestion was carried out for 20 minutes at 37°C in an environmental shaker with vigorous shaking (300rpm/min). The tissue digest was filtered through 500 µm nylon screen and the filtrate was washed with three successive centrifugations and resuspensions in cold HBSS medium. This filtrate was transferred to a sterile petri dish with a black base and examined under a dissection microscope. Islets visible as yellowish white spheres were handpicked carefully by finely drawn pasture pipettes and aseptically transferred to HBSS. The islets prepared by this method were used for all other experiments.

The islets isolated by the above mentioned method were resuspended in HEPES buffered HBSS with 4mM glucose and pre-incubated for 1hour at 37^0C (Howell & Taylor, 1968). The islet suspension was centrifuged at 4^0C at 500xg to remove inherent insulin. The pre-incubated islets were then washed thrice with cold 10mM Tris HCl buffer, pH 7.4 and finally resuspended in HBSS without glucose. 200µl of islet suspension was transferred to tubes containing 10^{-8} M, 10^{-7} M , 10^{-6} M, 10^{-5} M, 10^{-4} M concentrations of [^{3}H] DA. [^{3}H] DA concentrations were used along with 4mM and 20mM glucose. The final incubation volume was made up to 0.5ml. The tubes were incubated for 2hours at 37^0C in a shaking water bath.

The tubes were centrifuged after incubated for one hour at 1,500xg for 10min at 4^0C. The supernatant was aspirated out and pellet washed superficially with 0.2ml of HBSS twice to remove free [^{3}H] DA. The pellet was digested with 100µl of 1M KOH overnight and counted in a liquid scintillation counter with Cocktail-T to measure the [^{3}H] DA uptake.

Effect of Norepinephrine on dopamine uptake by pancreatic islets *in vitro*

The islets prepared as per the above mentioned procedure were transferred to tubes containing 10^{-8} M, 10^{-4} M concentrations of [^{3}H] DA and 10^{-8} M, 10^{-4} M concentrations of NE.

[^{3}H] DA concentrations were used along with 4mM and 20mM glucose. NE was used to study the [^{3}H] DA uptake along with glucose in this experiment. The

final incubation volume was made up to 0.5ml. The tubes were incubated for 2hours at 37^0C in a shaking water bath.

The tubes were centrifuged after incubated for one hour at 1,500xg for 10min at 4^0C. The supernatant was aspirated out and pellet washed superficially with 0.2ml of HBSS twice to remove free [^{3}H] DA or NE. The pellet was digested with 100µl of 1M KOH overnight and counted in a liquid scintillation counter with Cocktail-T to measure the [^{3}H] DA uptake.

In vitro insulin secretion in the presence of different concentrations of dopamine, its antagonist and agonist

The isolated islets were incubated for 1hour at 37^0C with 10^{-8} M, 10^{-7} M , 10^{-6} M, 10^{-5} M, 10^{-4} M concentrations of DA and two different concentrations of glucose i.e., (i) 4mM glucose and (ii) 20mM glucose. Cells after incubation and centrifugation at 1,500xg for 10 min at 4^0C, the supernatant were transferred to fresh tubes for insulin assay by radioimmunoassay.

Similarly the islets were incubated with combinations of DA and its antagonists, dopamine agonists to study the effect of DA and its receptors on glucose induced insulin secretion using radioimmunoassay.

Studies were also done in islets incubated for 1hour at 37°C with combinations of DA and NE at different concentrations to know the effect of NE on the role of DA in insulin secretion.

RADIOIMMUNO ASSAY OF INSULIN

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 are 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 the experiments from the secretion studies 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 3hours. The second antibody was added (50μl) along with 500μl of PEG. The tubes were then vortexed and incubated for 20minutes and they were centrifuged at 1500xg for 20minutes. The supernatant was aspirated out and the radioactivity in the pellet was determined in a gamma counter.

A standard curve was plotted with %B/B_o on the Y-axis and insulin concentration/ml on the X-axis of a log-logit graph. %B/B_o 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 were determined from the standard curve plotted using MultiCalc™ software (Wallac, Finland).

EXPRESSION STUDIES OF DOPAMINE D_2 RECEPTOR IN DIFFERENT BRAIN REGIONS OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS.

REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

Preparation of RNA

RNA was isolated from the different brain regions of control, diabetic and insulin treated diabetic t experimental rats using the Tri reagent from Sigma Aldrich.

Isolation of RNA

Tissue (25-50 mg) homogenates were made in 0.5 ml Tri Reagent and was centrifuged at 12,000x*g* for 10 minutes at 4^{0}C. The clear supernatant was transferred to a fresh tube and it was allowed to stand at room temperature for 5 minutes. 100μl of chloroform was added to it, mixed vigorously for 15 seconds and allowed to stand at

room temperature for 15 minutes. The tubes were then centrifuged at 12,000x*g* for 15 minutes at 4^0C. Three distinct phases appear after centrifugation. The bottom red organic phase contained protein, interphase contained DNA and a colourless upper aqueous phase contained RNA. 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. The tubes were centrifuged at 12,000x*g* for 10 min at 4^0C. RNA precipitate forms a pellet on the sides and bottom of the tube. The supernatants were removed and the RNA pellet was washed with 500µl of 75% ethanol, vortexed and centrifuged at 12,000x*g* for 5 min at 4^0C. 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 were 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.

RT-PCR Primers

The following primers were used for dopamine D_2 receptors and β-actin mRNA expression studies.

Primers	Gene
5'- GCC AAA CCA GAG AAG AAT GG -3' 5'- GAT GTG CGT ATG AAG GAA GG-3' PRODUCT SIZE: 500bp	DA D_2
5'- CAA CTT TAC CTT GGC CAC TAC C -3' 5'- TAC GAC TGC AAA CAC TCT ACA CC -3' PRODUCT SIZE: 150bp	β-ACTIN

RT-PCR of dopamine D_2 receptors and β-actin

RT-PCR was carried out in a total reaction volume of 20µl reaction mixture in 0.2ml tubes. RT-PCR was performed on an Eppendorf Personal thermocycler. cDNA synthesis of 2mg RNA was performed in a reaction mixture containing MuMLV reverse transcriptase (40U/reaction), 2mM dithiothreitol, 4 units of human placental RNAse

inhibitor, 0.5μg of random hexamer and 0.25mM (dNTPS dATP, dCTP, dGTP and dTTP). The tubes were then incubated at 42^0C for one hour. Then reverse transcriptase, MuMLV, was inactivated by heating at a temperature of 95°C.

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was carried out in a 20μl volume reaction mixture containing 4μl of cDNA, 0.25mM dNTPS - dATP, dCTP, dGTP and dTTP -, 0.5units of Taq DNA polymerase and 10 picomoles of specific primers. The three primers used have the same annealing temperature.

Following is the thermocycling profile used for PCR

94^0C -- 5 min --- Initial Denaturation

94^0C -- 30 sec --- Denaturation

56^0C -- 30 sec --- Annealing 36 cycles

72^0C -- 30 sec --- Extention

72^0C -- 7 min --- Final Extension

Analysis of RT-PCR product

The Polymerase Chain Reaction product was loaded on a 2.0% agarose gel with ethidium bromide. Bromophenol blue was used as the indicator dye. 60V current was used for all the run. The image was captured using an Imagemaster gel documentation system (Pharmacia Biotec) and the bands were densitometrically analysed using Total Lab software. Dopamine D_2 receptor mRNA expression in the brain regions-CS, CC, HYPO and BS of control, diabetic and insulin treated diabetic rats were analysed.

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).

RESULTS

BLOOD GLUCOSE LEVEL AND BODY WEIGHT OF EXPERIMENTAL RATS

Streptozotocin administration led to a significant increase ($p<0.001$) in blood glucose of diabetic group. Insulin treatment was able to significantly reduce ($p<0.001$) the increased blood glucose level to normal when compared to diabetic group (Table-1). Onset of diabetes caused a decrease in body weight ($p<0.001$) (Table-1). Insulin treatment normalized the body weight to normal in diabetic rats ($p<0.001$) (Table-1).

HISTOLOGICAL STAINING IN THE BRAIN REGIONS OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS

Sections of tissues from different brain regions corpus striatum, hypothalamus, and cerebral cortex were stained with periodic acid staining and studied under light microscopy. These regions uniformly exhibited the accumulation of glycogen granules in diabetic rats which were absent in control rats (Fig: 4,5,6). In diabetic rats 14 day insulin treatment caused the lowering of these granules in the brain regions of CS, CC and HYPO (Fig: 4,5,6) indicating the mobilization of glycogen.

HISTOLOGICAL STAINING IN THE PANCREAS OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS

Pancreatic tissue section from control rats showed distinct nuclei with hematoxylin and eosin stain (Fig: 7). The pancreatic section from the diabetic rats showed distorted nuclei and cells (Fig: 7). Insulin treatment reduced the damage to the tissue

EFFECT OF DIFFERENT DOSES OF STREPTOZOTOCIN IN CORPUS STRIATAL DOPAMINE AND HOMOVANILLIC ACID

Dopamine content in the striatum increased significantly ($p<0.001$) after 48hours with an increase in the dose of streptozotocin (Fig: 10). Also, the content of HVA in the striatum decreased significantly ($p<0.001$) as DA content increased (Fig:11). There was also a concordant decrease in the turn over of DA to HVA with the increase in the dose of streptozotocin (Fig: 11).

DOPAMINE AND HOMOVANILLIC ACID CHANGES IN THE CORPUS STRIATUM AT DIFFERENT TIME INTERVALS AFTER STREPTOZOTOCIN INJECTION.

Dopamine content increased significantly ($p<0.001$) with time and showed a maximum at the end of 48hrs (Fig: 8). Similarly the homovanillic acid content decreased as time interval increased and showed the lowest value ($p<0.001$) at the end of 48hrs (Fig: 9). There was a significant decrease ($p<0.001$) in the turn over ratio of HVA from DA (Fig: 9).

DOPAMINE AND HOMOVANILLIC ACID CONTENTS IN DIFFERENT BRAIN REGIONS OF CONTROL, DIABETIC AND INSULIN TREATED RATS

Corpus striatum

There was a significant increase ($p<0.001$) in DA content of corpus striatum in 14-day diabetic rats (Table-2). Insulin treatment did not normalise the DA content and remained significantly high in 14 day insulin treated diabetic rats ($p<0.01$). Homovanillic acid the metabolite of DA was found to be significantly decreased ($p<0.05$) in the 14-day diabetic rats. Insulin treatment restored the HVA levels to control values when compared to diabetic rats ($p<0.01$). The turnover rate of HVA from DA was significantly decreased ($p<0.001$) in diabetic group when compared to control. This decreased turnover of HVA from DA was not reversed ($p<0.001$) by insulin treatment in diabetic rats to control values (Table: 2).

Hypothalamus

Dopamine content in the hypothalamus was significantly decreased in ($p<0.001$) 14 day diabetic rats (Table: 3). Insulin treatment did not completely restore the decreased DA content to control levels which remained significantly low ($p<0.001$). HVA also showed significant ($p<0.001$) decrease in 14-day diabetic rats. Insulin treatment did not restore the altered HVA levels to control values and they remained significantly low ($p<0.001$). The turnover rate of HVA to DA did not show any alteration in the hypothalamus during diabetes (Table: 3).

Cerebral cortex

Dopamine content increased significantly ($p<0.001$) in the cerebral cortex of diabetic rats when compared to control (Table-4). Insulin treatment did not normalize

the DA content to control levels and remained significantly high in 14 day insulin treated diabetic rats ($p<0.001$). Homovanillic acid, the metabolite of DA was found to be significantly increased ($p<0.001$) in the 14-day diabetic rats (Table-4). Insulin treatment did not restored the HVA levels to control values and remained significantly high ($p<0.001$) as in the diabetic group. The turnover rate of HVA from DA was significantly decreased ($p<0.01$) in diabetic group when compared to control. In insulin treated diabetic rats the turnover rate normalised when compared to untreated diabetic rats ($p<0.05$; Table-4).

Brain stem

Doapmine content in the brain stem decreased significantly ($p<0.001$) in diabetic rats compared when to controls (Table-5). Insulin treatment did not reverse the decreased DA ($p<0.05$). Homovanillic acid content of diabetic rats decreased significantly ($p<0.001$; Table-5). HVA levels were not normalized during the 14 day insulin treatment in diabetic rats ($p<0.001$). The decrease in DA with a proportional decrease in HVA content was observed. There was no alteration the turnover ratio of HVA from DA (Table-5).

PLASMA DOPAMINE AND OTHER MONOAMINE CONCENTRATIONS IN CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS

The plasma DA concentration in diabetic group was significantly decreased ($p<0.001$) when compared to control (Table-6). Insulin treatment significantly reversed ($p<0.001$) the altered DA content when compared to diabetic group. The HVA content in the plasma decreased significantly when compared to control in the diabetic group ($p<0.001$; Table-6). Insulin treatment brought about a reversal in the decreased HVA which was significant ($p<0.05$) when compared to the diabetic group but this reversal did not fully restored to control values and remained significantly ($p<0.01$) decreased. The HVA/DA turnover in diabetic group was significantly decreased ($p<0.001$) compared to control. Insulin treatment significantly reversed the turn over rate ($p<0.01$) to control values (Table-6).

The Norepinephrine and EPI content in diabetic group also showed a significant increase ($p<0.001$) compared to control (Table-6). Insulin treatment significantly

reversed ($p<0.001$) the increased EPI content when compared to diabetic group, while the NE content remained high ($p<0.01$) when compared to control levels.

DOPAMINE, HOMOVANILLIC ACID AND OTHER MONOAMINE CONCENTRATIONS IN THE ADRENALS OFCONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS.

Dopmine concentrations in the adrenals during diabetes was significantly decreased ($p<0.001$) when compared to control (Table-7). Insulin treatment reversed ($p<0.05$) the altered DA content when compared to diabetic group. The HVA content decreased significantly in the diabetic group when compared to control ($p<0.001$) (Table-7). Insulin treatment did not restore the decreased HVA to control values and remained significantly decreased ($p<0.001$). There was no significant change in the turn over ratio from DA to HVA during diabetes.

The nonepinephrine and EPI content in diabetic group also showed a significant increase ($p<0.001$) compared to control (Table-7). Insulin treatment partially reversed the increased EPI and NE content which significantly decreased ($p<0.001$) when compared to diabetic group. But nonepinephrine and EPI content remained high ($p<0.01$) when compared to control levels on insulin treatment in diabetic rats. The turnover ratio of NE from DA showed a significant decrease ($p<0.001$) in 14day diabetic rats. Insulin treatment for 14-days in diabetic rats did not bring the altered ratio to control values and remained significantly decreased ($p<0.001$). The EPI/NE ratio in diabetic rats increased significantly ($p<0.001$) when compared to control and insulin treatment fully restored the elevated ratio to normal values when compared to diabetic group (Table-7).

PANCREATIC DOPAMINE AND HOMOVANILLIC ACID CONTENTS OF CONTROL, DIABETIC AND INSULIN TREATED RATS

Dopamine content of pancreas in diabetic group decreased significantly ($p<0.001$) when compared to control (Table-8). Treatment with insulin, did not completely reverse the decreased DA to control levels ($p<0.001$) but showed an increase in DA levels ($p<0.05$) when compared to diabetic. Homovanillic acid content in the pancreas decreased significantly ($p<0.001$) in 14-day diabetic rats when compared to controls, while insulin treatment did not fully restore it to control values. In comparison with

diabetic group the content in HVA showed a reversal ($p<0.05$) on insulin treatment but was significantly low ($p<0.001$) when compared to control. The turnover ratio from DA to HVA was significantly decreased ($p<0.001$) during diabetes when compared to control. Insulin treatment in 14 day diabetic rats did not fully restore ($p<0.001$) the turnover ratio to control levels.

BRAIN DOPAMINE RECEPTOR CHANGES IN CONTROL, DIABETIC AND INSULIN TREATED RATS

Corpus striatum

Scatchard analysis using [^{3}H] Dopamine

Scatchard analysis in corpus striatum of diabetic rats showed significant decrease ($p<0.001$) in B_{max} when compared to control (Table-9; Fig.-12). But the K_d did not show any change in diabetic group. Insulin treatment significantly reversed ($p<0.001$) the B_{max} to normal when compared to diabetic group. A decrease in B_{max} without any significant change in K_d shows a decrease in DA receptors without any change in its affinity during diabetes.

Displacement analysis of [^{3}H] Dopamine against Dopamine

The dopamine receptors in the corpus striatum did not show any change in affinity during diabetes. The competition binding did not show any change in affinity state fitting the equation to a one-site model in control, diabetic and insulin treated diabetic rats. (Table-10; Fig.-13). The Hill slope value was unity (-1.00) in diabetic group compared to control (-1.087) confirming the one-site model. There was no change in Log (EC_{50}) value in diabetic group when compared to control. Treatment with insulin in 14 day diabetic rats caused a decrease in affinity showing an increased K_i value with an increase in Log (EC_{50}) value when compared to control (Table-10; Fig.-13). The Hill slope value for the insulin treated groups was near unity (-0.944) fitting the equation on a single site model. These results confirm a decrease in affinity of DA receptors with alteration in their number in insulin treated diabetic rats.

Cerebral cortex

Scatchard analysis using [^{3}H] Dopamine

Scatchard analysis of [^{3}H] DA against DA in cerebral cortex of diabetic rats showed a significant increase ($p<0.001$) in B_{max} and K_d ($p<0.001$) compared to control (Table-11; Fig.-14). An increase in B_{max} and K_d signifies an up-regulation of DA accompanied with a decrease in its affinity. Insulin treatment to diabetic rats significantly reversed ($p<0.001$) the K_d to control value but B_{max} showed a partial reversal and remained significantly high ($p<0.001$) when compared to control.

Displacement analysis of [^{3}H] Dopamine against Dopamine

Dopamine receptor affinity decreased during diabetes in cerebral cortex fitting the equation to a single-site model as seen in control (Table-12; Fig.-15). This was confirmed by the Hill slope value which (-0.981) in diabetic group was near unity and in control had a hill slope value in unity (-1.128). The Log (EC_{50}) value in diabetes increased with a (Table-12; Fig.-15) a decrease in affinity i.e. increase in Ki value. Insulin treatment restored the values to near control levels. The restoration to control levels by insulin fitted the equation to a single-site model with the hill slope value in unity (-1.107) and the Log (EC_{50}) value to control values.

Hypothalamus

Scatchard analysis using [^{3}H] Dopamine

Scatchard analysis in hypothalamus of diabetic rats showed a significant increase ($p<0.001$) in the K_d with no change in the B_{max} when compared to control (Table-13; Fig.-16). This increase in K_d without any change in B_{max} shows a decrease in affinity of DA receptors for dopamine. Insulin treatment was able to significantly reversed ($p<0.001$) back the decreased K_d to control compared to diabetic group.

Displacement analysis of [^{3}H] Dopamine against Dopamine

Dopamine receptor displacement analysis in hypothalamus of diabetic rats showed an increase in Ki (Table-14; Fig.-17). The Hill slope value was near unity (-0.914) in diabetic group compared to control (-1.012) which fitted to a single site model. The Log (EC_{50}) value in diabetes increased with a (Table-14; Fig.-17) a decrease in

affinity when compared to control. Treatment with insulin reversed the increased Ki and Log (EC_{50}) to control values and the hill slope value was unity (-1.087) with a one-site model similar to control (Table-14; Fig.-17).

BRAIN DOPAMINE D_2 RECEPTOR CHANGES IN CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS

Corpus striatum

Scatchard analysis using [^{3}H] Spiperone

Scatchard analysis of dopamine D_2 receptors was done using [^{3}H] spiperone. Diabetic group showed a significant increase ($p<0.001$) in B_{max} without any significant change in K_d compared to control (Table-15; Fig.-18). The B_{max} remained significantly increased ($p<0.001$) during insulin treatment when compared to control. There was no change in K_d during these conditions.

Displacement analysis of [^{3}H] Spiperone against Spiperone

Dopamine D_2 receptors did not show any change in affinity in the striatum during diabetes. All the experimental groups fitted best to a one-site equation (Table-16; Fig.-19). This was confirmed by the Hill slope value that was unity in all the groups. There was no significant change in Ki and Log (EC_{50}) in any of these groups (Table-16; Fig.-19).

Scatchard analysis using [^{3}H] YM-09151-2

YM-09151-2 is a potent antagonist for dopamine D_2 receptor. In diabetic rats the B_{max} increased significantly ($p<0.001$) with no change in K_d when compared to control (Table-17; Fig.-20). Insulin treatment showed a partial restoration of the B_{max} in diabetic rats when compared to untreated diabetes ($p<0.001$). But 14day insulin treatment did not fully bring the elevated B_{max} to control levels and remained significantly high. There was no alteration in the K_d during diabetes and insulin treatment when compared control (Table-17; Fig.-20).

Displacement analysis of [^{3}H] YM-09151-2 against YM-09151-2

Dopamine D_2 receptor did not shown any change in affinity in the striatum of diabetic rats in the displacement analysis. All the experimental groups fitted best to a one-site equation (Table-18; Fig.-21). This was confirmed by the Hill slope value that was unity in all the groups. The Ki and Log (EC_{50}) in any of these groups remained unaltered indicating that there was no affinity change during diabetes or insulin treatment (Table-18; Fig.-21).

RT-PCR analysis of dopamine D_2 receptors

RT-PCR analysis revealed that the dopamine D_2 receptor mRNA expression increased in the corpus striatum during diabetes and this increase in expression did not change during insulin treatment (Fig: 22; Table: 19).

Cerebral cortex

Scatchard analysis using [^{3}H] YM-09151-2

Scatchard analysis of [^{3}H] YM-09151-2 against YM-09151-2 in cerebral cortex of diabetic rats showed a significant increase ($p<0.001$) in B_{max} with no change in K_d compared to control (Table-20; Fig.-23). An increase in B_{max} signifies an up-regulation D_2 receptors accompanied with no alterations in its affinity. Insulin treatment to diabetic rats did not reverse ($p<0.001$) the B_{max} that remained significantly high ($p<0.001$) when compared to control (Table-20; Fig.-23).

Displacement analysis of [^{3}H] YM-09151-2 against YM-09151-2

In the cerebral cortex the competition binding assay was carried out using various concentrations of unlabelled YM-09151-2 against [^{3}H] YM-09151-2. There was no change in affinity during diabetes and insulin treatment. All the experimental groups fitted best to a one-site model with the hill slope value within unity (Table-21; Fig.-24). There was no significant change in Ki and Log (EC_{50}) in any of these groups (Table-21; Fig.-24) indicating that there was no alterations in affinity of the receptors.

RT-PCR analysis of Dopamine D_2 receptors

The RT-PCR analysis in the cerebral cortex showed an increased expression of dopamine D_2 receptor mRNA during diabetes and in the case of insulin treatment the expression pattern remained high (Fig: 25; Table: 22).

Hypothalamus

Scatchard analysis using [^{3}H] YM-09151-2

Scatchard analysis of [^{3}H] YM-09151-2 in hypothalamus of diabetic rats showed a significant decrease in B_{max} ($p<0.001$) when compared to control (Table-23; Fig.-26). The K_d of diabetic rats showed a significant decrease ($p<0.001$) when compared to control. This decrease in K_d with a change in B_{max} shows an increase in affinity of D_2 receptors for dopamine with a change in its number. Insulin treatment did not completely reverse the changes that occurred during diabetes. The B_{max} and K_d increased significantly ($p<0.001$) when compared to diabetic but remained decreased when compared to control.

Displacement analysis of [^{3}H] YM-09151-2 against YM-09151-2

The dopamine D_2 receptor affinity increased significantly in the hypothalamus of diabetic rats in the displacement analysis (Table-24;Fig.-27). The Hill slope value was near unity (-1.000) in diabetic group compared to control (1.046) which fitted to a single site model. The Log (EC_{50}) value during diabetes decreased with a (Table-24; Fig.-27) an increase in affinity when compared to control. Treatment with insulin reversed the decreased Ki and Log (EC_{50}) partially but did not reach the control values. The hill slope value was near unity (-0.952) and fitted to a one-site model similar to that of control (Table-24; Fig. - 27).

RT-PCR analysis of Dopamine D_2 receptors

The RT-PCR analysis in the hypothalamus showed a decrease in the expression of dopamine D_2 receptor mRNA during diabetes when compared to control (Fig-28; Table: 25). Insulin treatment reversed the expression to control levels.

Brain stem

Scatchard analysis using [^{3}H] YM-09151-2

Scatchard analysis in diabetic rats showed a significant decrease ($p<0.01$) in B_{max} with a significant increase in K_d compared to control showing a decrease in affinity (Table-26; Fig.-29). The B_{max} was reversed to control values by insulin treatment. Insulin treatment lowered the K_d significantly when compared to diabetic ($p<0.001$) rats.

Displacement analysis of [^{3}H] YM-09151-2 against YM-09151-2

The dopamine D_2 receptor affinity decreased significantly the brain stem of diabetic rats in the displacement analysis. They fitted best to a one-site equation (Table-27; Fig.-30). This was confirmed by the Hill slope value that was near unity in all the groups. In diabetic group the Ki value increased shifting the affinity towards a lower affinity state. Treatment of diabetic rats with insulin caused a decrease in Ki value which was lower than the control, shifting the affinity to a higher affinity state. Log (EC_{50}) value was decreased during diabetes with a decrease in affinity. In insulin treated diabetic rats the Log (EC_{50}) increased as the affinity increased (Table-27; Fig.-30) showing a decreased Ki value when compared to control.

RT-PCR analysis of Dopamine D_2 receptors

RT- PCR analysis revealed a decreased expression of dopamine D_2 receptor mRNA during diabetes in the brain stem while in insulin treated rats the expression was reversed which was high when compared to control levels (Fig-31; Table: 28).

ALTERED PANCRETIC ISLET DOPAMINE RECEPTOR BINDING PARAMETERS IN CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS

Scatchard analysis using [^{3}H] Dopamine

A significant increase in the B_{max} of [^{3}H] DA binding ($p<0.001$) in the islet membrane preparation of diabetic rats were observed when compared to control. The K_d of the receptor in diabetic rats increased significantly ($p<0.001$) showing a decrease in affinity (Table-29; Fig.-31). Insulin treatment showed a partial reversal of the B_{max} which was significantly lesser ($p<0.001$) than the diabetic group. In comparison with the control the insulin treated rats showed a significant increase in B_{max} ($p<0.01$). The K_d

was restored to near control levels in insulin treated diabetic rats which was significantly lower ($p<0.001$) than the diabetic rats (Table-29; Fig.- 31).

Displacement analysis of [^{3}H] Dopamine against Dopamine

The competition curve for [^{3}H] DA fitted for a single- sited model in control, diabetic and insulin treated groups with Hill slope values near unity (Table: 30; Fig: 32). In diabetic rats both Log (EC_{50}) and Ki increased showing a shift in affinity from higher to lower state (Table: 30; Fig: 32). Insulin treatment caused a reversal of both Log (EC_{50}) and Ki partially, they were increased when compared to diabetic but were lower than the control.

ALTERED PANCRETIC ISLET DOPAMINE D_2 RECEPTOR BINDING PARAMETERS IN CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS

Scatchard analysis using [^{3}H] YM-09151-2

A significant decrease in the B_{max} of [^{3}H] YM-09151-2 binding ($p<0.001$) in the islet membrane preparation of diabetic rats were observed when compared to control. The K_d of the receptor in diabetic rats decreased significantly ($p<0.001$) showing an increase in affinity when compared to control (Table-31; Fig.-33). Insulin treatment showed a reversal in the K_d to control values ($p<0.001$). In comparison with the control B_{max} in insulin treated diabetic rats remained significantly decreased ($p<0.001$; Table-31; Fig.-33).

Displacement analysis of [^{3}H] YM-09151-2 against YM-09151-2

Dopamine D2 receptor displacement analysis using [^{3}H] YM-09151-2 fitted for a single- sited model in control, diabetic and insulin treated groups with Hill slope values near unity (Table-32; Fig.-34). In diabetic rats both Log (EC_{50}) and Ki decreased, showing an increase in affinity. Insulin treatment caused a partial reversal of both Log (EC_{50}) and Ki values.

[^{3}H] Dopamine uptake by pancreatic islets *in vitro*

Our results showed a significant increase in [^{3}H] DA uptake by pancreatic islets in the presence of 10^{-4}M [^{3}H] DA in 4mM and 20mM glucose incubated with cells. As the

concentration of DA decreased to 10^{-7} M [^{3}H] DA the uptake decreased significantly when compared to 10^{-4}M [^{3}H] DA in 4mM and 20mM glucose incubated with cells (Fig.-35). 4mM and 20mM glucose in the incubation medium can be considered equivalent to normal and diabetic states respectively. In cells incubated with 10^{-8} M [^{3}H] DA and different concentrations of glucose the uptake was similar to 10^{-5} M [^{3}H] DA uptake in the presence of glucose. These results show that there is a rapid uptake of high concentrations of DA into the pancreatic islets in the presence of 4mM and 20mM glucose concentration (Fig.-35). [^{3}H] Dopamine showed maximum uptake in the presence of glucose, 4mM and 20mM at 10^{-4}M concentration in the dose dependent study ($p<0.001$). Also, [^{3}H] Dopamine uptake was inhibited by 10^{-4} M NE significantly ($p<0.001$) while 10^{-8} M NE did not show any change (Fig.-36).

Effect of dopamine on glucose induced insulin secretion *in vitro*

The pancreatic islets were incubated with 10^{-8} M, 10^{-7}M, 10^{-6}M 10^{-5} M, 10^{-4} M DA in the presence of 4mM and 20mM glucose. Dopamine caused a dose dependent effect on glucose induced insulin secretion in the pancreatic islets (Fig: 37). 4mM and 20mM glucose in the incubation medium is considered equivalent to normal and diabetic states respectively. The secretion of insulin by 4mM glucose was significantly lesser ($p<0.001$) than the secretion induced by 20mM glucose. As the concentration of DA increased the level of insulin secretion decreased. In the presence of 10^{-8} M DA and 20mM glucose the insulin secretion was significantly high ($p<0.05$) when compared to 20mM glucose alone. The insulin secretion in the presence of 10^{-8} M DA and 20mM glucose was significantly higher than ($p<0.001$) when compared to 4mM glucose and 10^{-8} M DA. Dopamine concentrations 10^{-7}M, 10^{-6}M 10^{-5} M, 10^{-4} M in the presence of 20mM glucose showed significant decrease ($p<0.001$) on insulin secretion when compared to cells incubated with 20mM glucose only. Insulin secretion in the presence of 10^{-7}M, 10^{-6}M 10^{-5} M, 10^{-4} M of DA and 4mM glucose showed significant decrease when compared to cells incubated with 4mM glucose only. Dopamine concentration 10^{-4} M produced the maximum inhibition of insulin secretion in the presence of 4mM and 20mM glucose incubated with the pancreatic islets(Fig: 37). Thus, dopamine dose dependently inhibited the glucose induced insulin secretion.

Effect of dopamine antagonists in glucose induced insulin secretion

Dopamine action on glucose induced insulin secretion through its receptors, showed that butaclamol, a general antagonist of dopamine, at 10^{-4} M concentration blocked the stimulatory effect of 10^{-8} M DA concentration ($p<0.001$, Fig: 38).

Sulpiride, a potent dopamine D_2 receptor antagonist, at 10^{-4} M concentration significantly ($p<0.001$) blocked the stimulatory effect of 10^{-8} M DA and inhibitory effect of 10^{-4} M concentrationof DA in the presence of 4 mM and 20mM glucose (Fig: 38).

Effect of dopamine agonists in glucose induced insulin secretion

Dopamine D_2 receptor agonist bromocriptine (BRC) was incubated along with the pancreatic islets in the presence of 4mM and 20mM glucose concentrations. Bromocriptine at 10^{-8} M concentration had a lesser inhibitory effect on glucose induced insulin secretion in the presence of 20mM glucose ($p<0.001$) when compared to 10^{-4} M BRC (Fig: 39; $p<0.001$). Bromocriptine at 10^{-8} M in the presence of 4mM glucose significantly stimulated insulin secretion when compared to 4mM glucose without BRC (Fig: 39). 10^{-4} M BRC in the presence of 4mM glucose had no effect on insulin secretion.

7-OH DPAT an agonist for dopamine D_2 like receptor had an inhibitory effect on insulin secretion in both the concentrations of glucose when compared to control ($p<0.001$; Fig: 39).

Effect of norepinephrine on dopaminergic involvement in glucose induced insulin secretion

Norepinephrine in two concentrations (10^{-8} and 10^{-4} M) were added to DA (10^{-8} M and 10^{-4} M) in 4mM and 20mM glucose incubated with cells. It was observed that 10^{-8} M NE did not have any affect on the stimulatory effect of 10^{-8} M DA nor on the inhibitory effect of 10^{-4} M DA in both the glucose concentrations incubated with cells (Fig:40). But 10^{-4} M NE in presence of 10^{-8} M DA in 4mM and 20mM glucose significantly inhibited ($p<0.001$) the stimulatory effect of dopamine (Fig:40). A combination of 10^{-4} M NE and 10^{-4} M DA totally inhibited the glucose induced insulin secretion (Fig:40).

TABLE-1

BODY WEIGHT AND BLOOD GLUCOSE LEVELS OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS

ANIMAL STATUS	BODY WEIGHT (g)	BLOOD GLUCOSE (mg/dl blood)
CONTROL	296.00 ± 6.78	101.14 ± 1.64
DIABETIC	$218.00 \pm 8.60^{***}$	$553.90 \pm 4.71^{***}$
INSULIN TREATED DIABETIC	$272.00 \pm 6.63^{*‡‡}$	$109.46 \pm 8.83^{‡‡}$

Values are Mean ± S.E.M. of 4-6 separate experiments

$^{***}p<0.001$ with respect to control
$^{‡‡}p<0.001$ with respect to diabetic
$^{*}p<0.05$ with respect to control

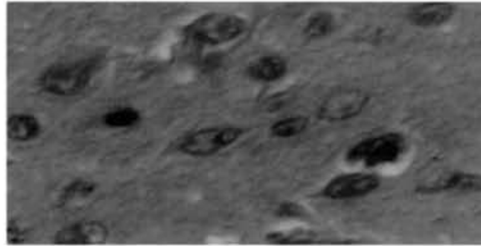
Figure: 4

Slides were prepared from processed striatum of control, diabetic and insulin treated diabetic rats stained with periodic acid stain and observed under light microscope (x100)

A) Control: no glycogen granules observed. Well defined cells in the corpus striatum are observed.
B) Diabetic: 14 day diabetic rat corpus striatum shows glycogen deposits that is stained blue. (⟶) The cellular contents are decreased.
C) Insulin treated diabetic: Insulin treatment for 14 days decreased the glycogen deposits. (⟶)

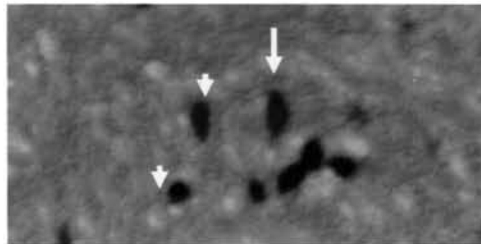
Figure-4
Histological sections of corpus striatum after periodic acid staining

a)



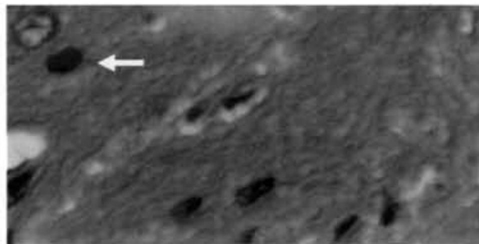
No glycogen granules

b)



Glycogen granules

c)



Glycogen granules
(Less number)

a) Control
b) Diabetic
c) Insulin treated

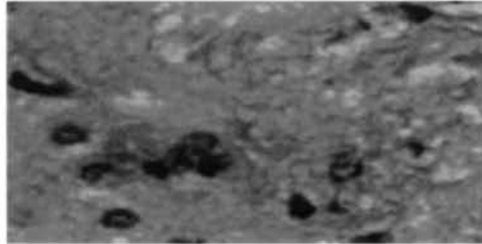
Figure: 5

Slides were prepared from processed hypothalamus of control, diabetic and insulin treated diabetic rats stained with periodic acid stain and observed under light microscope (x100)

A) Control: scattered one or two granules observed.
B) Diabetic: 14day diabetic rat hypothalamus shows accumulation of glycogen deposits that is stained blue (⟶)
C) Insulin treated diabetic: Insulin treatment for 14days decreased the glycogen deposit (⟶)

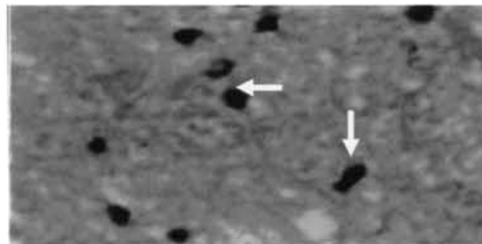
Figure-5
Histological sections of hypothalamus after periodic acid staining

a)



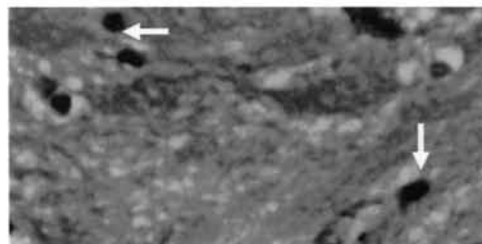
No glycogen granules

b)



Glycogen granules

c)



Glycogen granules
(Less number)

a) Control
b) Diabetic
c) Insulin treated

Figure: 6

Slides were prepared from processed cerebral cortex of control, diabetic and insulin treated diabetic rats stained with periodic acid stain and observed under light microscope (x100)

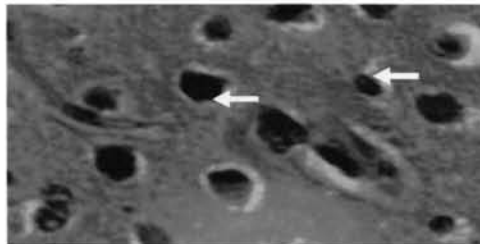
A) Control: No glycogen granules observed.
B) Diabetic: 14day diabetic rat cerebral cortex shows accumulation of glycogen deposits that is stained blue (⟶)
C) Insulin treated diabetic: Insulin treatment for 14days decreased the glycogen deposits (⟶)

Figure-6
Histological sections of cerebral cortex after periodic acid staining

a)

No glycogen granules

b)



Glycogen granules

c)

Glycogen granules
(Less numbers)

a) Control
b) Diabetic
c) Insulin treated

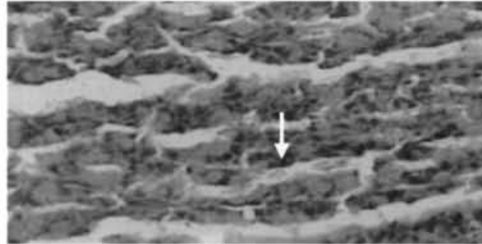
Figure: 7

Slides were prepared from processed pancreas of control, diabetic and insulin treated diabetic rats stained with hematoxylin eosin stain observed under light microscope (x 40)

A) Control: pancreatic tissue section from control rats with distinct nuclei (⟶)
B) Diabetic: pancreatic tissue section from diabetic rats showing distorted nuclei and the cells (⟶)
C) Insulin treated diabetic: pancreatic tissue section from insulin treated diabetic rats showing the reduced the damage to the tissue. (⟶)

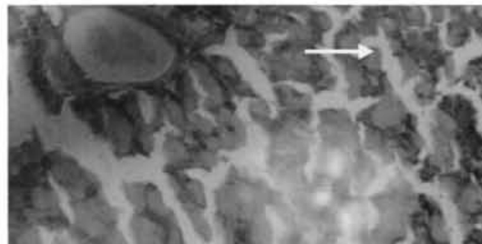
Figure: 7
Histological staining in the pancreas with hematoxylin and eosin stain

a)



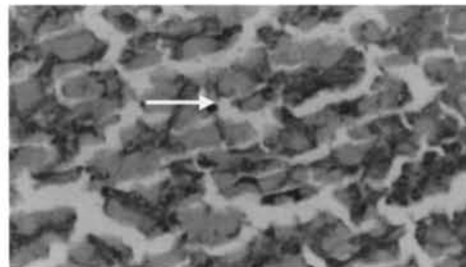
Pancreas which are nucleated

b)



Degenerated pancreas with depleted nucleus

c)



Pancreas showing less nucleus after insulin treatment

a) Control
b) Diabetic
c) Insulin treated

Figure: 8

Dopamine changes in the corpus striatum at various time intervals after administration of streptozotocin (65mg /kg body wt.)in rats.

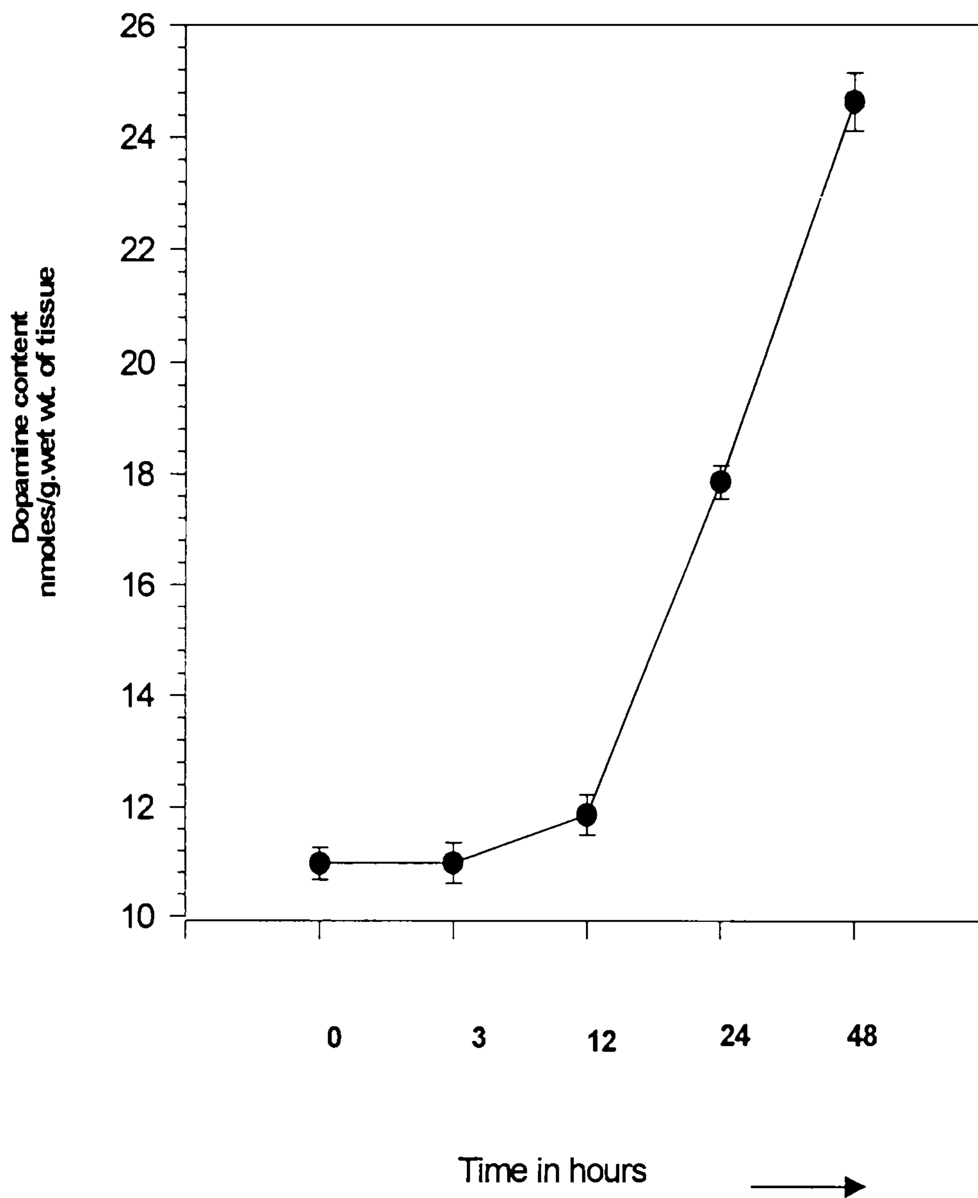
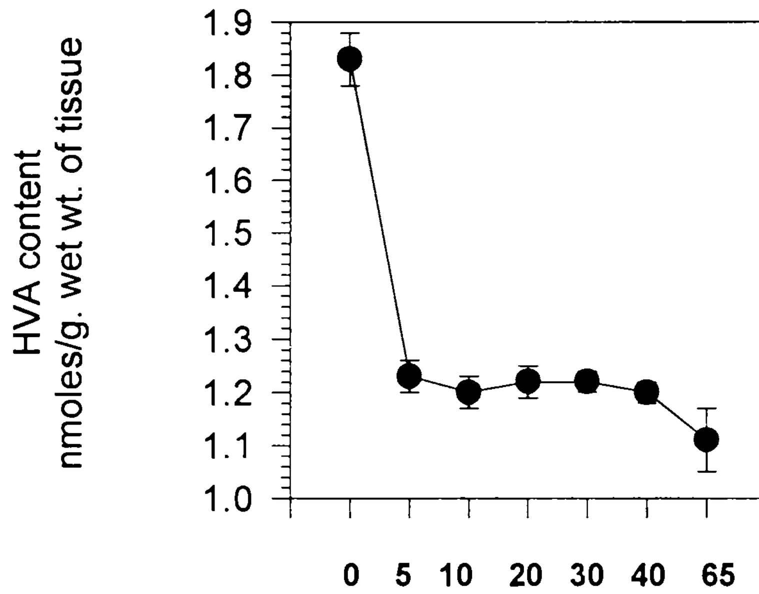


Figure: 9
Changes in homovanillic acid (HVA) and HVA/Dopamine(DA) ratio in the corpus striatum after the injection of different doses of streptozotocin



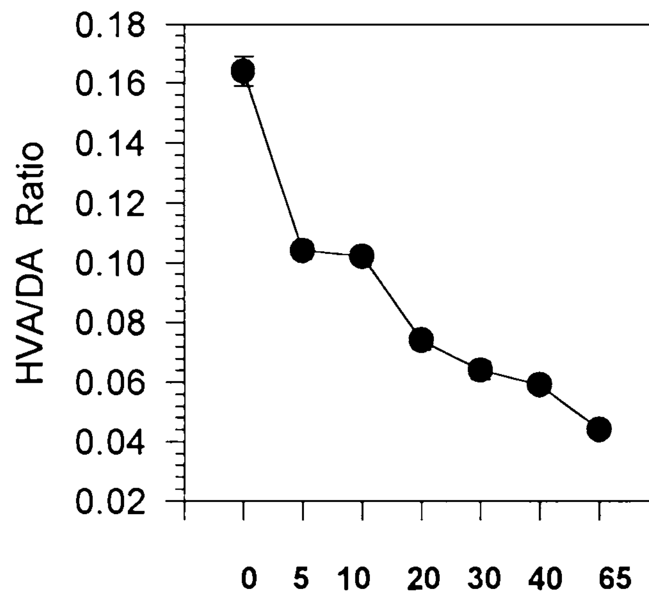


Figure: 10

Dopamine content in the corpus striatum of rats injected with various doses of streptozotocin

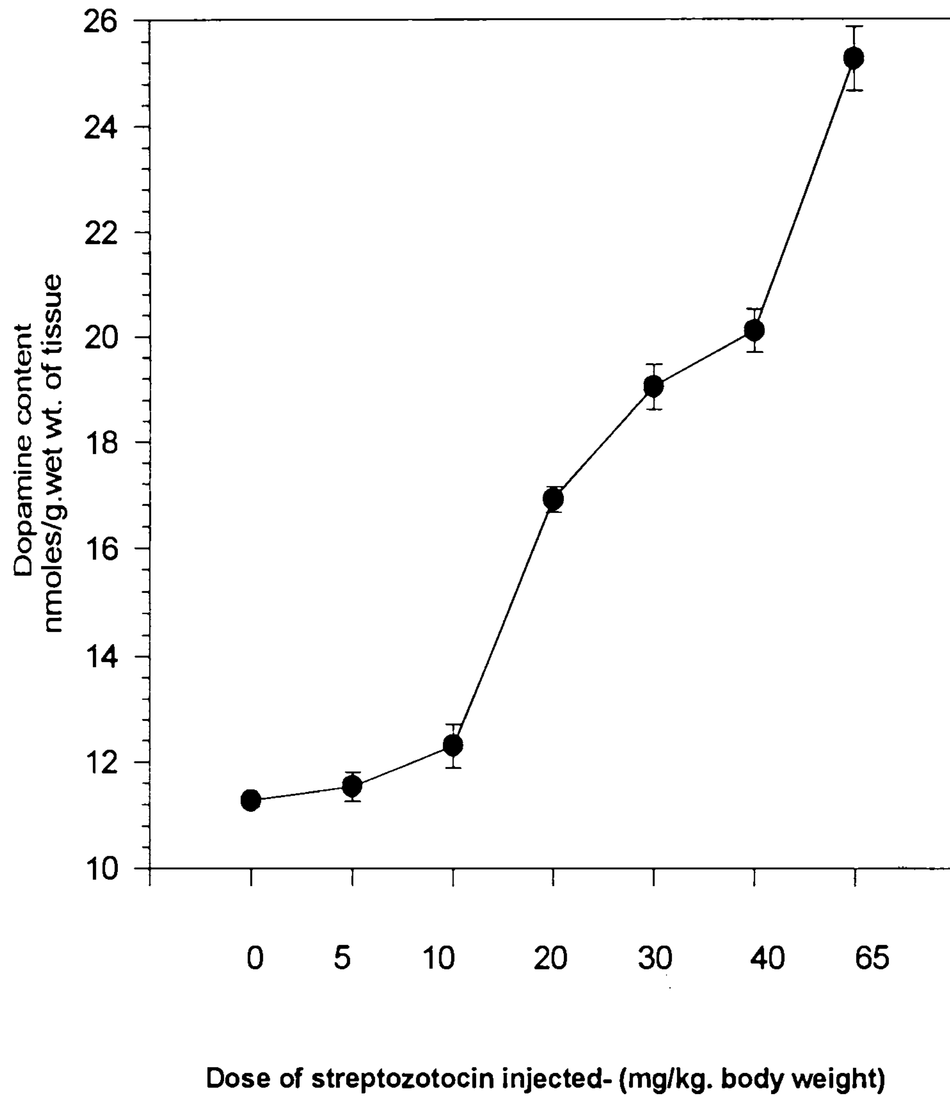
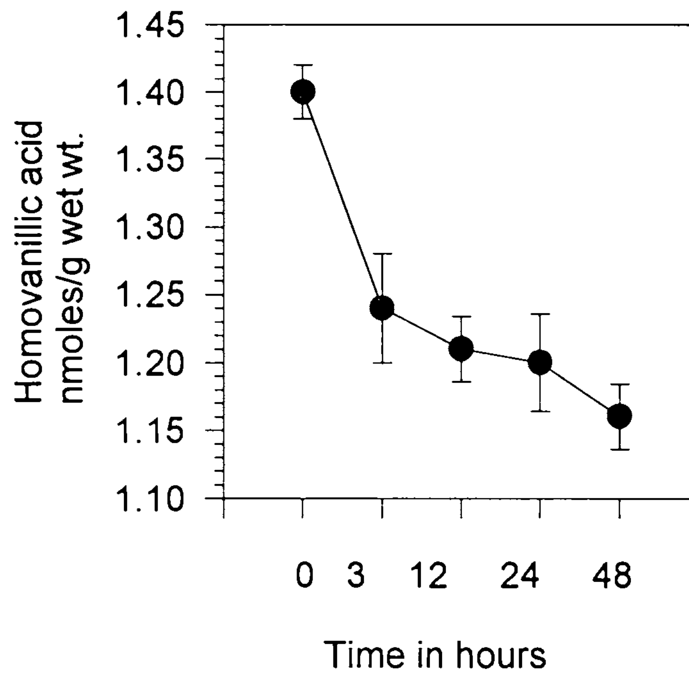


Figure: 11
Changes in homovanillic acid (HVA) and HVA/Dopamine(DA) ratio in the corpus striatum at various time intervals after streptozotocin (65 mg/ kg bodt wt.) injection



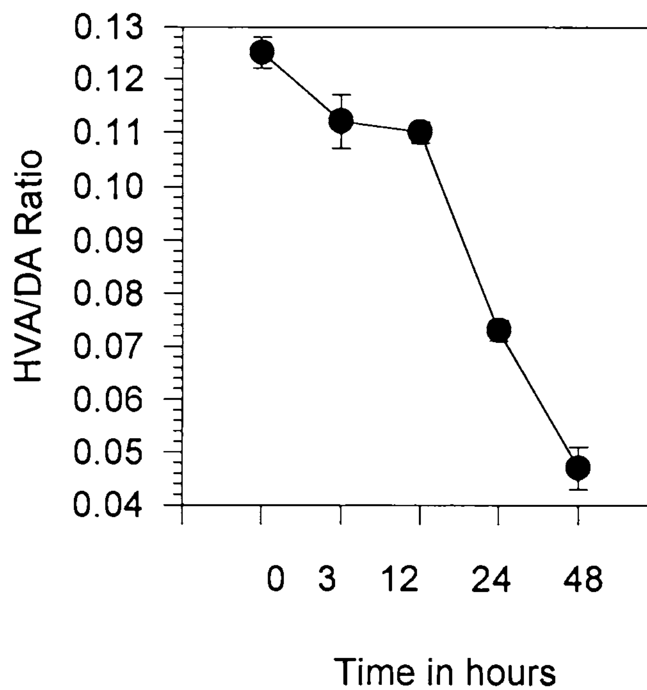


TABLE -2

DOPAMINE AND HOMOVANILLIC ACID CONTENTS IN THE CORPUS STRIATUM OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS (nmoles/ g. wet wt. of tissue)

ANIMAL STATUS	DOPAMINE	HVA	HVA/DA RATIO
CONTROL	12.31 ± 0.28	2.60 ± 0.025	0.160 ± 0.003
DIABETIC	$20.75 \pm 0.95^{***}$	$1.87 \pm 0.026^{*}$	$0.083 \pm 0.001^{***}$
INSULIN TREATED DIABETIC	$18.10 \pm 0.32^{***\dagger\dagger}$	$2.35 \pm 0.020^{\dagger\dagger}$	$0.092 \pm 0.005^{***}$

Values are Mean ± S.E.M. of 4-6 separate experiments
$^{***}p<0.001$ compared to control
$^{*}p<0.05$ compared to control
$^{\dagger\dagger}p<0.01$ compared to diabetic

DA: dopamine; HVA: homovanillic acid

TABLE-3

DOPAMINE AND HOMOVANILLIC ACID CONTENTS IN THE HYPOTHALAMUS OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS (nmoles/ g. wet wt. of tissue)

ANIMAL STATUS	DOPAMINE	HVA	HVA/DA Ratio
CONTROL	0.99 ± 0.041	0.26 ± 0.034	0.27±0.010
DIABETIC	0.32 ± 0.026***	0.10 ± 0.010***	0.33±0.010
INSULIN TREATED DIABETIC	0.69 ± 0.024*** +++	0.17 ± 0.065***	0.24±0.056

Values are Mean ± S.E.M. of 4-6 separate experiments

***p<0.001 when compared to control

+++p<0.001 when compared to diabetic

DA: dopamine; HVA: homovanillic acid

TABLE-4

DOPAMINE AND HOMOVANILLIC ACID CONTENTS IN THE CEREBRAL CORTEX OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS (nmoles/g. wet weight of tissue)

ANIMAL STATUS	DOPAMINE	HVA	HVA/DA RATIO
CONTROL	0.54 ± 0.130	0.28 ± 0.014	0.55 ± 0.100
DIABETIC	$3.02 \pm 0.045^{***}$	$0.96 \pm 0.035^{***}$	$0.32 \pm 0.020^{**}$
INSULIN TREATED DIABETIC	$2.02 \pm 0.110^{***\ +++}$	$0.91 \pm 0.043^{***}$	$0.45 \pm 0.030^{+}$

Values are Mean ± S.E.M. of 4-6 separate experiments

$^{***}p<0.001$ compared to control

$^{**}p<0.01$ compared to control
$^{+++}p<0.01$ compared to diabetic
$^{+}p<0.05$ compared to diabetic

DA: dopamine; HVA: homovanillic acid

TABLE -5

DOPAMINE AND HOMOVANILLIC ACID CONTENTS IN THE BRAIN STEM OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS (nmoles/ g. wet wt. of tissue)

ANIMAL STATUS	DOPAMINE	HVA	HVA/DA RATIO
CONTROL	0.54±0.017	0.41±0.023	0.76±0.025
DIABETIC	0.29±0.010***	0.20±0.030***	0.68±0.060
INSULIN TREATED DIABETIC	0.31±0.010***	0.26±0.034***	0.84±0.131

Values are Mean ± S.E.M. of 4-6 separate experiments

***$p<0.001$ when compared to control

DA: dopamine; HVA: homovanillic acid

TABLE-6

EPINEPHRINE, NOREPINEPHRINE, DOPAMINE AND HOMOVANILLIC ACID LEVELS IN THE PLASMA OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS (nmoles/ ml plasma)

ANIAML STATUS	Epinephrine	Norepinephrine	Dopamine	HVA	HVA/DA Ratio
CONTROL	2.62±0.25	1.22±0.070	0.8±.0.036	0.57±0.028	0.71±0.030
DIABETIC	$10.85\pm0.57^{***}$	$4.40\pm0.12^{***}$	$0.30\pm0.027^{***}$	$0.19\pm0.060^{***}$	$0.32\pm0.040^{***}$
INSULIN TREATED DIABETIC	$4.34\pm0.20^{*}$ +++	$2.56\pm0.19^{**}$	$0.51\pm0.010^{***+++}$	$0.35\pm0.012^{**+}$	$0.68\pm0.030^{+++}$

Values are Mean ± S.E.M. of 4-6 separate experiments

***P<0.001 when compared to control
**p<0.01 when compared to control
*p<0.05 when compared to control
+++p<0.001 when compared to diabetic
+p<0.05 when compared to diabetic

DA: dopamine; HVA: homovanillic acid

TABLE-7

EPINEPHRINE, NOREPINEPHRINE, DOPAMINE, HOMOVANILLIC ACID CONTENTS IN THE ADRENALS OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS
(nmoles /g.wet wt. of tissue)

ANIMAL STATUS	EPINEPHRINE	NOREPINEPHRINE	DOPAMINE	HVA	EPI/NE RATIO	NE/DA RATIO	HVA/DA RATIO
CONTROL	123.17±2.77	89.24±0.544	11.36±0.812	3.47±0.231	0.740±0.028	0.124±0.011	3.28±0.123
DIABETIC	341.60±7.77***	223.65±0.678***	3.76±0.278***	1.18±0.072***	0.929±0.011***	0.017±0.002***	3.19±0.167
INSULIN TREATED DIABETIC	170.76±3.89$^{*** +++}$	112.01±0.246$^{*** +++}$	6.39±0.204$^{*** +}$	1.97±0.129$^{*** +}$	0.67±0.041^{+++}	0.057±0.002$^{*** ++}$	3.31±0.146

Values are Mean ± S.E.M. of 4-6 separate experiments

*** $p<0.001$ when compared to control

\+ $p<0.05$ when compared to control
++$p<0.01$ when compared to diabetic
+++ $p<0.001$ when compared to diabetic

DA: dopamine; HVA: homovanillic acid; NE : norepinephrine; EPI: epinephrine

TABLE-8

DOPAMINE AND HOMOVANILLIC ACID CONTENTS IN THE PANCREAS OF CONTROL, DIABETIC AND INSULIN TREATED DIABETIC RATS (nmoles/ g. wet wt. of tissue)

ANIMAL STATUS	DOPAMINE	HVA	HVA/DA RATIO
CONTROL	2.07± .0048	1.03± 0.06	0.49±0.013
DIABETIC	0.84± 0.036***	0.22± 0.013***	0.26±0.013***
INSULIN TREATED DIABETIC	1.02 ± 0.035 $^{***+}$	0.33±0.013^{***+}	0.35±0.015^{***+}

Values are Mean ± S.E.M. of 4-6 separate experiments

***P<0.001 when compared to control
+++P<0.001 when compared to diabetic
+P<0.05 when compared to diabetic

DA: dopamine; HVA: homovanillic acid

Figure-12

Scatchard analysis of [^{3}H] dopamine binding against butaclamol in the corpus striatum of control, diabetic and insulin treated diabetic rats

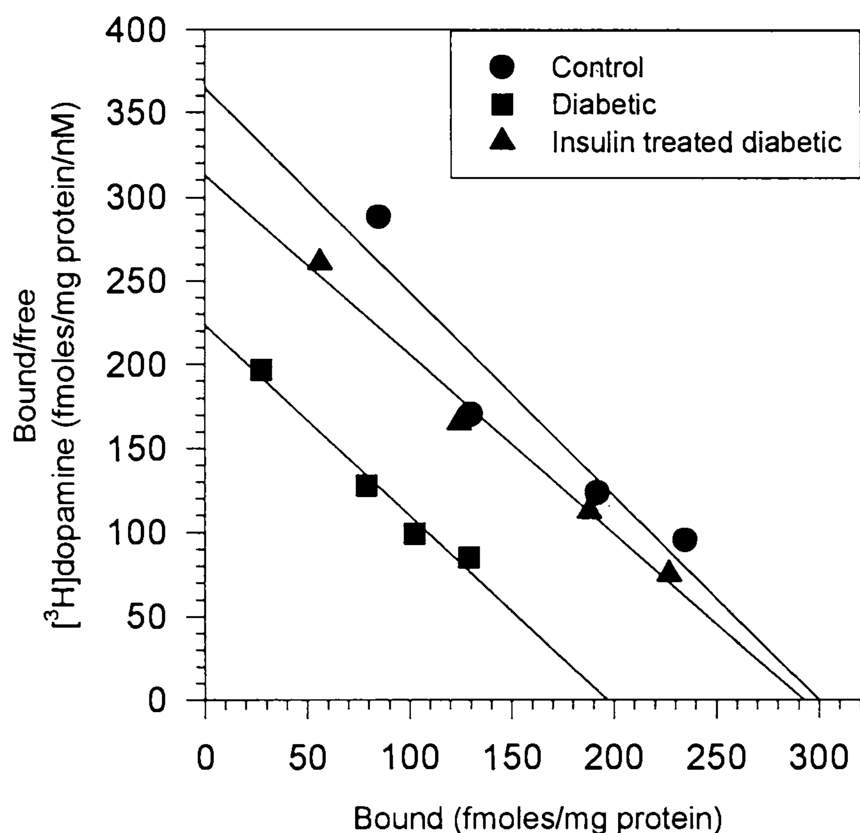


Table-9

[^{3}H] dopamine binding parameters in the corpus striatum of control, diabetic and insulin treated diabetic rats

Animal status	Bmax (fmoles/mg protein)	Kd(nM)
Control	301±1.25	0.83±0.025
Diabetic	196±0.82***	0.89±0.048
Insulin treated diabetic	294±3.08+++	0.95±0.023**

Values are a Mean ± S.E.M. of 4-6 separate experiments

**p<0.01 when compared to control

***P<0.001 when compared to control

+++p<0.001 when compared to diabetic

Table: 10

Binding parameters of [^{3}H] dopamine against dopamine in corpus striatum of control, diabetic and insulin treated diabetic rats

Animal status	Best fit model	Log(EC_{50})	Ki	Hill Slope
Control	One site	-7.496	$2.452x10^{-8}$	-1.087
Diabetic	One site	-7.501	$2.460x10^{-8}$	-1.000
Insulin treated diabetic	One site	-7.544	$2.025x10^{-7}$	-0.944

Figure: 13

Displacement of [^{3}H] dopamine with dopamine in the corpus striatum of rats

Values are mean of 3-5 separate experiments.

Data were fitted with an iterative nonlinear regression software (prism, Graph Pad, San Diego, CA).
Ki- the affinity of the receptor for the competing drugs.
EC_{50} is the concentration of the competitor that competes for half specific binding.

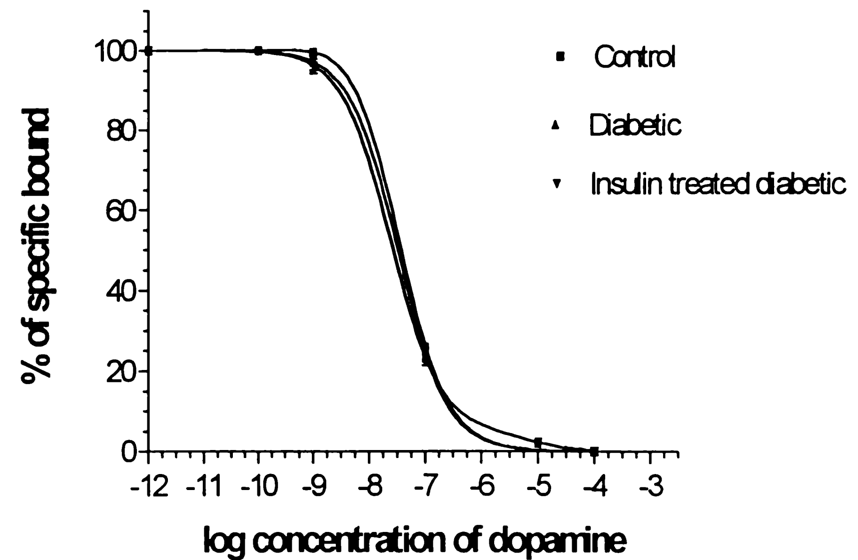


Figure-14

Scatchard analysis of [^{3}H] dopamine binding against butaclamol in the cerebral cortex of control, diabetic and insulin treated diabetic rats

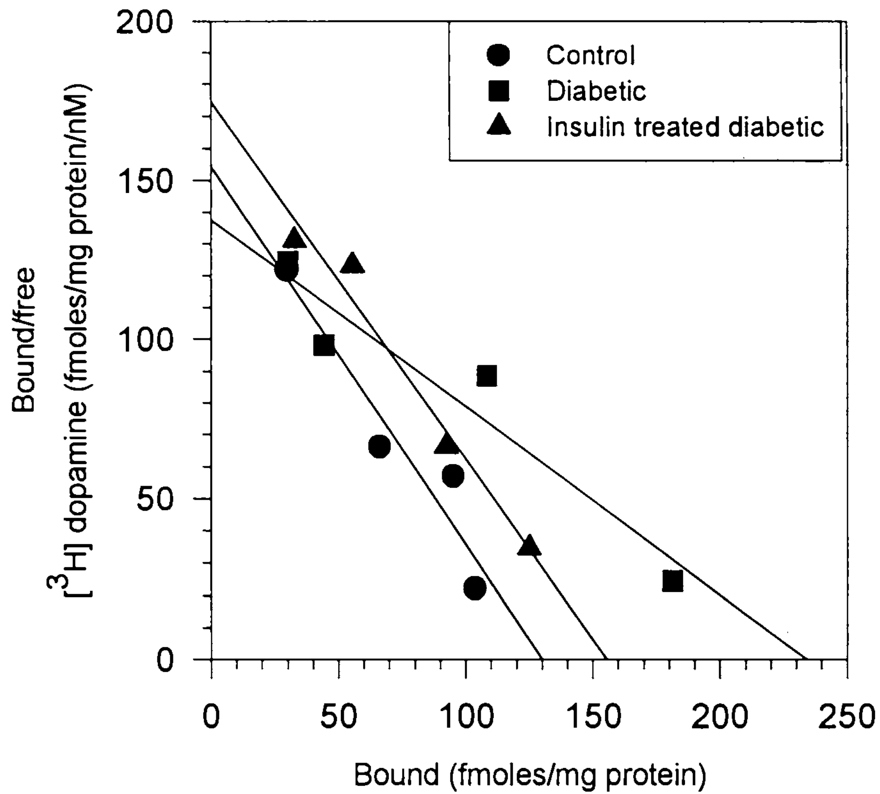


Table: 11

[^{3}H] dopamine binding parameters in the cerebral cortex control, diabetic and insulin treated diabetic rats

Animal status	Bmax (fmoles/mgprotein)	Kd (nM)
Control	129.13±1.20	0.94±0.027
Diabetic	234.19±1.01***	1.71±0.030***
Insulin treated diabetic	156.46±1.38$^{***\ +++}$	1.01±0.036

Values are mean ± of 4-6 separate experiments

***P<0.001 when compared to control

$^{+++}$p<0.001 when compared to diabetic

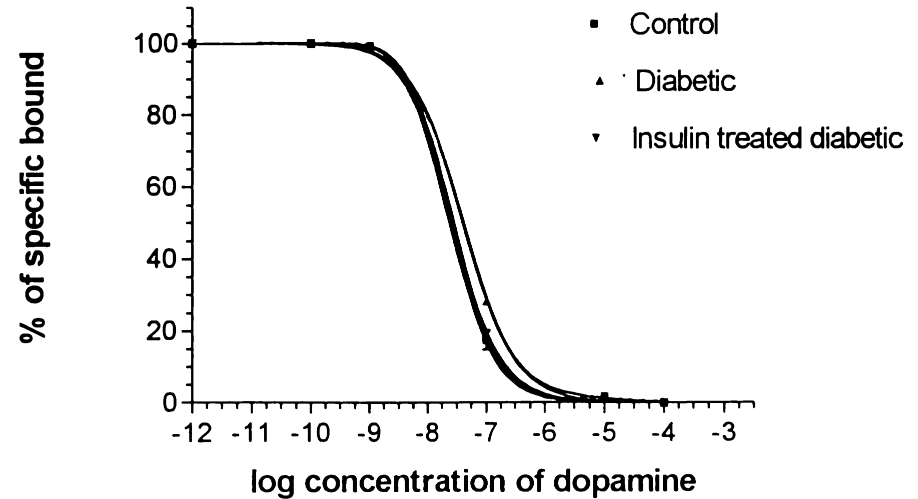
Table: 12

Binding parameters of [^{3}H] dopamine against dopamine in cerebral cortex of control, diabetic and insulin treated diabetic rats

Animal status	Best fit model	Log(EC_{50})	Ki	Hill slope
Control	One site	-7.621	$1.892x10^{-8}$	-1.128
Diabetic	One site	-8.366	$3.415x10^{-7}$	-0.981
Insulin treated diabetic	One site	-7.530	$2.173x10^{-8}$	-1.107

Figure: 15

Displacement of [^{3}H] dopamine with dopamine in the cerebral cortex of rats



Values are mean of 3-5 separate experiments.

Data were fitted with an iterative nonlinear regression software (prism, Graph Pad, San Diego, CA).

Ki- the affinity of the receptor for the competing drugs.

EC_{50} is the concentration of the competitor that competes for half specific binding.

Figure-16

Scatchard analysis of [^{3}H] dopamine binding against butaclamol in the hypothalamus of control, diabetic and insulin treated diabetic rats

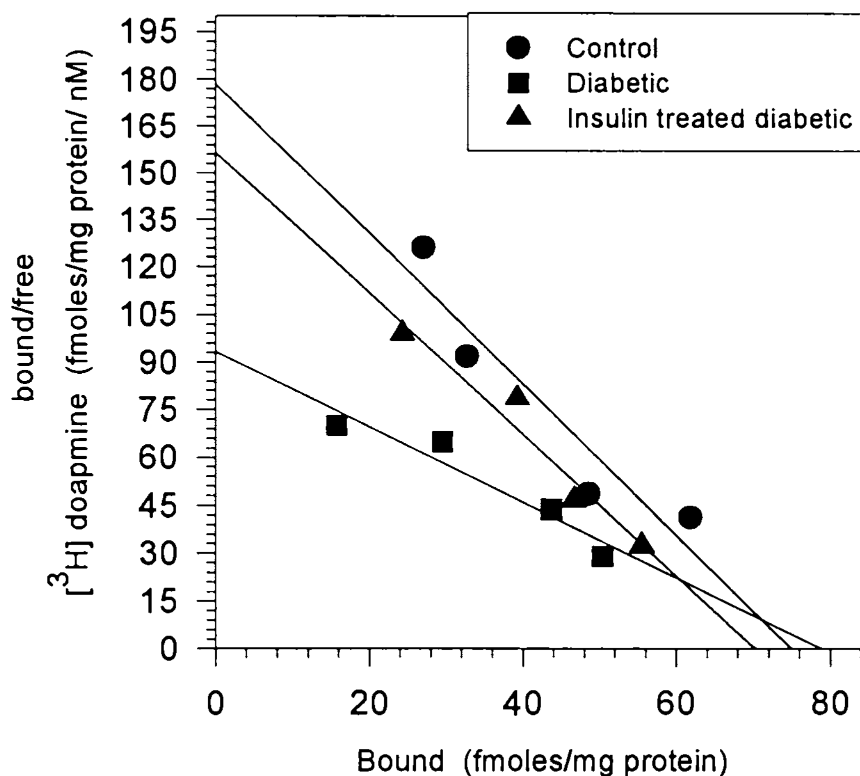


Table-13

[^{3}H] dopamine binding parameters in the hypothalamus of control, diabetic and insulin treated diabetic rats

Animal status	Bmax (fmoles/mg protein)	Kd (nM)
Control	75.03±1.80	0.423±0.045
Diabetic	78.50±2.04	0.844±0.023***
Insulin treated diabetic	70.00±2.17	0.440±0.063+++

Values are Mean ± S.E.M of 4-6 separate experiments

***$P<0.001$ when compared to control

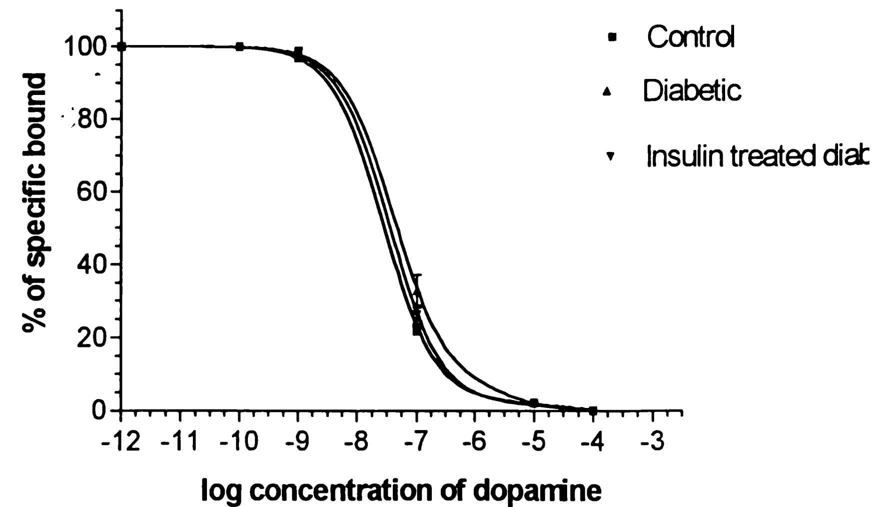
+++$p<0.001$ when compared to diabetic

Table: 14

Binding parameters of [^{3}H] dopamine against dopamine in hypothalamus of control, diabetic and insulin treated diabetic rats

Animal status	Best fit model	Log(EC_{50})	Ki	Hill Slope
Control	One site	-7.547	1.785×10^{-8}	-1.012
Diabetic	One site	-7.353	2.827×10^{-7}	-0.914
Insulin treated diabetic	One site	-7.449	2.067×10^{-8}	-1.087

Figure: 17
Displacement of [^{3}H] dopamine with dopamine in the hypothalamus of rats



Values are mean of 3-5 separate experiments

Data were fitted with an iterative nonlinear regression software (prism, Graph Pad, San Diego, CA).
Ki- the affinity of the receptor for the competing drugs.
EC_{50} is the concentration of the competitor that competes for half specific binding.

Figure-18

Scatchard analysis of [^{3}H] spiperone binding against butaclamol in the corpus striatum of control, diabetic and insulin treated diabetic rats

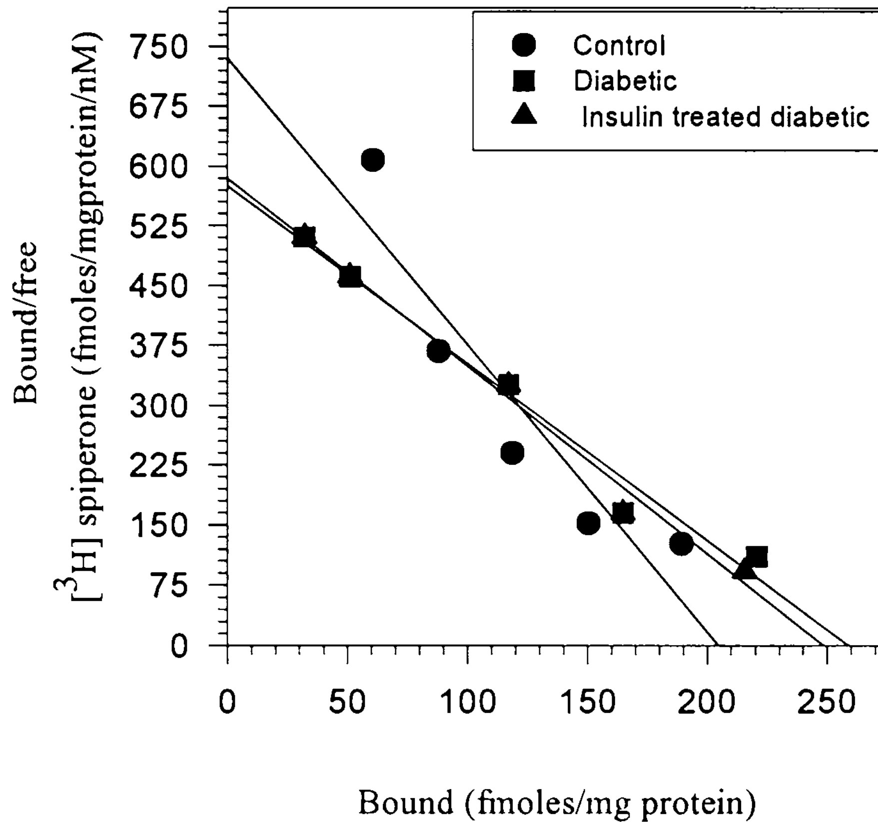


Table-15

[^{3}H] Spiperone binding parameters in the corpus striatum of control, diabetic and insulin treated diabetic rats

Animal status	Bmax (fmoles /mg protein)	Kd (nM)
Control	200.6±0.47	0.34±0.020
Diabetic	257.5±2.22***	0.40±0.019
Insulin treated diabetic	241.7±1.70***	0.37±0.015

Values are a Mean ± S.E.M. of 4-6 separate experiments

***P<0.001 when compared to control

Table: 16

Binding parameters of [^{3}H] spiperone against spiperone in corpus striatum of control, diabetic and insulin treated diabetic rats

Animal status	Best fit model	Log(EC_{50})	Ki	Hill Slope
Control	One site	-7.495	2.044×10^{-8}	-1.090
Diabetic	One site	-7.435	2.253×10^{-8}	-0.971
Insulin treated diabetic	One site	-7.412	2.170×10^{-8}	-1.017

Figure: 19

Displacement of [^{3}H] Spiperone with Spiperone in the corpus striatum of rats

Values are mean of 3-5 separate experiments

Data were fitted with an iterative nonlinear regression software (prism, Graph Pad, San Diego, CA).

Ki- the affinity of the receptor for the competing drugs.

EC_{50} is the concentration of the competitor that competes for half specific binding.

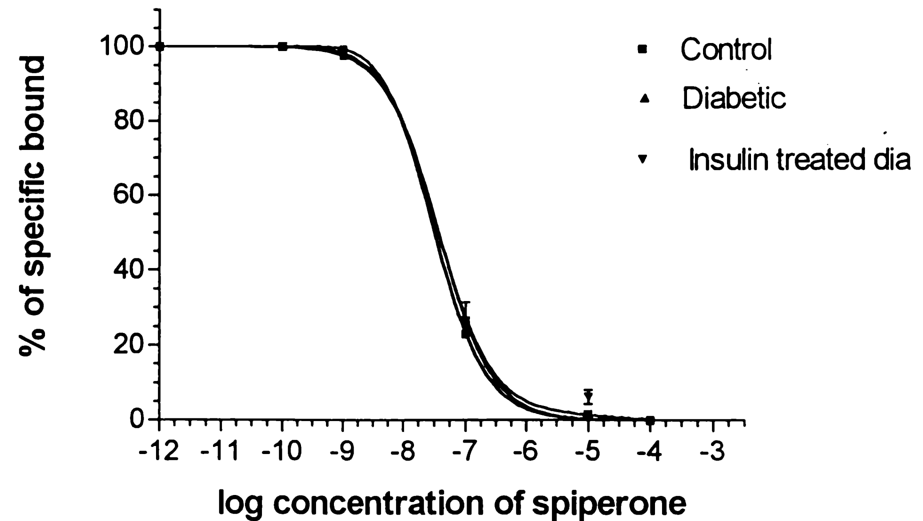


Figure-20

Scatchard analysis of [^{3}H] YM-09151-2 binding against sulpiride in the corpus striatum of control, diabetic and insulin treated diabetic rats

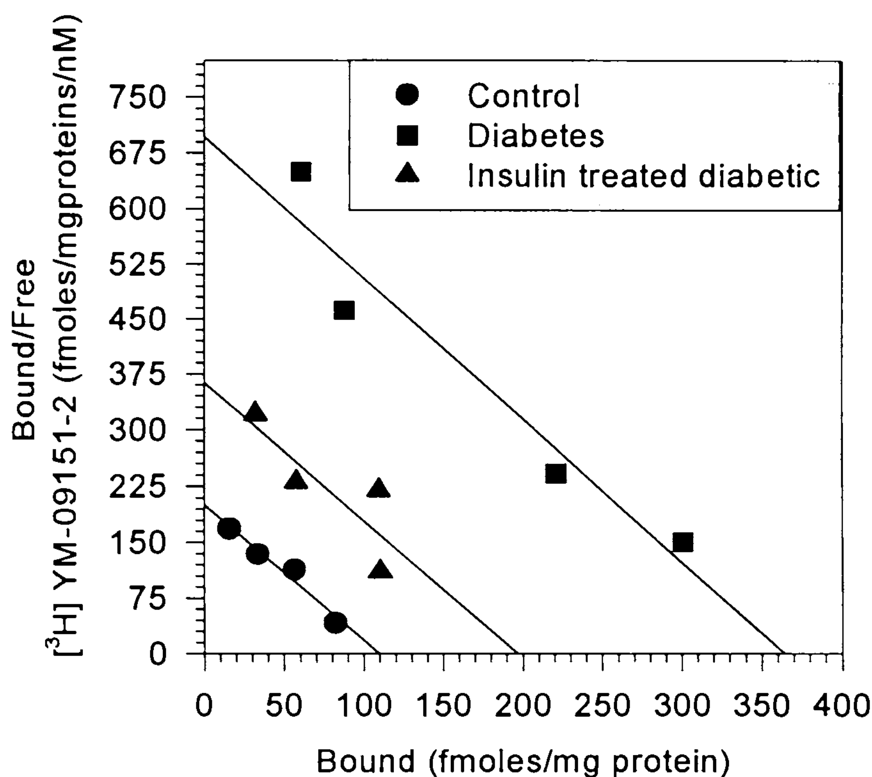


Table-17

[^{3}H] YM-09151-2 binding parameters in the corpus striatum of control, diabetic and insulin treated diabetic rats

Animal status	Bmax (fmoles/mg protein)	Kd(nM)
Control	110±0.71	0.55±0.017
Diabetic	363.5±2.01***	0.51±0.013
Diabetic insulin treated	195±1.10+++ ***	0.54±0.044

Values are a Mean ± S.E.M. of 4-6 separate experiments
***P<0.001 when compared to control
+++P<0.001 when compared to diabetic

Table: 18

Binding parameters of [^{3}H] YM-09151-2 against YM-09151-2 in corpus striatum of control, diabetic and insulin treated diabetic rats

Animal status	Best fit model	Log(EC_{50})	Ki	Hill Slope
Control	One site	-7.421	2.608×10^{-8}	-1.255
Diabetic	One site	-7.439	2.446×10^{-8}	-1.008
Insulin treated diabetic	One site	-7.404	2.698×10^{-8}	-1.116

Figure: 21
Displacement of [^{3}H] YM-09151-2 with YM-09151-2 in the corpus striatum of rats

Values are mean of 3-5 separate experiments

Data were fitted with an iterative nonlinear regression software (prism, Graph Pad, San Diego, CA).
Ki- the affinity of the receptor for the competing drugs.
EC_{50} is the concentration of the competitor that competes for half specific binding.

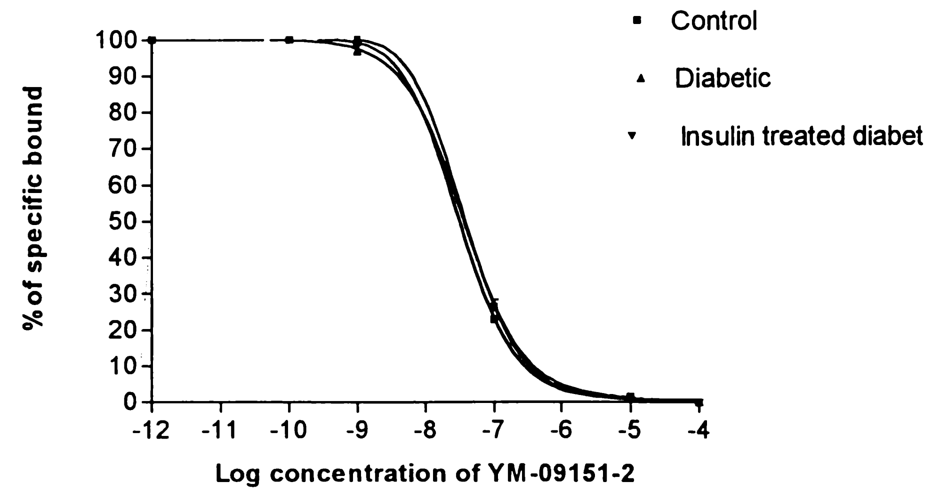


Figure-22
RT-PCR amplification product of dopamine D_2 receptor mRNA from the corpus striatum of control, diabetic and insulin treated diabetic rats

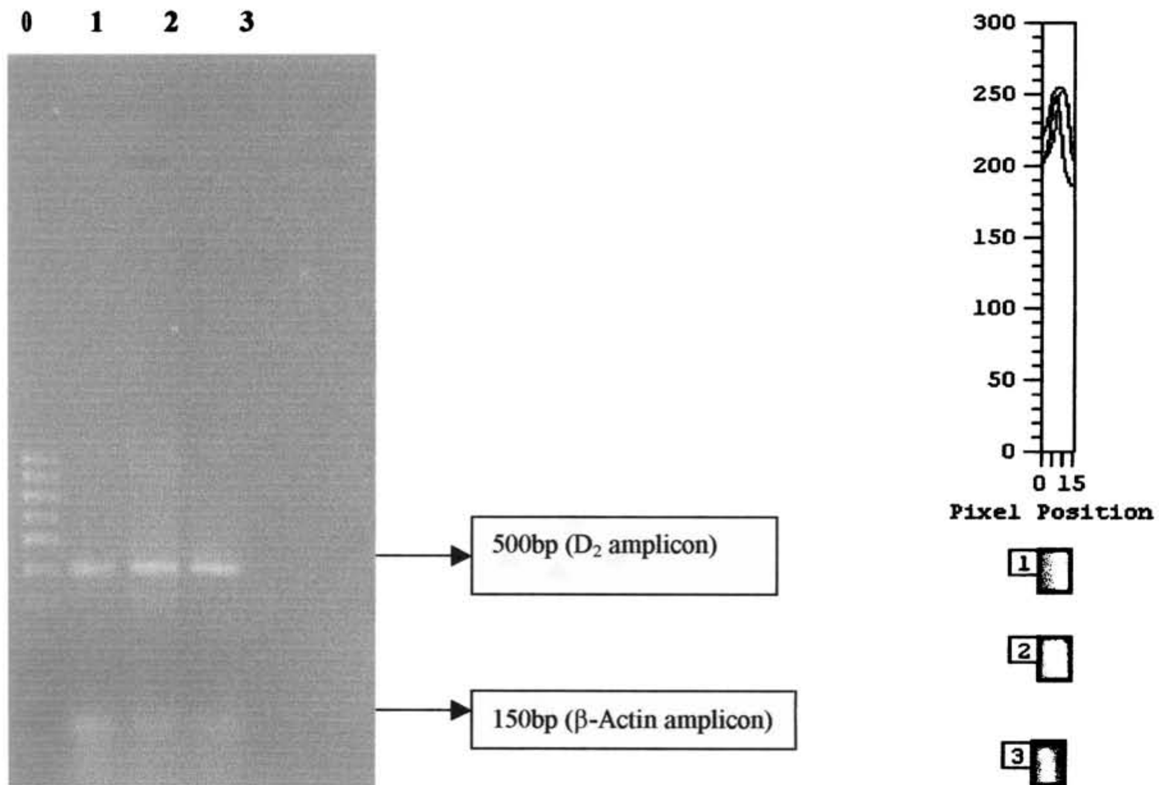


Table: 19

Band properties of dopamine D_2 receptor mRNA RT-PCR amplicon

Lane No.	Raw Volume	Area	Peak
1	44,251.00	192	247.62
2	48,248.00	192	254.96
3	45,077.00	192	251.42

0-100bp marker
1-Control
2-Diabetic
3-Insulin treated diabetic rat

Figure-23

Scatchard analysis of [^{3}H] YM-09151-2 binding against sulpiride in the cerebral cortex of control, diabetic and insulin treated diabetic rats

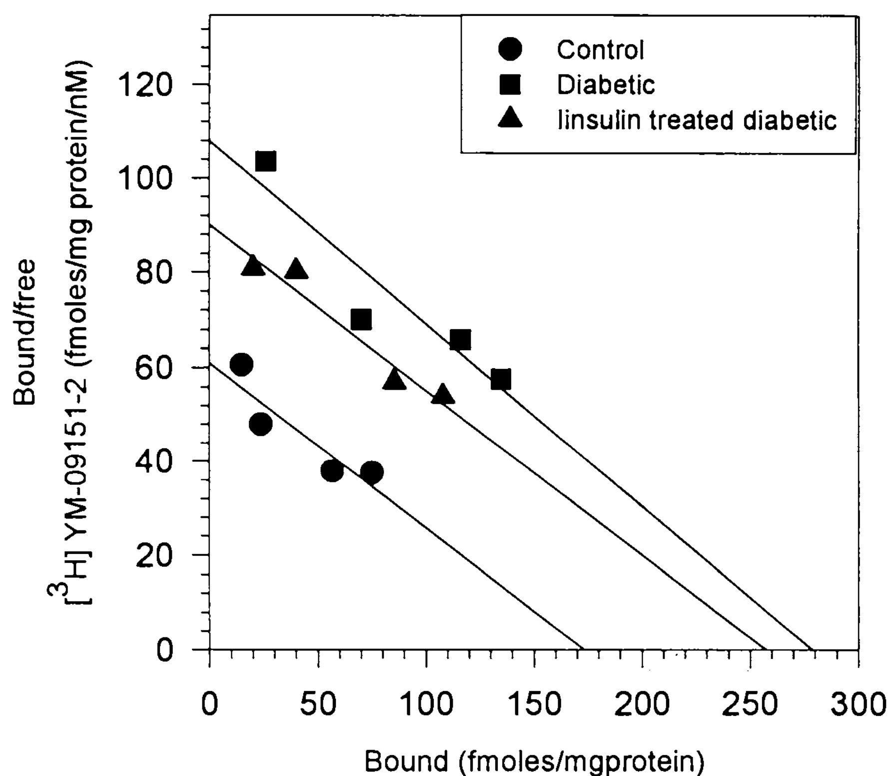


Table-20

[^{3}H] YM-09151-2 binding parameters in the cerebral cortex of control, diabetic and insulin treated diabetic rats

Animal Status	Bmax (fmoles/mg protein)	Kd (nM)
Control	172.00±3.60	2.87±0.069
Diabetic	280.00±9.04***	2.60±0.101
Insulin treated diabetic	256.00±9.51***	2.84±0.025

Values are mean ± of 4-6 separate experiments
***P<0.001 when compared to control

Binding parameters of [^{3}H] YM-09151-2 against YM-09151-2 in cerebral cortex of control, diabetic and insulin treated diabetic rats

Animal status	Best fit model	Log(EC_{50})	Ki	Hill slope
Control	One site	-7.041	6.973×10^{-8}	-1.019
Diabetic	One site	-7.051	7.008×10^{-8}	-0.970
Insulin treated diabetic	One site	-7.080	7.314×10^{-8}	-1.000

Figure: 24

Displacement of [^{3}H] YM-09151-2 with YM-09151-2 in the cerebral cortex of rats

Values are mean of 3-5 separate experiments.

Data were fitted with an iterative nonlinear regression software (prism, Graph Pad, San Diego, CA).

Ki- the affinity of the receptor for the competing drugs.

EC_{50} is the concentration of the competitor that competes for half specific binding.

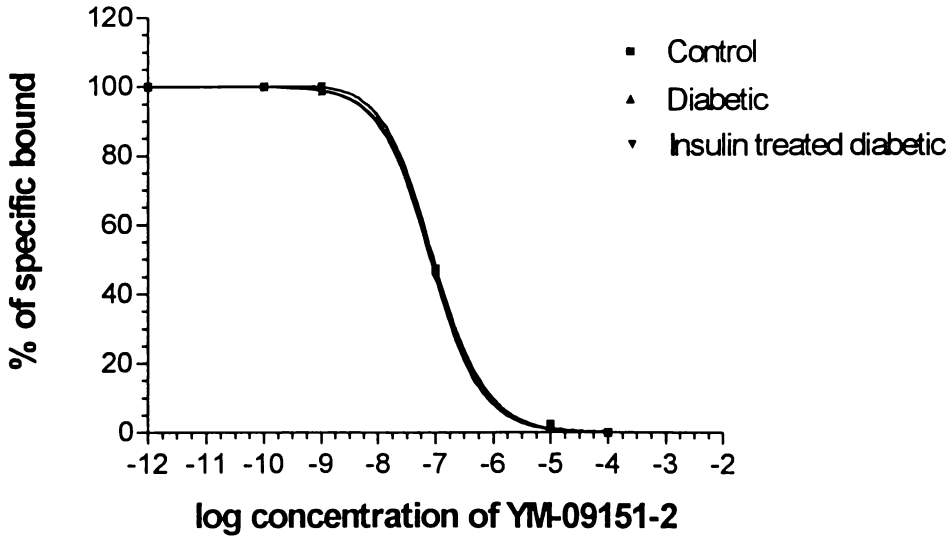


Figure-25

RT-PCR amplification product of dopamine D_2 receptor mRNA from the cerebral cortex of control, diabetic and insulin treated diabetic rats

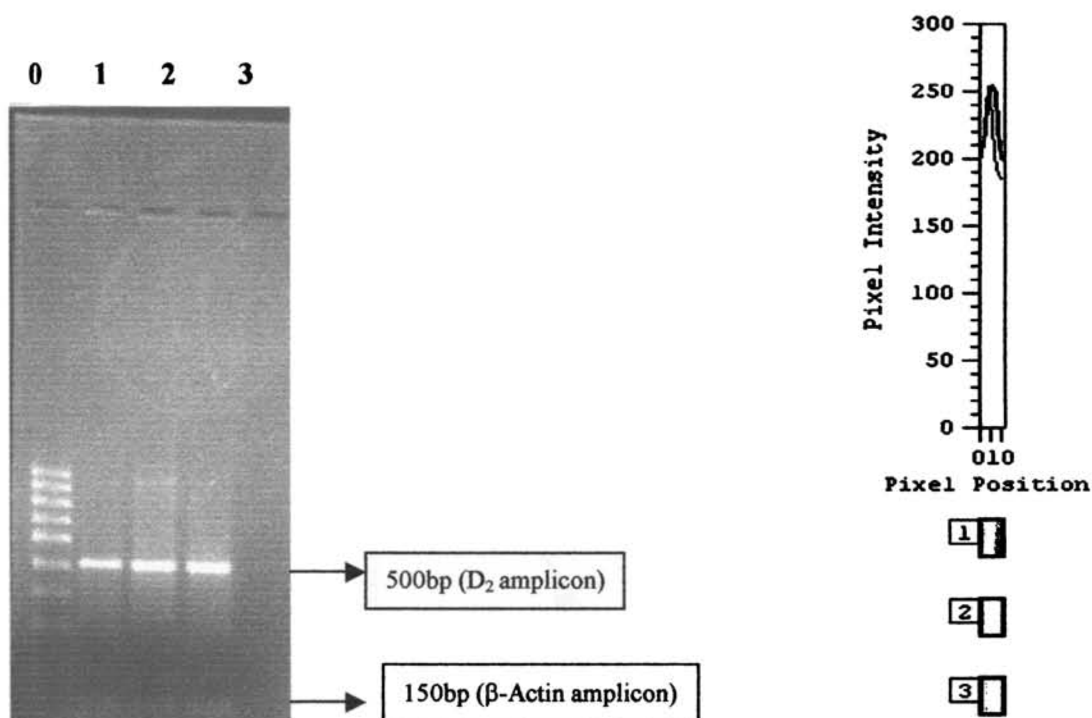


Table:22

Band properties of dopamine D2 receptor mRNA RT-PCR amplicon

Lane No	Raw Volume	Area	Peak
1	20,811	168	250.59
2	26,690	168	253.91
3	25,023	168	252.50

0-100bp marker
1-Control
2-Diabetic
3-Insulin treated diabetic

Figure-26

Scatchard analysis of [^{3}H] YM-09151-2 binding against sulpiride in the hypothalamus of control, diabetic and insulin treated diabetic rats

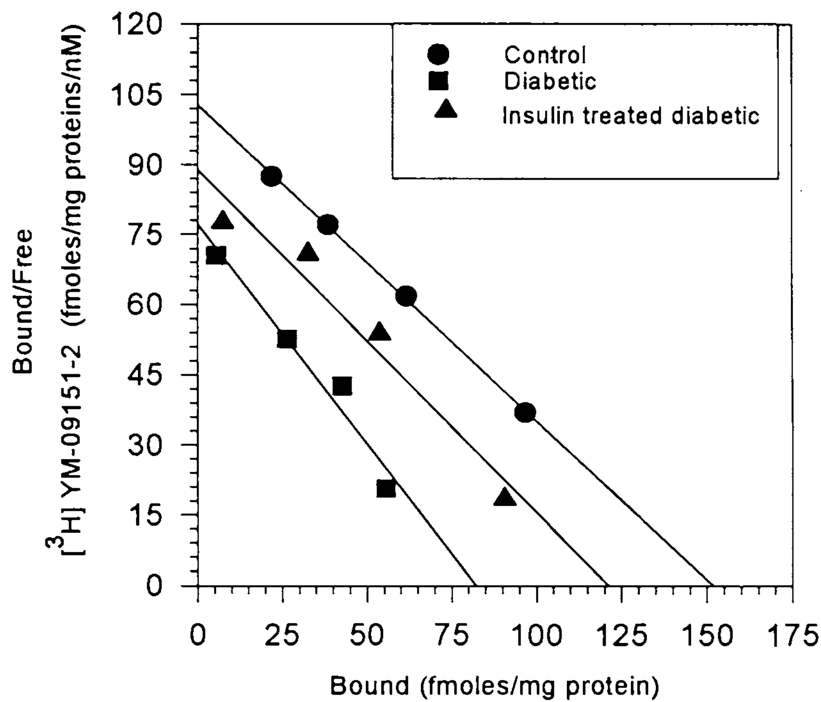


Table-23

[^{3}H] YM-09151-2 binding parameters in the hypothalamus of control, diabetic and insulin treated diabetic rats

Animal status	Bmax (fmoles/mgprotein)	Kd (nM)
Control	151.20±1.034	1.48±0.0125
Diabetic	82.0±1.014***	1.08±0.0122***
Insulin treated diabetic	112.20±1.163***+++	1.27±0.0200** +++

Values are Mean ± S.E.M of 4-6 separate experiments

**p<0.01 when compared to control

***P<0.001 when compared to control

+++p<0.001 when compared to diabetic

Table: 24

Binding parameters of [^{3}H] YM-09151-2 against YM-09151-2 in hypothalamus of control, diabetic and insulin treated diabetic rats

Animal status	Best fit model	Log(EC_{50})	Ki	Hill slope
Control	One site	-7.672	$1.664x10^{-8}$	-1.046
Diabetic	One site	-7.837	$1.246x10^{-8}$	-1.000
Insulin treated diabetic	One site	-7.767	$1.371x10^{-8}$	-0.952

Figure: 27

Displacement of [^{3}H] YM-09151-2 with YM-09151-2 in the hypothalamus of rats

Values are mean of 3-5 separate experiments

Data were fitted with an iterative nonlinear regression software (prism, Graph Pad, San Diego, CA).

Ki- the affinity of the receptor for the competing drugs.

EC_{50} is the concentration of the competitor that competes for half specific binding.

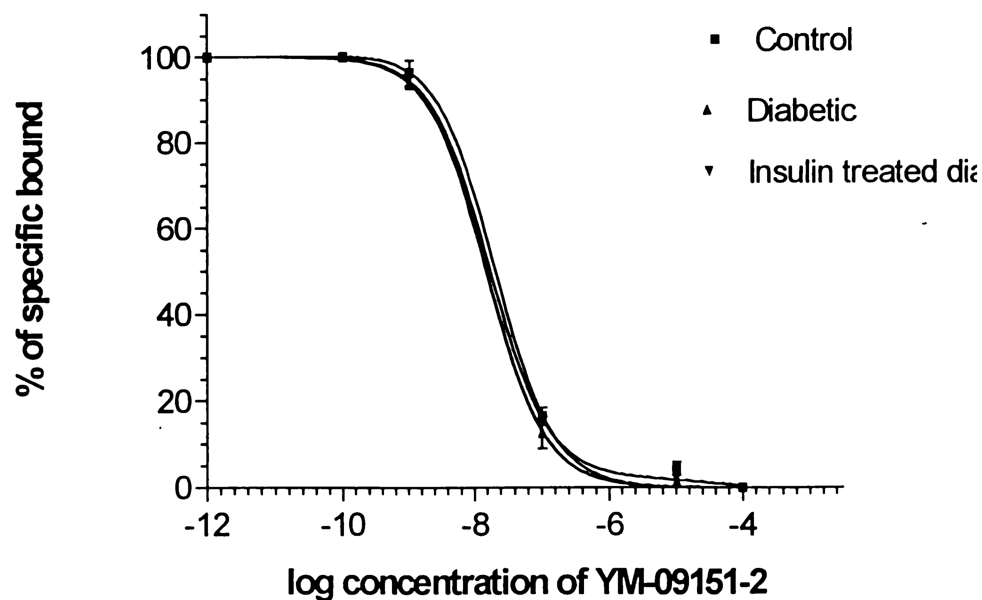


Figure-28
RT-PCR amplification product of dopamine D_2 receptor mRNA from the hypothalamus of control, diabetic and insulin treated diabetic rats

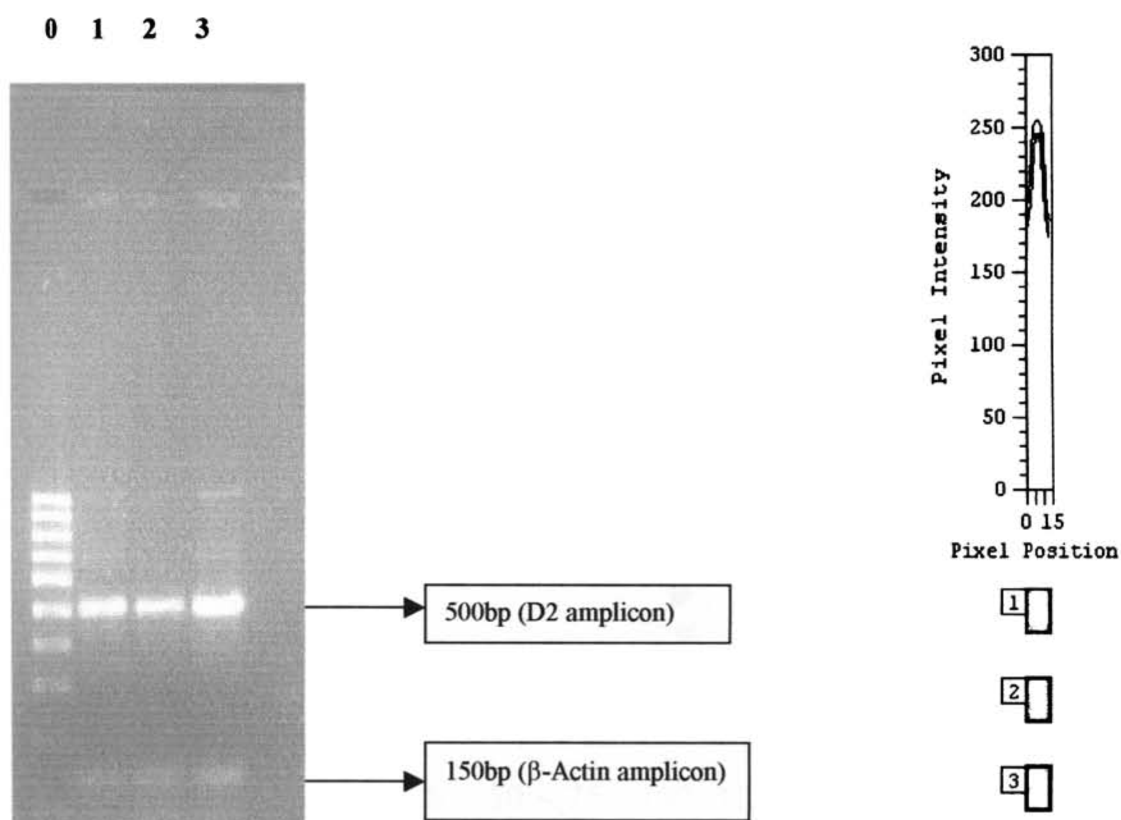


Table:25
Band properties of dopamine D_2 receptor mRNA RT-PCR amplicon

Lane No	Raw Volume	Area	Peak
1	39,862.00	168	245.54
2	35,199.00	168	246.21
3	42,185.00	168	254.21

0-100bp marker
1-Control
2-Diabetic
3-Insulin treated

Figure-29

Scatchard analysis of [^{3}H] YM-09151-2 binding against sulpiride in the brain stem of control, diabetic and insulin treated diabetic rats

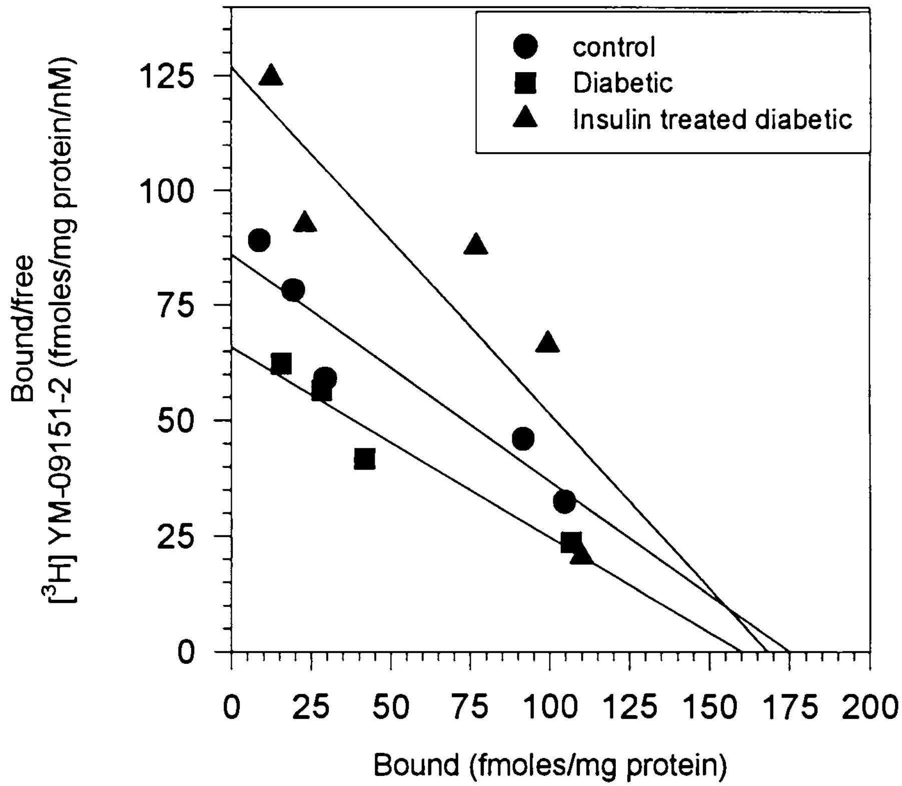


Table-26
[^{3}H] YM-09151-2 binding parameters in the brain stem of control, diabetic and insulin treated diabetic rats

Animal status	Bmax (fmoles/mg protein)	Kd (nM)
Control	174.25±0.85	2.10±0.044
Diabetic	160.4±4.16**	2.42±0.19***
Insulin treated diabetic	168.25±0.85	1.31±0.011†††

Values are a Mean ± S.E.M. of 4-6 separate experiments
**p<0.01 when compared to control
***P<0.001 when compared to control

Table: 27

Binding parameters of [^{3}H] YM-09151-2 against YM-09151-2 in the brain stem of control, diabetic and insulin treated diabetic rats

Animal status	Best fit model	Log(EC50)	Ki	Hill slope
Control	One site	-7.514	$2.735x10^{-8}$	-1.000
Diabetic	One site	-7.604	$2.255x10^{-7}$	-0.970
Insulin treated diabetic	One site	-7.319	$4.030x10^{-8}$	-1.012

Values are mean of 3-5 separate experiments.

Data were fitted with an iterative nonlinear regression software (prism, Graph Pad, San Diego, CA).
Ki- the affinity of the receptor for the competing drugs.
EC_{50} is the concentration of the competitor that competes for half specific binding.

Figure: 30

Displacement of [^{3}H] YM-09151-2 with YM-09151-2 in the brain stem of rats

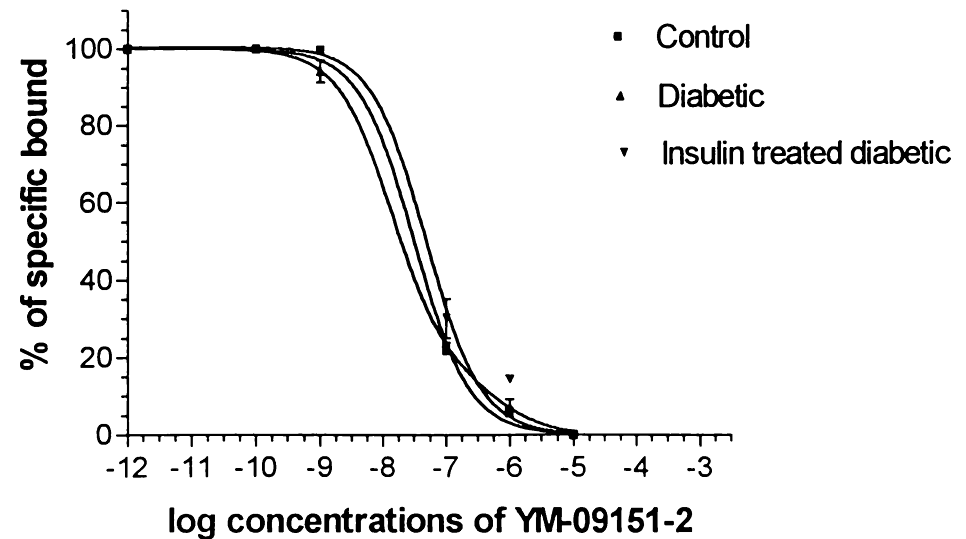


Figure-31
RT-PCR amplification product of dopamine D_2 receptor mRNA from the brain stem of control, diabetic and insulin treated diabetic rats

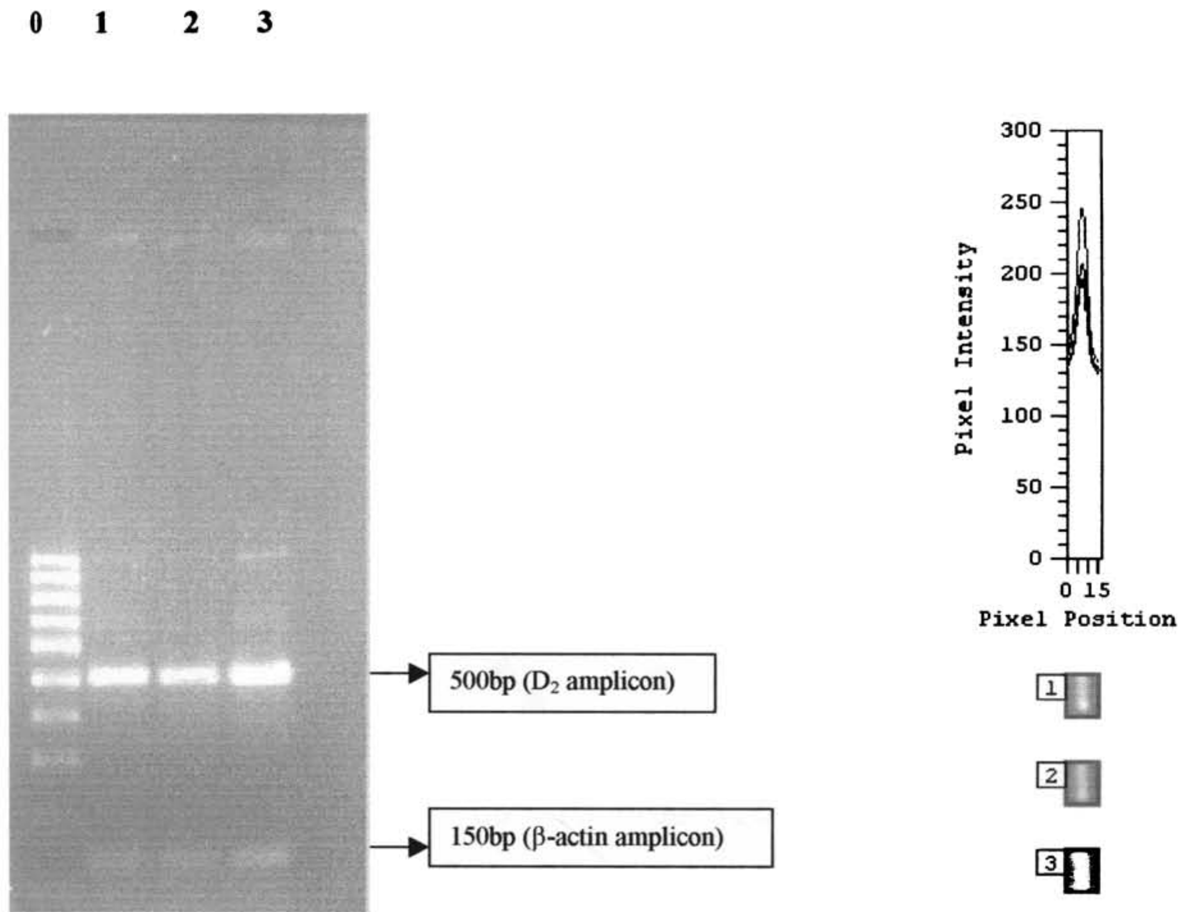


Table: 28
Band properties of dopamine D_2 receptor mRNA RT-PCR amplicon

Lane No	Raw Volume	Area	Peak
1	38,789.00	216.0	207.42
2	36,923.00	216.0	196.08
3	41,622.00	216.0	245.25

0 -100bp marker
1 -Control
2 -Diabetic
3 -Insulin treated diabetic

Figure-32

Scatchard analysis of [^{3}H] dopamine binding against butaclamol in the pancreatic islets of rats

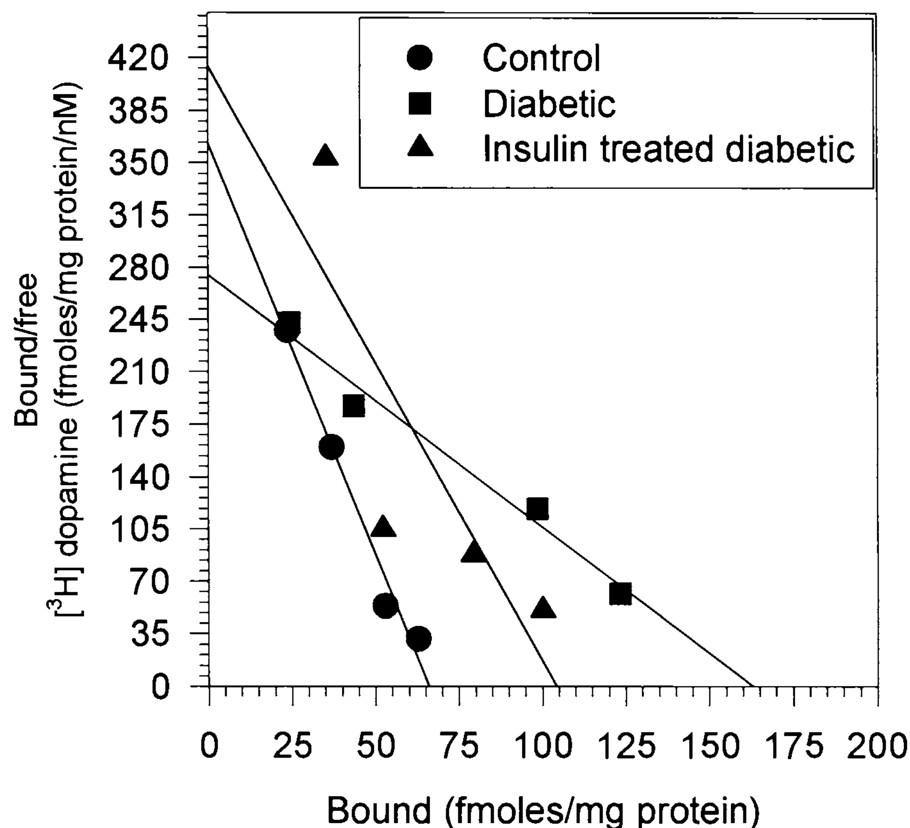


Table-29

[^{3}H] dopamine binding parameters in the pancreatic islets of control, diabetic and insulin treated diabetic rats

Animal status	Bmax (fmol/mg protein)	Kd (nM)
Control	66.0±1.50	0.193±0.011
Diabetic	162.3±11.70***	0.590±0.030***
Insulin treated diabetic	103.5±1.63**+++	0.251±0.025+++

Values are a mean ± S.E.M. of 4-6 separate experiments

***p<0.001 when compared to control

+++p<0.001 when compared to diabetic

**p<0.01 when compared to control

Table: 30

Binding parameters of [^{3}H] Dopamine against dopamine in pancreatic islets of control, diabetic and insulin treated diabetic rats

Animal status	Best fit model	Log(EC50)	Ki	Hills Slope
Control	One site	-6.292	1.292×10^{-7}	-1.002
Diabetic	One site	-5.687	1.112×10^{-6}	-0.965
Insulin treated diabetic	One site	-6.093	2.699×10^{-7}	-0.950

Figure : 32
Displacement of [^{3}H] dopamine-with dopamine in the pancreatic islets of rats

Values are mean of 3-5 separate experiments

Data were fitted with an iterative nonlinear regression software (prism, Graph Pad, San Diego, CA).
Ki- the affinity of the receptor for the competing drugs.
EC_{50} is the concentration of the competitor that competes for half specific binding.

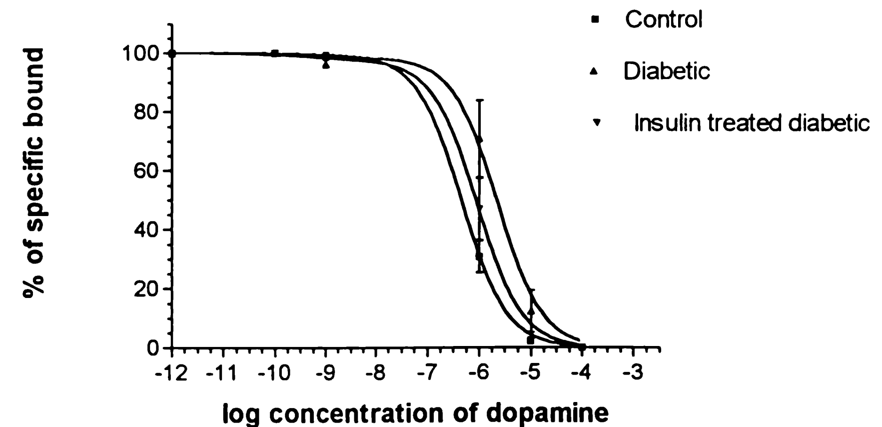


Figure-33

Scatchard analysis of [^{3}H] YM-09151-2 binding against sulpiride in the pancreatic islets of control, diabetic and insulin treated diabetic rats

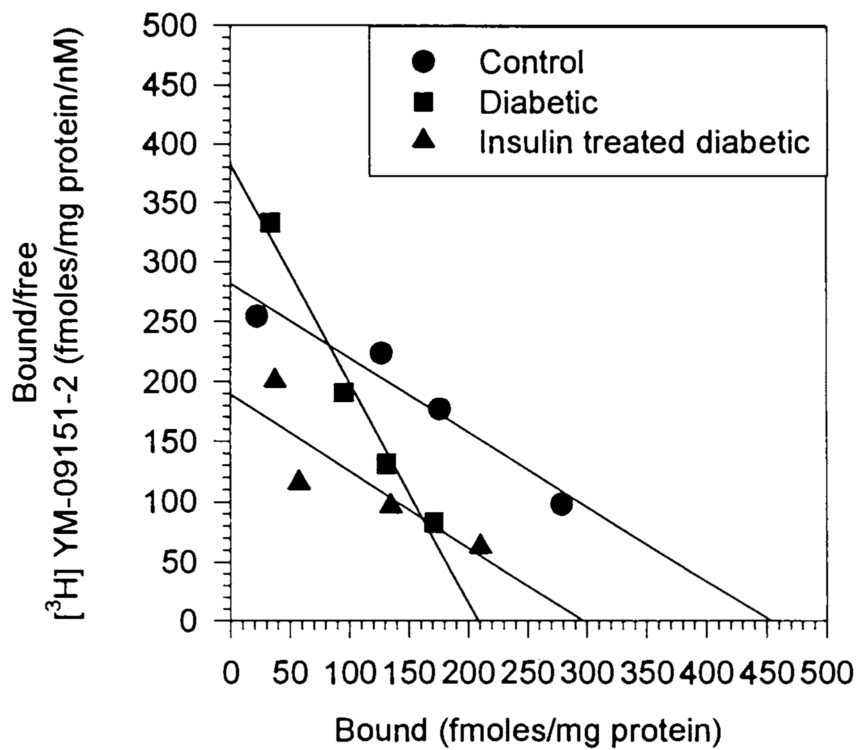


Table-31
[^{3}H] YM-09151-2 binding parameters in the pancreatic islets of control, diabetic and insulin treated diabetic rats

Animal status	Bmax (fmol/mg protein)	Kd (nM)
Control	450±3.98	1.61±0.015
Diabetic	207±5.52***	0.55±0.040***
Insulin treated diabetic	297±4.14^{***+++}	1.57±0.045'''

Values are mean ± S.E.M. of 4-6 separate experiments
***$p<0.001$ when compared to control
+++$p<0.001$ when compared to diabetic

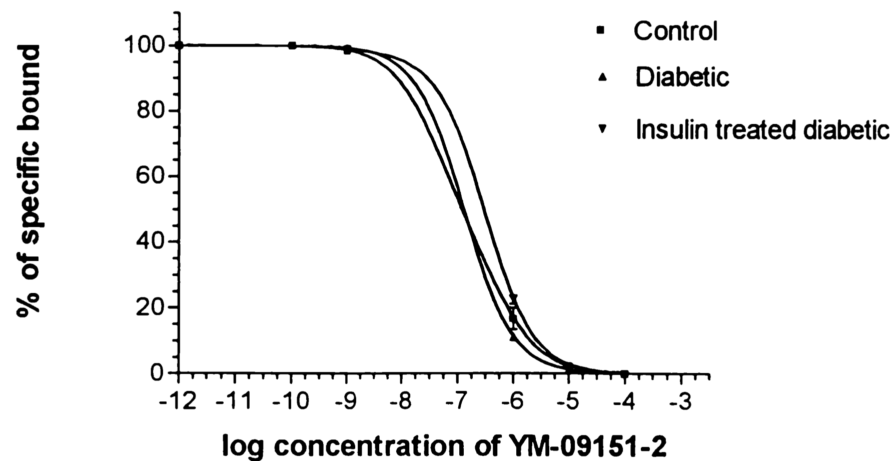
Table:32

Binding parameters of [^{3}H] YM-09151-2against YM-09151-2 in pancreatic islets of control, diabetic and insulin treated diabetic rats

Animal status	Best fit model	Log(EC50)	Ki	Hills Slope
Control	One site	-6.727	1.430×10^{-7}	-0.980
Diabetic	One site	-6.903	6.486×10^{-8}	-1.012
Insulin treated diabetic	One site	-6.552	2.130×10^{-7}	-0.960

-

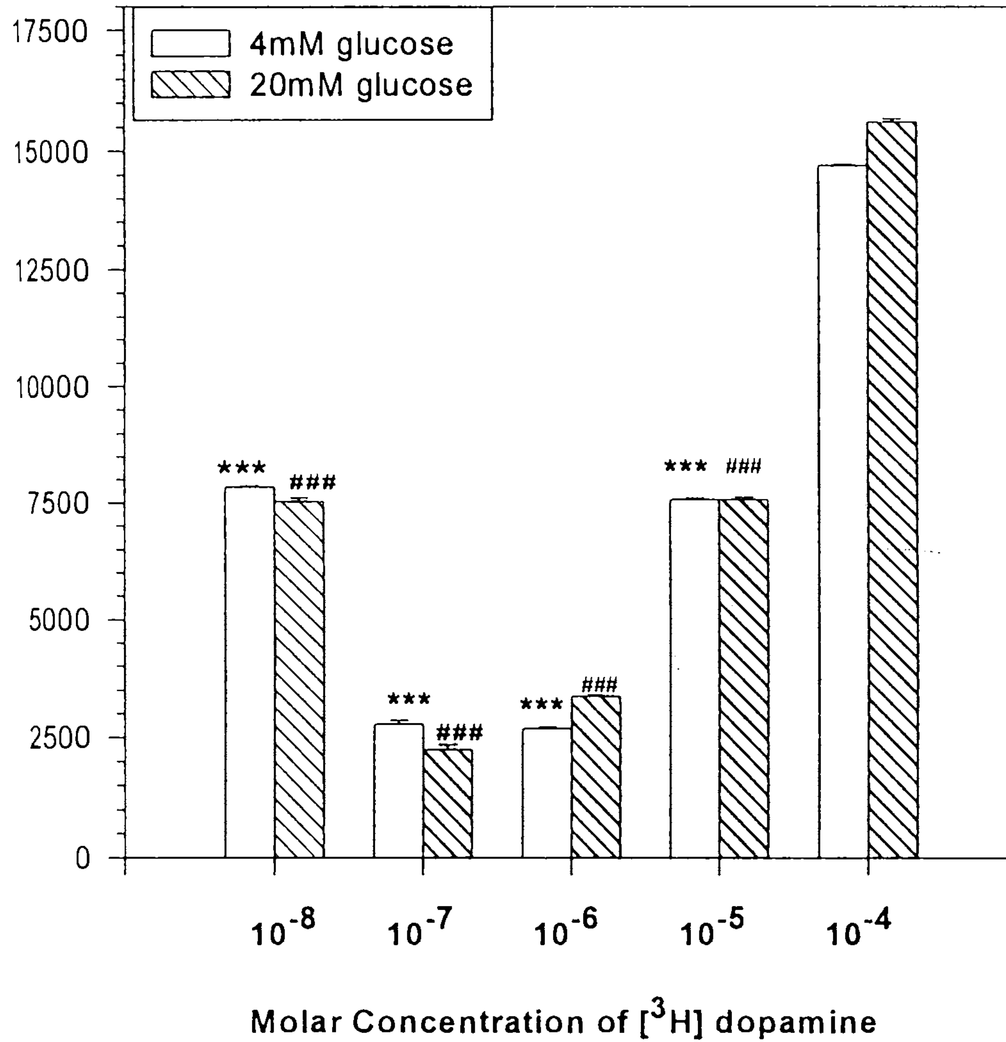
Figure: 34
.Displacement of [^{3}H] YM-09151-with YM-09151-2 in pancreatic islets of rats



Values are mean of 3-5 separate experiments.

Data were fitted with an iterative nonlinear regression software (prism, Graph Pad, San Diego, CA)
Ki- the affinity of the receptor for the competing drugs.
EC_{50} is the concentration of the competitor that competes for half specific binding.

Figure:35

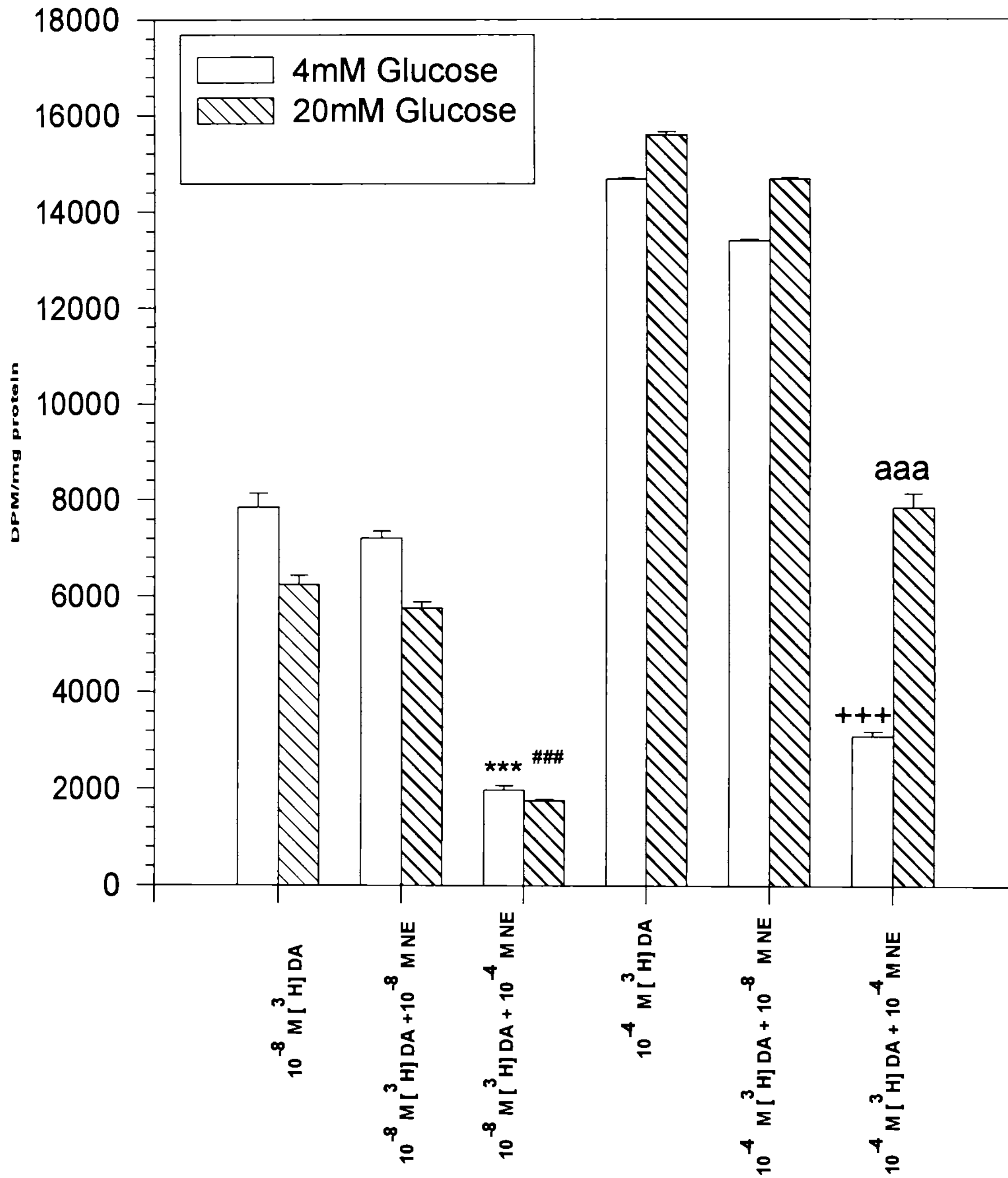


*** $p<0.001$ when compared with 10^{-4}M [^{3}H] dopamine+ 4mM glucose
$p<0.001$ when compared with 10^{-4}M [^{3}H] dopamine+20mM glucose

DA: Dopamine

Figure: 36

ct of Norepinephrine on [^{3}H] Dopamine uptake in pancreatic islets *in vitro*



*p<0.001 when compared to 10^{-8} M [^{3}H] + 4mM glucose

#p<0.001 when compared to 10^{-8} M [^{3}H]+20mM glucose

+p<0.001 when compared to 10^{-4}M [^{3}H]DA +4mM glucose

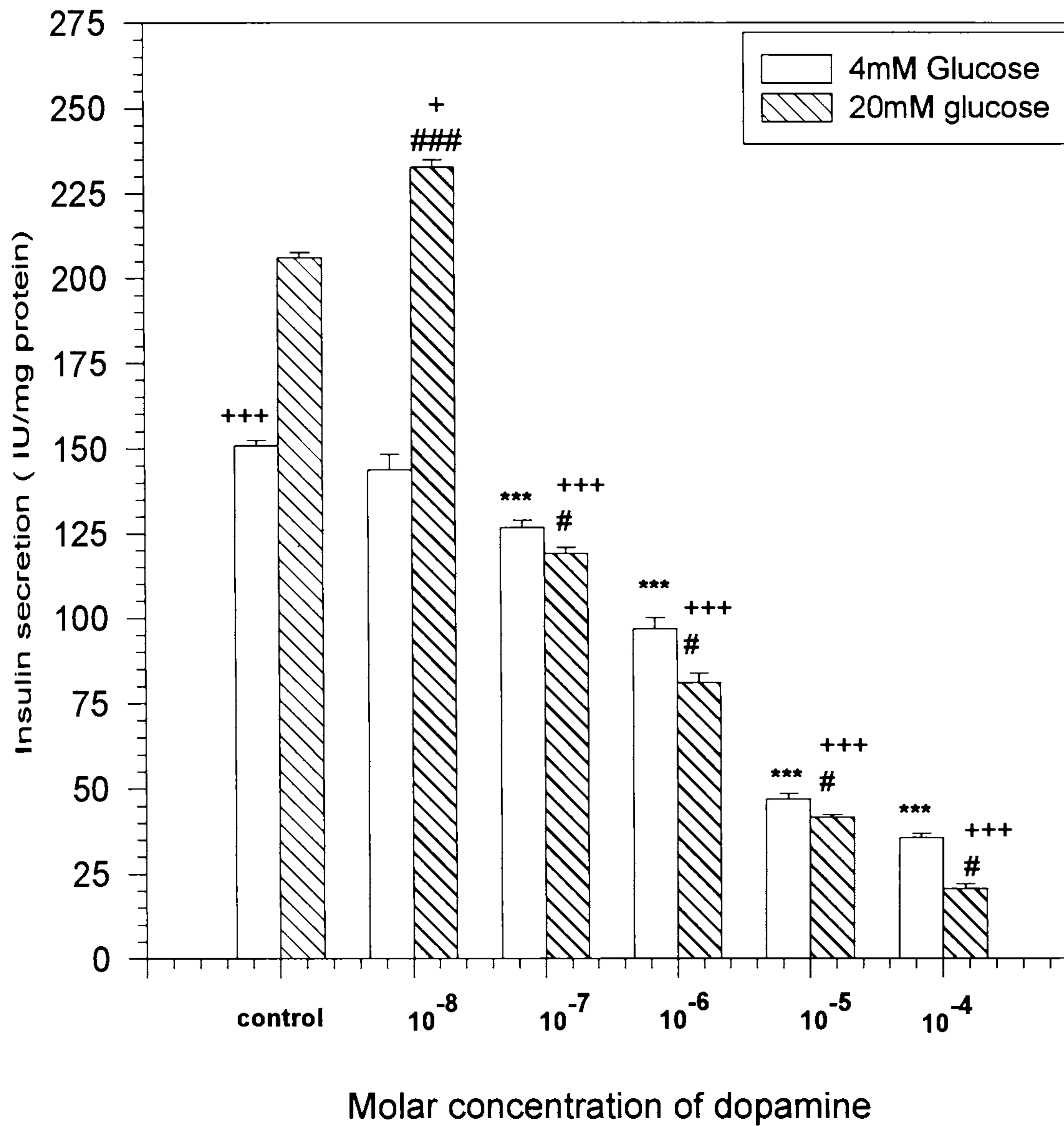
ap<0.001 when compared to 10^{-4} M [^{3}H]DA + 20mM Glucose

DA: Dopamine

NE: Norepinephrine

Figure: 37

Effect of Dopamine (10^{-8} to 10^{-4} M) on glucose induced insulin secretion from pancreatic islets *in vitro*



DA: Dopamine

*** $p<0.001$ when compared to 4mM glucose

+++$p<0.00$ when compared to 20mM glucose

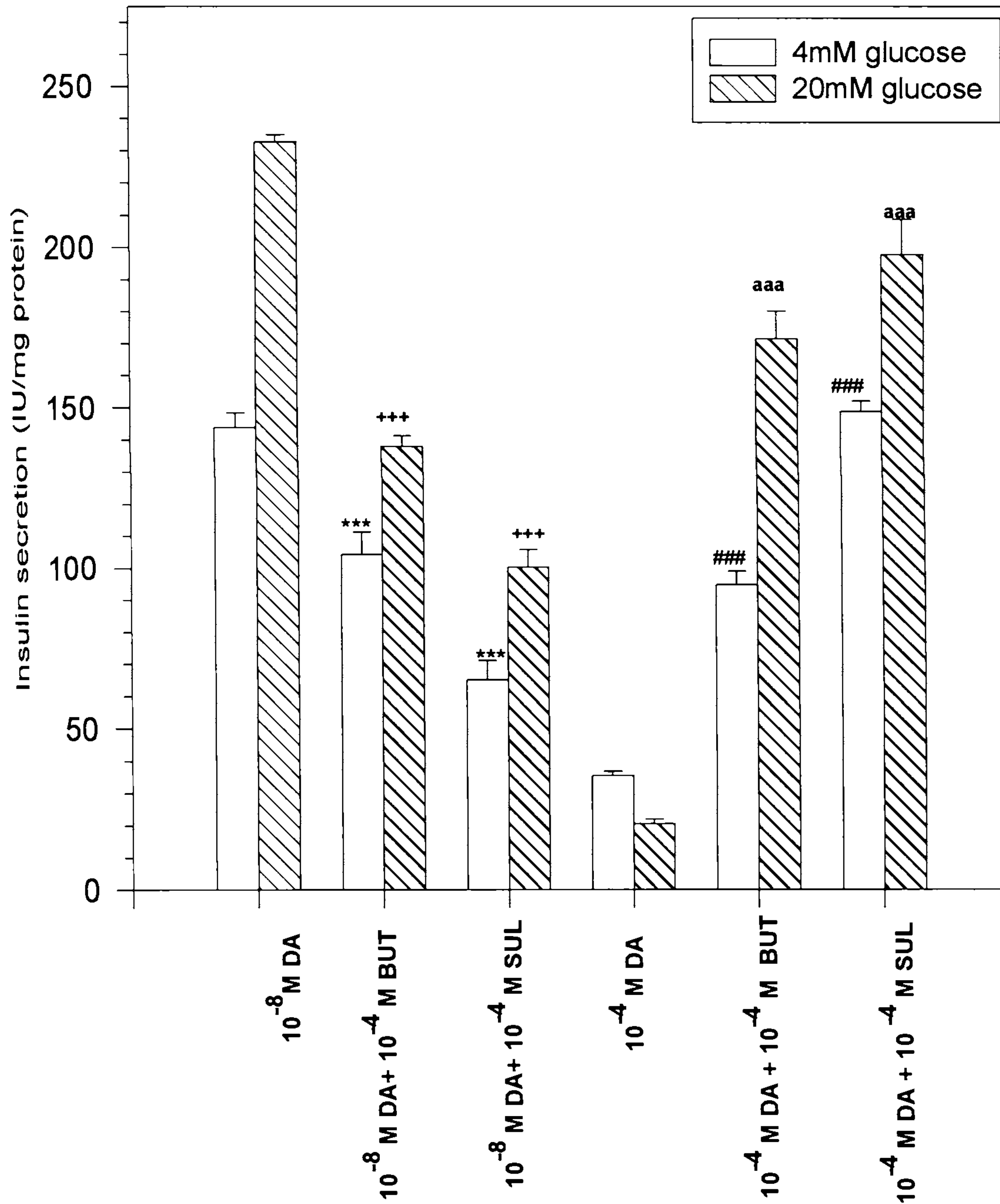
+$p<0.05$ when compared to 20mM glucose

# $p<0.05$ when compared to 4M glucose $+10^{-7}$ M, 10^{-6} M, 10^{-5} M,10^{-4}M DA

$p<0.001$ when compared to 4mM glucose $+10^{-8}$ M DA

Figure: 38

Effect of dopamine antagonists on dopamine (10^{-8} M & 10^{-4} M) induced glucose (4mM and 20mM) induced insulin secretion from pancreatic islets *in vitro*

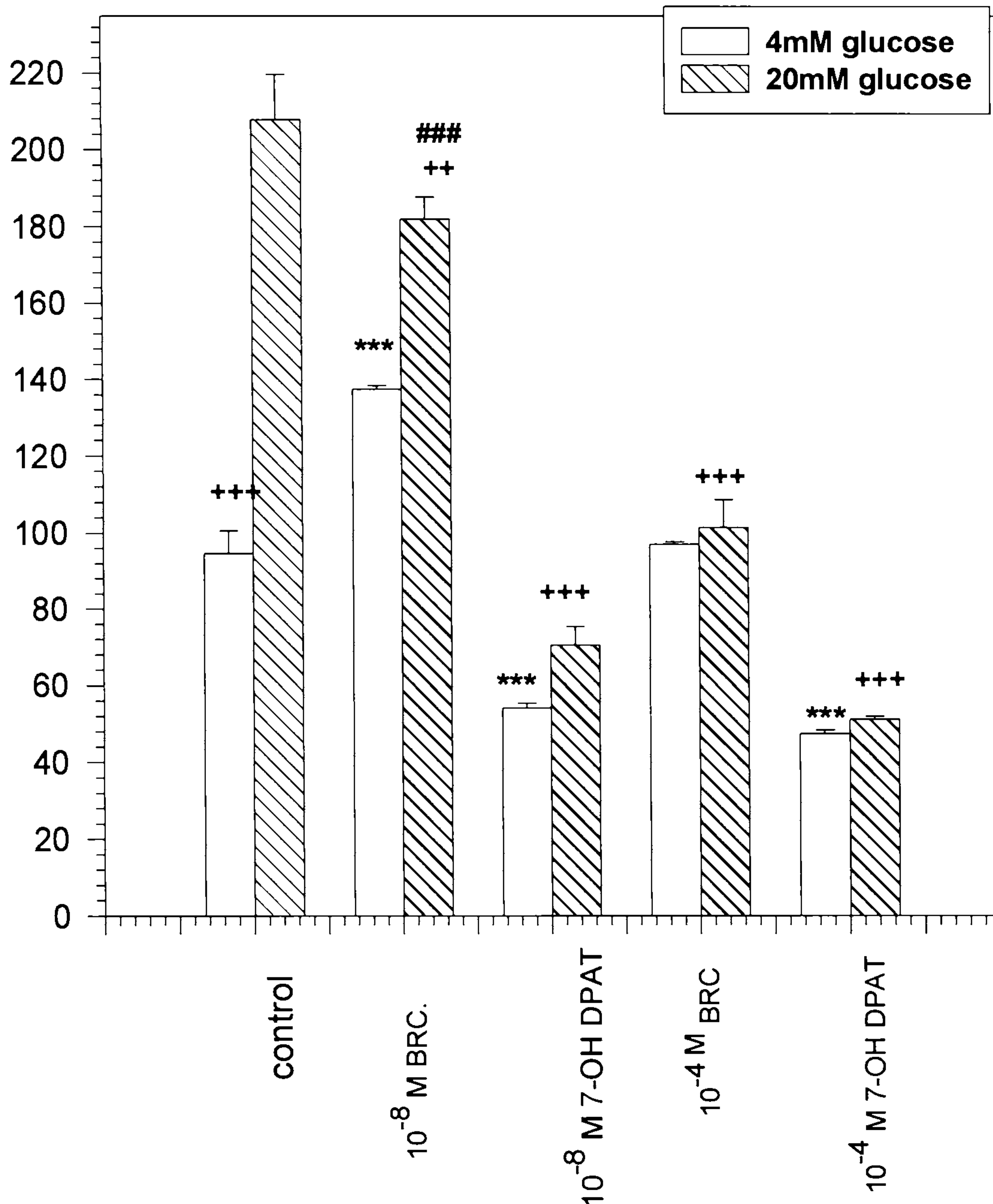


BUT: Butaclamol
SUL: Sulpiride
DA : Dopamine

***$p < 0.001$ when compared with 4mM glucose+ 10^{-8} M DA
+++$p < 0.001$ when compared with 20mM glucose+10^{-8} M DA
$p<0.001$ when compared with 4mM glucose +10^{-4} M DA
aaa $p<0.001$ when compared with 20mM glucose+10^{-4} M DA

Figure:39

Effect of dopamine agonists, bromocriptine and 7-OH-DPAT in ose (4mM and 20mM) induced pancreatic insulin secretion *'in vitro'*

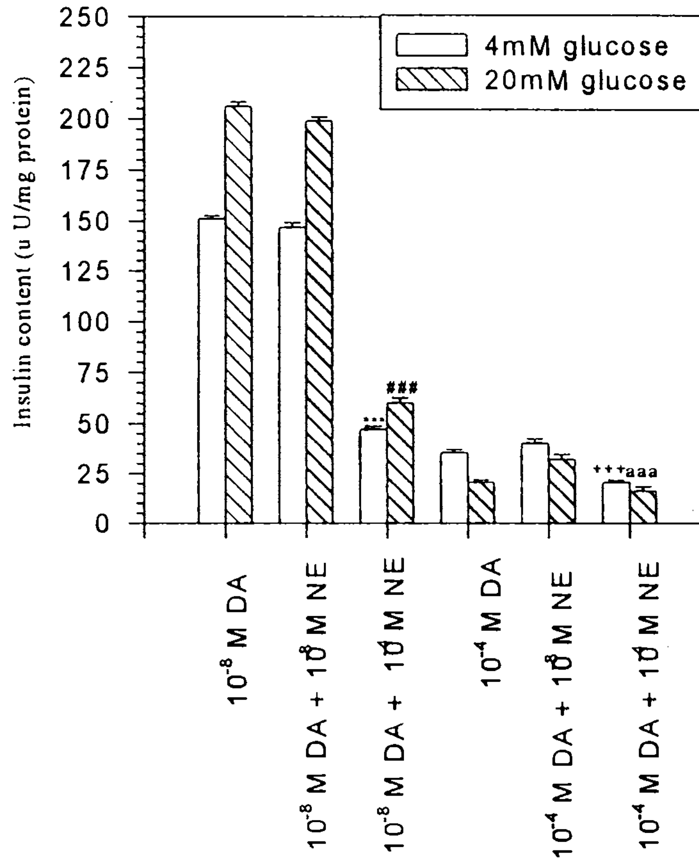


*** $p<0.001$ when compared to 4mM glucose
+++$p<0.001$ when compared to 20mM glucose
++ $p<0.01$ when compared to 20mM glucose
$p<0.001$ when compared to 10^{-8} M BRC + 4mM glucose

7-OH DPAT: 7- hydroxy-2-(DI-n-propyl-amino)Tetralin

BRC: Bromocriptine

Figure:40
Effect of norepinephrine on the role of dopamine in pancreatic islet glucose induced insulin secretion *in vitro*



Values are Mean ± S.E.M. of 4-6 separate determinations
***$p<0.001$ when compared to 4mM glucose +10^{-8}M DA
$p<0.001$ when compared to 20mM glucose + 10^{-8}M DA
+++ $p<0.001$ when compared to4mM glucose + 10^{-4}M DA
aaa $p<0.001$when compared to 20mM glucose + 10^{-4}M DA

NE: Norepinephrine
DA: Dopamine

DISCUSSION

Diabetes Mellitus is a metabolic disorder that not only causes a decrease in efficiency of the pancreatic β cells to secrete insulin but also is accompanied by altered monoamine levels and their turnover rates in the CNS (Bhattacharya and Saraswathi, 1991, Garris, 1990, Lackovic *et al*, 1990). It is characterized by hyperphagia, polydypsia and activation of the hypothalamic pituitary axis (HPA) producing (Mohan Kumar, *et al*., 2003) a marked increase in food and water intake. Hyperglycemia is reported to be a major factor that damages the CNS monoaminergic activity as a result of neuronal degeneration in different regions of the brain. Onset of diabetes has been reported to inhibit the firing of dopaminergic neurons (Saller, 1984) with alteration in its metabolism. The magnitude and duration of dopamine signalling during diabetes is reported to be altered as a result of decreased activity of DA transporter (DAT) causing a low clearance of DA (Galli, *et al*., 2002; Figlewicz, *et al*., 1996; 2003). Hyperglycemia as a result of destruction in the pancreatic islets during diabetes is suggested to have an important role in the impairment of dopamine and other neurotransmitter functions.

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 increased glycogenolysis, decreased glycogenesis, increased gluconeogenesis, impaired glucose transport across membranes and almost complete suppression of the conversion of glucose into fatty acids via acetyl-CoA. During diabetes there is decrease in body weight as a result of altered metabolic function. Insulin treatment normalised the increased blood glucose level and decreased body weight to control values.

BRAIN MORPHOLOGICAL CHANGES DURING DIABETES

Microscopic sections from the brain regions using periodic acid stain in the CS, CC and HYPO revealed that there was an accumulation of glycogen granules in 14 day diabetic rats. Previous reports had only cited the accumulation of glycogen granules in the hypothalamus of 12 months diabetic rats (Bestetti & Rossi, 1980). Glycogen granules are reported to be absent or present in minimal quantities in the neuronal cytoplasm in normal conditions as these cells are reported to posses enzymes

involved in glycogen synthesis. Hyperglycemia is reported to cause an increased activity of these enzymes. Our results in the CS CC and HYPO of 14 day diabetic rats showed dense glycogen accumulation suggesting an increased formation of glycogen with destruction in the cells. This indicates the degenerative change in these brain regions which occurs in the early days of diabetes itself. Previous reports suggest that accumulation occured in 12 months diabetic rats (Bestetti & Rossi, 1980). Insulin treatment mobilized the accumulated glycogen but did not completely improve the degeneration.

PANCREATIC MORPHOLOGICAL CHANGES DURING DIABETES

Pancreatic tissue section from control rats showed cells with distinct nuclei with hematoxylin and eosin stain. In the diabetic pancreatic section distorted nuclei and cells were observed. The damage in the pancreas as a result of diabetes resulted in hyperglycemia which we observed by the increase in blood glucose levels. Treatment with insulin brought a significantly elevated blood glucose level to control values. Onset of hyperglycemia caused severe progressive cell destruction Streptozotocin induced diabetes is reported to cause marked degeneration in the pancreas (Bora & Srivastava, 1985; Ani, *et al.*, 1996). Destruction in the pancreas is suggested to cause insulities as a result of lack of insulin. Insulin treatment reduced the damage to the tissue

CENTRAL NERVOUS SYSTEM ALTERATIONS OF DOPAMINE AND HOMOVANILLIC ACID DURING DIABETES

Several experimental models have been described which provide information on the etiology of IDDM. Sterptozotocin (STZ) is a toxic agent selective to pancreatic β-cells that induces IDDM by causing the β-cell destruction (Like & Rossini, 1976; Paik, *et al.*, 1980).

In experimental models various doses of STZ have been used to induce diabetes and 65mg have been found to produce (Sumiyoshi, *et al.*, 1996) maximum hyperglycemia. DA content in the striatum was affected by different doses of STZ. As the dose increased there was an increase in DA content and 65mg of STZ gave the maximum elevated DA content. When different doses of STZ were injected, an increase

in DA content was observed as a result of damage in the pancreatic islet population. The substantia nigra (SN) is an autonomic area in the central nervous system which plays an important role in controlling structure and activity of pancreatic islets. Lesions in the substantia nigra not only resulted in reduced size and number of islets cell populations but also decreased the content of insulin and glucagon in the pancreas (Smith & Davis, 1983). It has been established that central nervous system cell groups projecting into the pancreatic vagal motor neurons received inputs from the adrenergic, noradrenergic and serotonergic neurons from the lower brain stem and a dopaminergic input from paraventricular nucleus of hypothalamus (Lowey, *et al.*, 1994). Lesions in these brain regions are reported to affect the pancreatic islet cell population and growth. Similarly STZ damage of the islets disturbed the central dopaminergic connections altering the dopamine metabolism. Alteration in HVA has always been considered as an index of DA metabolism in the brain (Eisenhofer, *et al.*, 1991; 1993; Roth, *et al.*, 1983). In our results a significant decrease in HVA content on STZ injection indicates that the dopaminergic alterations are due to damages in the pancreatic islets. There was also a decrease in the conversion of DA to HVA as a result of STZ injection showing a decrease in the metabolism of dopamine.

In the time dependent study, DA content in the striatum began showing signs of increase 12 hrs after the injection of STZ and increased significantly at the end of 48 hrs. Dopamine content is reported to increase in the brain regions after 48 hrs of STZ injection (Onegova, *et al.*, 1980). A concordant decrease in peripheral insulin levels after injection of STZ has also been reported which suggest the importance of the feedback mechanism between the metabolic disturbances as a result of insulin insufficiency and the changes in the monoamines level in the brain regions (Onegova, *et al.*, 1980). The islet destruction by STZ has a role in causing stress to the brain by increasing the levels of DA during the onset of diabetes. The homovanillic acid content and the turnover ratio decreased as the content of DA increased. The decrease in HVA was observed 3 hrs after STZ injection when DA content did not show any change. This alteration in the DA metabolism indicates that damage in the dopaminergic system occurs as a result of the pancreatic islet destruction caused by the onset of diabetes. Thus central DA system and their alterations has a role in the etiology of diabetes.

Corpus Striatum

In the corpus striatum there was a significant increase in the dopamine content during diabetes. An increase in dopamine level in the striatum as a result of hyperglycemia attributes to the decreased release of dopamine (Lim & Lee, 1995). There was also a corresponding decrease in the HVA content during diabetes. The turn over ratio of DA to HVA also decreased during diabetes. Diabetes is observed to cause a decrease in dopamine metabolism (Kwok & Juorio, 1986). D-glucose is reported to depress the firing of dopamine containing neurons located in the substantia nigra by reducing the efflux of dopamine from the striatum (Saller, 1984). Similarly hyperglycemia during diabetes is reported to impair dopaminergic functions causing the accumulation of striatal DA inhibiting its efflux. Brain tyrosine concentration is decreased in diabetes (Frenstorm, 1983). Tyrosine is the precursor amino acid for the synthesis of dopamine. In diabetic condition the decrease in endogenous tyrosine concentration is reported to cause a reduction in the affinity of the enzyme tyrosine hydroxylase. The equilibrium in the tyrosine and tyrosine hydroxylase levels are reported to be much lower in diabetic rats. Diabetes is reported to cause a decrease in the accumulation of L-DOPA due to the inhibition of DOPA decarboxylase activity (Trulson & Himmel, 1983). The tyrosine hydroxylase enzyme mRNA expression in dopaminergic cells decreased during diabetes in the ventral tegmental area/substantia nigra compacta (VTA/SNc). Also, a decrease in the dopamine transporter protein (DAT) in the striatum is reported during diabetes which is also a primary factor for the elevation in the concentration of dopamine and decreased production of its metabolites (Figlewiz, *et al.*, 1996).

The enzymes involved in the synthesis and metabolism of dopamine are reported to be decreased during diabetes (Trulson & Himmel, 1983). There was a significant increase in striatal DA content during diabetes which resulted in the notable reduction of HVA. D-glucose is reported to suppress the dopaminergic transmission and firing in the brain, lowering the DA metabolism decreasing the metabolite content. Saller, (1984) studied the changes in the HVA content at various time periods in the striatum and found that four days after the alloxan administration there was an elevation of HVA content which decreased 21 days later and declined consistently after 42days. Our results showed

that the decrease in the level of striatal HVA observed in 14 day diabetic rats due to a decrease in the metabolism of DA. Dopamine metabolites DOPAC and HVA accumulation was decreased in STZ treated diabetic rats (Trulson and Himmel, 1983). Administration of probenecid, an inhibitor of HVA, in STZ diabetic rats had no effect on the metabolite accumulation that remained decreased, while in normal rats caused significant accumulation of DOPAC and HVA.

Insulin therapy did not normalize the elevated dopamine levels in the striatum. There are reports that insulin therapy during diabetes does not normalize the elevated dopamine content and the reduced DA turnover rate (Sally, *et. al.*1991). Insulin is reported to be a key regulator in ameliorating the dopamine levels and its metabolism. Reports gathered have shown that a complete recovery is not attained by insulin treatment during diabetes (Gupta, *et al.*, 1992; Bellush & Reid, 1991). We found that insulin treatment for 14 days in diabetic rats had partial effect on the decreased DA metabolism. Though insulin treatment did not reverse the elevated DA, it brought the decreased HVA content to control levels during diabetes in the striatum. Previous reports suggested that 4-6 weeks of insulin treatment caused a reversal of altered dopamine content as insulin normalized its metabolism (Kwok & Juorio, 1986). Homovanillic acid is implicated as an important marker for DA metabolism in the central and peripheral nervous system (Eisenhofer, *et al.*, 1991, 1993; Roth *et al.*, 1983). The decreased HVA with significantly low turnover ratio during diabetes is due to hyperglycemia. It has been suggested that excessive production of glucose results in hyperglycemia during diabetes impairing the metabolism of dopamine and other neurotransmitters (Girard, *et al.*, 1995). This hyperglycemic state during diabetes is due to the increased gluconeogenic pathway which is physiologically less sensitive to the inhibition by insulin (Girard, *et al.*, 1995). Insulin induced dopamine release that was not fully effective in diabetic rats could also be related to the peripheral insulin resistance exhibited by them (Cohen, *et al.*, 1991). Striatal dopamine release is affected by changing substantia nigra (SN) glucose levels. This response may well reflect the known effect of glucose on $K_{(ATP)}$ channel activity on both SN DA neurons and GABA axon terminals in the substantia nigra. These interactions could provide a mechanism whereby glucose modulates motor activity involved in food intake (Levin, 2000). Diabetic rats manifested an altered behavioral and

neurochemical response suggesting a dysfunctional biosynthetic capacity for DA as a result of a decreased neurotransmission (Ahmad & Merali, 1989). The striatal dopamine content was elevated with a corresponding decrease in its metabolism during diabetes was only partially restored to control levels with insulin treatment in 14day diabetic rats.

Hypothalamus

Dopaminergic action is important in the regulation of the hypothalamic-pitutary hormone release. Hypothalamic dopamine content decreased during diabetes. Also, dopamine and its receptors are implicated in the satiety and hunger aspects and body weight maintenance. The central vagal connection with dopaminergic innervation is reported to reach the pancreatic islets through the parahypothalamic ventricular (PHV) nucleus while aderenergic and serotonergic innervations reach the pancreas through the brain stem (Smith & Davis, 1983; Lowey, *et al.*, 1994). A decrease in DA in the hypothalamus during diabetes is caused due to the reduction in the low synthetic rate of dopamine as tyrosine levels decrease (Fernstrom, *et al.*, 1983; Leu, *et al.*, 1986). Altered dopamine is reported to affect the feeding pattern, as food intake is accompanied by DA release which differs significantly in the hypothalamus of obese and lean Zucker rats. The reduction in dopamine, norepinephrine and epinephrine levels in the hypothalamus suggests a low metabolism of monoamines (Bellush & Henley, 1990). They are responsible for the development of thermoregulatory deficits when exposed to cold environment (Leu, *et al.*, 1986). Dopamine is considered as a hormone of the hypothalamus involved in the secretion of prolactin. It has an inhibitory effect on the release of prolactin from the anterior pituitary.

The decrease in dopamine content reduced the hypothalamic HVA with no alterations in turnover ratio of HVA from DA. This indicates a low synthesis of dopamine in the hypothalamus during diabetes. Insulin treatment for 14 days caused partial improvement of the DA and HVA content. The turnover ratio was near control values in all the groups. The decrease in DA and HVA content could be due to the depletion of dopamine resulting in a dysfunctional biosynthetic capacity for DA during diabetes (Ahmad & Merali, 1989; Merali, *et al.*, 1988). This finding bear importance

since hypothalamus is reported to play a role in behavioral and physiological changes associated with diabetes.

Cerebral Cortex

Extracellular dopamine originates from DA and NE neurons in the prefrontal cortex (PFC). Recent reports suggested that extracellular DA release in the cortex depend on NE rather than DA innervation. In the cortex DA acts not only as NE precursor but also as co-transmitter (Gessa *et al.*, 2001). The co-release of NE and DA seems to be controlled by α_2 adrenergic receptors located on NE nerve terminals.

Cortical dopamine content increased with an increase in HVA content during diabetes. But the turnover rate of HVA from DA decreased during diabetes which indicates a decrease in the metabolism of dopamine turnover rate. Though there was a significant increase in both DA and HVA content, the metabolismof dopamine decreased as a result of hyperglycemia during diabetes. An increased DA and HVA content in the CC during diabetes is reported previously (Gupta, *et al.*, 1992, Yan, *et al.*, 1991). Cerebral cortical dopamine metabolism is reported to decrease because increased glucose during diabetes (Kwok & Juorio, 1986) affects the dopaminergic activites such as working, memory, and stress response (Tam & Roth., 1997). During diabetes a lack of tyrosine affect markedly the physiology and functions of these DA neurons. The overall deficit in the availability of the precursor amino acid tyrosine which has been previously reported has an influence in the functioning of DA neurons. Insulin treatment did not reverse the elevated DA and HVA to control values. This in compliance with previous reports that a complete recovery has never been attained by insulin therapy (Gupta, *et al.*, 1992). The possible reason could be that during diabetes the alternative metabolic pathways supply glucose to provide energy. These pathways are reported to be resisting the inhibition by insulin causing only partial recovery (Girard, *et al.*, 1995). This increased dopamine with a decreased turnover during diabetes in the cerebral cortex is associated with metabolic disturbance and behavioural changes. Efflux of DA in the prefrontal cortex is reported to stimulate hunger and food intake (Ahn & Phillips, 2002). Diabetes is marked by hyperphagia causing excessive food intake. Hyperglycemia causes the reuptake of DA into the brain cells which could possibly stimulate a

hyperosmolar state that results in dehydration causing polydypsia (Hirata, *et al.*, 1992).

Brain Stem

Dopamine and HVA content in the brain stem decreased during diabetes correspondingly. A significant increase in the NE content in the brain stem from previous reports (Task, *et al*, 1992; Jackson, *et al.*, 1997, 1999) suggested the decrease in DA could be because of the increased turnover to NE. This is important as the turnover to NE causes an increased sympathetic stimulation. This has important relevance in insulin secretion from the pancreatic islets as the increased sympathetic stimulation could inhibit the insulin secretion.

ALTERATIONS IN DOPAMINE AND HVA CONTENT IN THE PLASMA, AND ADRENALS OF CONTROL, DIABETIC AND INSULIN TREATED RATS

Dopamine concentration in the plasma during diabetes decreased significantly. This is in concurrence with earlier reports that showed that plasma DA decreased during diabetes (Chandrashekar-Reddi, *et al.*, 1994). The plasma concentration of the DA is used as an indicator of central nervous system dopaminergic activity (Esler, *et al.*, 1991). This decrease in plasma DA concentration indicates that diabetes causes an alteration in the overall dopaminergic function and activity. Peripheral plasma level of HVA, the deaminated and o-methylated metabolite of dopamine, is often used as an indicator of central nervous system dopaminergic activity (Esler, *et al* 1993). Our results show that HVA decreased significantly underlying the decreased metabolism of DA during diabetes. Regional HVA production is associated with the metabolism of dopamine in sympathetic nerves and it is at a rate which appears to be influenced by sympathetic nervous system. Also the turnover ratio of HVA from DA was also decreased during diabetes. The decreased DA with concordant decrease in HVA and the turnover ratio has immense importance as the plasma NE and EPI levels increased significantly. This increase in NE and EPI levels agrees with previous reports from our laboratory and others are due to central and peripheral increase in the sympathetic stimulation during diabetes (Jackson, *et al.*, 1997; Chaouloff, *et al.*, 1990a; Chandrashekar-Reddi, *et al.*, 1994). In 14 day diabetic rats the turnover ratio of NE from DA also decreased as there was a significant decrease in DA. The activity of dopamine-β-hydroxylase (DBH) is reported

to be increased in blood from diabetic rats (Berkowitz & Head, 1978). Thus, the increased activity of DBH causes an increased conversion of DA to NE which triggers the sympathetic nervous system. Insulin treatment for 14 days showed improvements in the concentrations of DA, NE, EPI and HVA. The turnover ratio of HVA from DA was restored to control values reflecting changes in brain DA metabolism.

The dopamine and HVA contents in the adrenals decreased significantly indicating an overall decrease in the metabolism of DA. Increased NE and EPI content in the adrenal medulla during diabetes is observed as a result of the decreased dopamine content. Most of the NE released is efficiently removed by neuronal and extraneuronal uptake (Eisenhofer, *et al.*, 1992). Evidences suggest that in the periphery DA serves not only as a precursor for active compounds released from sympathetic nerves and the adrenal medulla is suggested to act as an autocrine or paracrine regulator of local organ function (Eisenhofer, *et al.*, 1995). The increase in the sympathetic tone is because of the increase in the NE and EPI levels in diabetic rats. Reports show a decrease in the NE levels in the adrenal medulla during diabetes (Patel, *et al.*, 1997). We observed a decreased turnover of NE from DA during diabetes which could be as a result of degeneration of the adrenals. A decreased turn over is being reported in the adrenal medulla as a result of its degeneration during diabetes (Patel, *et al.*, 1997). Also, it has been suggested that the sympathetic tone is differentially altered in the peripheral tissues and in the adrenals there is a decreased turnover during diabetes (Patel, *et al.*, 1997). Thus a decreased DA and HVA content with an increased NE and EPI levels during diabetes increased the synpathetic stimulation. At the same time 14 day streptozotocin diabetes indicated a lower turnover of NE from DA suggesting an overall damage in the sympatho-adrenal system causing peripheral neuropathy.

Brain Dopamine receptor alterations during diabetes

Diabetes mellitus is often accompanied with emotional, behavioral, mood disturbances and centrally mediated neurological complications (Salkovic & Lackovic, 1992). Striatal dopamine receptors were markedly decreased with no change in affinity during diabetes with the accumulation of DA in the striatum and a decreased HVA metabolism. Striatal dopamine firing during diabetes is decreased affecting

dopaminergic functions (Saller, 1984). The decreased dopamine receptor density during diabetes is related to the decreased locomotor activity in STZ-induced diabetic rats (Kobayashi, *et al.*, 1990; Shimomura, *et al.*, 1990). This finding correlates with our present data suggesting that the disturbances in the central dopaminergic receptors during STZ- induced diabetes affects dopamine related functions. An increase in DA receptors is reported to cause an increased DA receptor sensitivity during diabetes (Lazovsky, *et al.*, 1981) due to long term blockade of DA receptors or lesions in the striatal DA receptors. The firing of DA neurons projecting from the substantia nigra to the striatum is reported to be rapidly suppressed by hyperglycemia leading to the hypofunction of dopamine receptors (Saller, 1984).

The decreased DA receptors during diabetes that we report in the striatum is a major cause in affecting dopamine related functions. There are hypothesis that suggests activities related to the functional capacities of DA receptors like stereotypy, ambulation, behaviour are diminished due to hyperglycemia (Lazovsky, *et al.*, 1981). Also a decrease in DA receptors during diabetes may result in hyporesponsiveness (Kamei, *et al.*, 1998). It is suggested that in alloxan treated rats with the onset of diabetes causes metabolic changes such as weight loss and dehydration are reported to occur which modify the DA metabolism (Omar, *et al.*, 1985).

Insulin treatment effectively restored the decreased density to control levels but there was a decrease in the affinity of the receptors. The decrease in the affinity of the receptors during insulin treatment may be a compensatory mechanism in restoring the decreased dopaminergic function to normal state which is in compliance with previous reports.

The dopamine receptors in the hypothalamus did not alter in number during diabetes but there was a decrease in affinity in both Scatchard and displacement analysis. The Log (EC_{50}) value in diabetes increased with an increase in Ki suggesting a decrease in affinity state. Studies based on the reports from the anterior pituitary of alloxan treated diabetic rats showed no significant changes in the DA receptors and there was no modification in the binding affinity (Omar, *et al.*, 1985). The pancreatic vagal motor neurons receive dopaminergic input from paraventricular nucleus of hypothalamus (Lowey, *et al.*, 1994). This demonstrates the importance of CNS dopamine in the

pancreatic hormone secretion and in glucose homeostasis. Thus, hypothalamic dopamine receptors and their alterations are important during diabetes. Functions related to hypothalamus like increased water uptake and thermoregulatory deficits are suggestive of dopaminergic alterations directly or indirectly causing alterations in various autonomic, somatosensory, and motor neural functions of STZ-diabetic rats (Leu, *et al.*, 1986).

The decrease in the DA receptor affinity during diabetes in the hypothalamus could be an important factor in the impairment of regulation of food intake and body weight. Our result in the hypothalamus suggests that decrease in the affinity of receptors during diabetes is linked to hyperglycemia. An alteration in the sensitivity of the receptors during diabetes has been previously reported causing a difference in the modulation of innervating DA systems. As the normal responses occurring in hypothalamic catecholamine metabolism after the consumption of food are modified by the presence of diabetes (Glanville & Anderson, 1986). Insulin treatment normalized the decreased affinity to control values. Insulin deficit and hyperglycemia affect hypothalamic DA receptor functions.

In the cerebral cortex the alterations in the DA receptors during diabetes showed an increase in the receptor density without any change in affinity. Dopamine in the cerebral cortex is thought to be involved in functions like motor functions, memory, and stress response (Tam & Roth, 1997). This indicates the long term blockade of DA receptors or damage in the cortical dopaminergic neurons as a result of hyperglycemia. Our studies have shown that [^{3}H] DA binding is significantly increased in the cerebral cortex during diabetes. Increased DA receptor sensitivity and altered dopaminergic transmission has been implicated in the pathogenesis of schizophrenia (Lazovsky, *et al.*, 1981). Our data suggest that elevated glucose causes the reduction of dopamine content leading to a compensatory increase in its receptors.

Thus the alterations in the dopamine receptors in the different brain regions had a differential effect during diabetes. Diabetes alters the sensitivity of the dopaminergic receptors and that altered response of the dopaminergic system could be indirectly involved in the modulation of nociception in diabetic rats possibly through the enhancement and/or deactivation of the endogenous met-enkephalinergic system (Kolta, *et al.*, 2002). These alterations are of immense importance as chronic hyperglycemia

diminishes central dopaminergic function and increased dopamine sensitivity would be a compensatory adjustment to a reduced central dopaminergic activity.

Brain dopamine D_2 receptor alterations during diabetes

Striatal dopamine D_2 receptor density was significantly increased during diabetes. Previously [^{3}H] spiroperidol binding to dopamine D_2 receptors have been reported to be increased during diabetes (Trulson & Himmel 1983). Dopamine D_2 receptors were increased significantly during diabetes and insulin treatment did not reverse the increased number of receptors to control levels. Dopamine D_2 receptor antagonist [^{3}H] YM-09151-2 was also used for the receptor binding in the corpus striatum.. The B_{max} increased during diabetes and did not reverse during insulin treatment. This shows that during diabetes the dopamine D_2 receptors are significantly increased in the striatum and insulin treatment has only a partial effect in normalising the altered levels. The difference in the binding of [^{3}H] Spiperone and [^{3}H] YM-09151-2 is consistent with previous reports that [^{3}H] spiperone binds to dimers of the dopamine D_2 receptors and [^{3}H] YM-09151-2 binds to receptor monomers (Marzella, *et al.*, 1997) inspite of both binding only to high affinity sites. Previous reports suggest that both the compounds have different affinities for the same dopamine D_2 receptors. The increased B_{max} during diabetes is comparable to those changes observed after lesions of dopaminergic neurons or after chronic administration of dopamine receptor blockers. Excessive glucose or hyperglycemia is reported to deplete the dopamine metabolism and a decreased dopamine synthesis rate is suggestive to cause an increase in the receptor number of dopamine D_2 resulting in its increased number. This is reported to affect both the nigrostriatal and mesolimbic dopamine systems. Striatal dopamine D_2 receptor primarly represents a population of dopamine D_2 sites (Marzella, *et al.*, 1997). Striatal dopamine D_2 receptors are reported to be involved in the modulation of morphine-induced antinocipception in diabetic mouse (Kamei & Saitoh, 1996). During diabetes it has been documented that the sensitization of these receptors and their increased number results in a decreased locomotory and ambulatory activity (Kobayashi, *et al.*, 1990; Shimomura, *et al.*, 1990).

Dopamine through its dopamine D_1 receptor stimulates adenylyl cyclase and inhibits adenylyl cyclase activity through its dopamine D_2 receptors. Dopamine D_1 stimulated cAMP production was markedly increased in diabetic rats, whereas ability of dopamine D_2 receptor action to reduce cAMP formation was almost abolished during diabetes (Gorio, *et al.*, 1989). An imbalance between Gs -proteins and Gi/Go protein mediated efficacy of Gs activity as a result of the loss of Gi/Go inhibitory functions has been found in the striatum and other tissues of diabetic animals (Salkovic & Lackovic, 1992). Doapmine D_2 receptors exert their function activating Gi proteins in the brain. Regulation of the inhibitory G protein-calcium channel complex involves pertusis toxin (PTX) sensitive and insensitive G proteins (Wiley, *et al.*, 1998). Concomitantly with such transductional alteration detected in chronic diabetes, caused a marked increase of the striatal content of met-enkephalin, which is known to utilize Gi/Go proteins for inhibition of adenylyl cyclase. Diabetes causes the activation of mitogen activated protein kinase (MAP kinase) p38 as an early step in the signal pathway to dysfunction in experimental diabetic neuropathy (Agthong & Tomlinson, 2002). Increased content of dopamine and elevated B_{max} of dopamine D_2 receptors in the corpus striatum could cause a transductional defect in diabetic animals leading to diabetic neuropathy.

Diabetic neuropathy is the most common secondary complication of diabetes mellitus. Evaluation of the effect of levosulpiride, a selective antagonist for D_2 dopamine receptors, on the glycemic control of IDDM performed on 40 long-standing subjects with clinical signs of autonomic neuropathy and delayed gastric emptying improved glycemic control (Prando, *et al.*, 1997). The effect of bromocriptine, a potent dopamine D_2 receptor agonist on intraocular pressure in diabetic patients with autonomic neuropathy revealed that it exerts an ocular hypotensive action through presynaptic dopamine receptors (Gale, *et al.*, 1991).

Dopamine D_2 receptor gene expression increased in the striatum during diabetes as a result of the decreased transmission of dopamine. Hyperglycemia depresses the dopaminergic function and firing. Therefore a decreased dopaminergic activity is always suggested to increase the dopamine D_2 receptors. An increase in the expression of dopamine D_2 receptors gene results in the increased number.

Dopamine D_2 receptor cDNA is described to exist in two isoforms (short and

long) as a result of alternative splicing of the same gene that encodes for the receptor. They are represented as D_{2S} and D_{2L}. The longer form designated as D_{2L} is the predominant form, although there is some variability among brain regions in the relative proportions of the two forms (Higgins, *et al.*, 1991; Sealfon, *et al.*, 1991, Todd, *et al.*, 1996). Our RT-PCR data showed that the long D_{2L} form expressed in all conditions and showed increase in expression during diabetes and insulin treatment. A lesion in the striatum is reported to increase the expression of dopamine D_{2L} receptor gene (Zang, *et al.*, 1994).

In the cerebral cortex also we report an increase in the B_{max} of dopamine D_2 receptors with out any change in the affinity during diabetes. It is suggested that chronic treatment with selective dopamine D_1 or dopamine D_2 receptor blockers induces a receptor-specific increase or decrease of DA receptors (Spano, *et al.*, 1987). There fore hyperglycemia is reported to bring about an increase in the number of dopamine D_2 receptors during diabetes.

The dopamine neurons projecting to the prefrontal cortex (PFC) are thought to be involved in various motor and behavioural functions (Tam & Roth, 1997). This increased number of dopamine receptors could account for the behavioural supersensitivity to dopamine agonist as a result of damage in the dopamine functions (Cresse, *et al.*, 1976). Diabetes mellitus is also reported to be one important factor for tardive dyskinesia caused by the chronic treatment with neuroleptic antipsychotic drugs which exert their action through the dopamine D_2 receptors (Meltzer, *et al.*, 1996). The increased binding of dopamine D_2 receptors in the cerebral cortex with no change in affinity during diabetes has a relevance to the alterations in dopaminergic homeostasis affecting its function.

The dopamine D_2 receptor mRNA in the cerebral cortex increased during diabetes and remained high after the treatment with insulin. Our receptor studies show an increase in the receptor number with a decrease in affinity. Therefore such a receptor expression pattern in the cerebral cortex may be due to differential translational regulation of the dopamine D_2 receptor mRNA. Cortical dopamine D_2 receptor expression has never been previously reported in diabetes. Dopamine D_{2L} receptor mRNA expression was increased during diabetes in the cerebral cortex. Lesions in the corpus striatum is

reported to cause an increased expression of long isoform of dopamine D_{2L} receptor mRNA (Higgins, *et al.*, 1991, Sealfon, *et al.*, 1991; Todd, *et al.*, 1996).

Thus, dopamine D_2 receptors during diabetes are increased in the striatum and cerebral cortex with an accumulation of DA. Dopamine D_2 receptors are reported to regulate the release of dopamine from dopaminergic neurons originating in the ventral tegmental area as well as in the substantia nigra (Stoof, *et al.*, 1987). Hyperglycemia during diabetes could damage the DA D_2 receptors, decreasing the DA related functions in the striatum and other brain regions.

In the hypothalamus of diabetic rats the binding of [^{3}H] YM-09151-2 to dopamine D_2 receptors decreased significantly with an increase in affinity. The regional difference in the receptor status is relevant to the role which dopamine plays during various physiological and behavioural activites. Unis, *et al.*, (1998) reported that [^{3}H] YM-09151-2 binds to the dopamine D_2 high affinity receptors. The decrease number of dopamine D_2 receptors in the hypothalamus could result in the sensitization of its receptors leading to a shift into the higher affinity state. In the intra lateral hypothalamic area (Intra-LHA) blockade of dopamine D_2 receptors by specific antagonist in tumor bearing (TB) and non tumor bearing (NTB) rats increased food intake indicating the involvement of dopamine D_2 receptors in feeding mechanisms (Zhang, *et al.,* 2001). Thus during diabetes the decrease in dopamine D_2 receptor number or B_{max} could disturb hypothalamic functions. Impairment of dopamine D_2 receptor is an important factor that leads to hyperphagic and polydypsic condition as DA participates in regulating meal size (Oler, *et al.*, 1997). Dopamine –acetylcholine (DA-Ach) interaction within the lateral hypothalamus (LH) is involved in the regulation of locomotion, feeding behaviour and reinforcement (Hoebel, *et al.*, 2000, Baptista, *et al.*, 1990). The cholinergic stimulation of these activities is regulated by DA through D_2 receptors in the hypothalamus. Thus dopamine in the hypothalamus is related to sensory input, feeding reflexes, food reward or memory processes (Hernandez & Hoebel, 1988). In the hypothalamus co-administration of dopamine D_1 and dopamine D_2 agonists inhibit the feeding effect mediated by the action on neuropeptide Y (NPY) (Kuo, 2002). This is effective in the reduction of food intake in diabetic rats, revealing the efficiency of dopamine D_1/ D_2 agonist in the improvement of hyperphagia in diabetic animals. Dopamine D_2 receptor

mRNA expression was in concordant with the receptor data showing a decrease in the expression of mRNA during diabetes and insulin treatment in diabetic rats caused an increase in the expression when to control levels. A decrease in dopamine D_2 receptors are reported in obese Zucker rats which contribute to the specific feeding pattern in obese rats represented by an increased meal size and decreased meal number (Zhang, *et al.*, 2002). Insulin is reported to regulate the re-uptake of catecholamine transporters. Intracerebroventricular injection of insulin is reported to cause an increased mRNA expression of DAT (Figlewicz, *et* al., 1994). We report an increased expression of dopamine D_2 receptor mRNA during insulin treatment in diabetic rats. Our receptor analysis in the hypothalamus showed that insulin treatment did not fully restore the decreased receptor number and increased affinity to control level. The increased expression during insulin treatment in the hypothalamus could be a mechanism to normalise the decreased number to control levels. Thus, decrease in dopamine D_2 receptors in the hypothalamus due to lesions rising as a result of hyperglycemia.

Brain stem is an important part of the brain in monitoring the glucose status and the regulation of feeding (Guillod, *et al.*, 2003). In the brain stem also there was a significant decrease in the dopamine D_2 receptor density with a decrease in the affinity resulting in an overall down regulation of the receptor. Brain stem dopamine D_2 receptors have never been reported previously. During diabetes the significant increase in NE and EPI (Tasaka, *et al,* 1992; Jackson, *et al.*, 1997; 1999) could bind to α_2 adrenergic receptors increasing the sympathetic nerve discharge could inhibit insulin secretion from the pancreatic islets. From our data we suggest that the increased activation of sympathetic stimulation during diabetes as a result of increased NE and EPI is because of a decreased DA content in the brain stem with a decrease in the down regulation of dopamine D_2 receptors. This down regulation of dopamine D_2 receptors in the brain stem could have a possible role in the regulation of insulin secretion by releasing EPI and NE from the adrenal medulla that leads to the inhibition of insulin secretion in the pancreas. In the brain stem there was a decrease in the expression of dopamine D_2 receptor mRNA as a result of diabetes which increased further on insulin treatment. This could be due to the differential transcriptional regulation during diabetes. Insulin treatment brought the B_{max} to control values but there was an increase in affinity

of the receptors. Insulin is reported to have a modulatory effect on CNS dopamine and insulin injection is suggested to cause an increase in dopaminergic function (Figlewicz, *et al.*, 1996, 1994). It has been reported that damages in the brain can cause an alterations in the expression of the dopamine D_{2L} isoform which is expressed in the *in vivo* condition (Higgins *et al.*, 1991; Sealfon, *et al.*, 1991; Todd *et al.*, 1996).

Hypothalamic- pituitary –axis and dopaminergic functions during diabetes

The hypothalamus is involved in the monitoring of glucose status and the regulation of feeding and hunger (Guillod, *et al.*, 2003). Dopamine content during diabetes decreased in the hypothalamus with a decrease the number of dopamine D_2 receptors and corresponding decrease in the dopamine D_2 receptor gene expression. Diabetes activates the HPA axis producing (Mohan Kumar, *et al.*, 2003) a marked increase in food intake and water intake which is completely reversed by insulin treatment. Dopaminergic neurons are the direct targets for insulin action which participate in the reward seeking behaviour (Figlewicz, *et al.*, 2003). Therefore during diabetes the decreased availability of dopamine could affect these functions. We report a decreased dopamine content and dopamine D2 receptors in the hypothalamus during diabetes. This could decrease dopaminergic signalling in the hypothalamus. Diabetes is reported to decrease the dopamine transporter thus reducing the dopaminergic signaling affecting dopamine related functions (Galli, *et al.*, 2002). Dopamine D_2 receptor disruption is reported to impair body growth and the somatotroph population (Becu-Villalobos, *et al.*, 2002). We report a decreased b_{max} of dopamine D_2 receptor during diabetes which could impair the body growth. Thus, a decreased dopamine with a decreased dopamine D_2 receptor expression and receptor number in the hypothalamus as a result of diabetes affects the metabolic functions of the hypothalamus.

Dopaminergic alteration during diabetes in the pancreas and its significance in glucose metabolism

Pancreatic islets are sited to contain dopamine in the secretory granules along with serotonin and calcium (Ahren & Lundquist, 1985). There was a significant decrease in the turnover ratio of HVA from DA in the pancreas during diabetes. Pancreas is an important source of non-neuronal dopamine in the body and that this dopamine has a role

in protecting the intestinal mucosa (Hoffman, *et al.,*' 1996 Early sympathetic islet neuropathy (eSIN) is reported to occur selectively in the islet during diabetes in diabetic rats (Taborsky, *et al.*, 2002) as a result of monoamine alterations. Increased NE in the islets could possibly be because of the increased uptake and decreased degradation. The increased NE and EPI content with a decreased DA and HVA levels in the adrenal medulla anf plasma during diabetes was observed. Most of the NE released is efficiently removed by neuronal and extraneuronal uptake (Eisenhofer *et al.,* 1992). Evidences suggest that in the periphery DA serves not only as a precursor for active compounds released from sympathetic nerves and the adrenal medulla but also is suggested to act as an autocrine/ paracrine regulator of local organ function (Eisenhofer, *et al.*, 1995). The central nervous system cell groups project into the pancreatic vagal motor neurons receive adrenergic, noradrenergic and serotonergic inputs from the lower brain stem and a dopaminergic input from paraventricular nucleus of hypothalamus demonstrating the importance of CNS dopamine in the pancreatic hormone secretion and glucose homeostasis (Lowey, *et al.*, 1994). Thus, dopamine content in the pancreas showed marked decrease with a decrease in its metabolism. This could be related to the sympathetic tone that is increased during diabetes as a result of an increased NE and EPI levels in the plasma, pancreas and the adrenals. The metabolic clearance rate of DBH is a major factor accounting for the increase in DBH activity in the streptozotocin-diabetic rat (Stolk JM, *et al.*, 1982, (Watanabe & Nagatsu, 1991). Hyperglycemia could possibly decrease the metabolism of DA present in the pancreatic secretory granules that would affect the pancreatic islet function. As findings suggest that an endogenous alterations in these hypothalamic monoamines may contribute to islet dysfunction, which is characteristics of diabetes (Cincotta, *et al.*, 1999).

The dopamine receptors showed an increased B_{max} during with a decrease in its affinity in the pancreatic islets. This was confirmed by both Scatchard and displacement analysis. The decreased dopamine content and metabolism as a result of hyperglycemia could be a cause of the increase in the receptor parameters in the pancreatic islets causing the sensitization of these receptors. Pancreatic dopamine receptors have not been a focus of studies till date except for a few reports (Imamura, *et al.,* 1990). It has been observed that the sympathetic alpha receptor and dopamine D_1 was distributed on the B-cells, the

sympathetic β_2 receptors on the D-cell and the dopamine D_2 on the varicosity of the sympathetic β_2 neuron. Dopamine binding sites are in the pancreatic arcinar cells are suggested to be receptors that mediate the action of dopamine on cAMP accumulation (Ribet, *et al.*, 1986). Studies in the past indicate that dopamine directly affects pancreatic islet B and D cell function. Dopamine suppresses somatostatin secretion predominantly through activation of dopaminergic receptors, whereas it suppresses insulin release through an alpha adrenergic mechanism and stimulates glucagon release through a β adrenergic mechanism (Gerich, *et al.*, 1982). Dopamine acts on specific dopamine receptors related to the exocrine pancreatic secretion and sulpiride was found to be a potent dopamine antagonist in the canine exocrine pancreas (Honda, *et al.*, 1980). The increased dopamine receptors in the pancreatic islets could be as a result of the decreased dopamine turnover and as a result of the increased adrenergic activity that is damaged during diabetes.

Binding of [^{3}H] YM-09151-2 for dopamine D_2 receptors in the pancreatic islets decreased significantly with an increase in affinity. The Log (EC_{50}) value during diabetes decreased with an increase in affinity (Table 32, Fig.-34). This is similar to what we obtained in the hypothalamus for dopamine D_2 receptor. The dopamine D_2 receptors in the pancreatic islets demonstrate a down regulation in receptor number with an increase in affinity. The damage caused as a result of hyperglycemia with an increased sympathetic stimulation in the pancreatic islets could be a possible cause for the decreased activity of dopamine D_2 receptors in the pancreatic islets during diabetes. Dopamine D_2 receptor alterations will possibly have an effect on the pancreatic islet population causing a decrease in insulin secretion.

Alterations in the dopamine and dopamine D_2 receptors during diabetes

Dopamine receptors decreased significantly in the striatum while the dopamine D_2 receptors increased in the number. In the cerebral cortex, dopamine DA and dopamine D_2 receptors showed an increase and the affinity of DA receptors decreased. The hypothalamic dopamine DA receptor number did not alter but there was a decrease in affinity while the dopamine D_2 receptors decreased with an increase in affinity. In the brain stem the dopamine D_2 receptors decreased with a decrease in affinity showing an

overall down regulation. Thus from our study we conclude that the altered dopamine receptors and dopamine D_2 receptors binding observed in brain region of diabetic rats increases the sympathetic stimulation. Altered dopamine is reported to mediate an increased sympathetic nerve discharge (Bauhelal & Mir, 1993). During diabetes in the pancreatic islets the decrease in dopamine and its turn over increased dopamine receptors. The dopamine D_2 receptors decreased with an increase in affinity. The overall decrease in the dopaminergic function is as a result of the the increased EPI and NE release from the adrenals into circulation and pancreas could lead to an inhibition of insulin release.

Effect of norepinephrine on dopamine uptake in the pancreatic islets.

Dopamine is stored in the secretory granules of the pancreatic islets along with serotonin and calcium (Ahren & Lundquist, 1985). The uptake studies using [^{3}H] DA in the pancreatic islets revealed that DA uptake was maximum in the presence of 10^{-4} [^{3}H] DA in both the concentrations of glucose (4 & 20mM) and the uptake decreased with a decrease in DA concentrations. These results indicate that in the presence of glucose there is an uptake of DA into the pancreatic islets. At high concentrations DA is always taken up into the pancreatic islets in both the concentrations of glucose. This could have an implication in insulin secretion as high concentrations of DA in the presence of glucose stimuli causes a reduction in insulin secretion. Dopamine is reported to modulate insulin secretion in the pancreatic islets with changes in calcium efflux (Carpinellie, *et al.*, 1994). Possibly a high DA concentration in the islets is essential in maintaining the equilibrium during insulin secretion. The function of islet β cells is controlled by a glucose sensor that operates at physiological glucose concentrations and acts in synergy with signals originating from hypothalamic neurons. Evidence exists that the extra pancreatic cells producing and secreting these neuro endocrine signals also exhibit a glucose sensor and an ability to integrate nutrient and neuro hormonal messages (Pipeleers, *et al.*, 2001). From our uptake studies in the pancreatic islets we suggest that the DA is involved in glucose induced insulin secretion.

We observed that NE at low concentration did not have any effect on the [^{3}H] DA uptake while at high concentration inhibited the uptake of DA in the presence of 4mM and 20mM glucose. Thus, high concentration of NE blocked the uptake of DA into

the pancreatic islets and this could affect the role of DA in glucose induced insulin secretion. Increased NE level is reported to inhibit the pancreatic islet function (Sheen, *et al.*, 2001). The blockade of DA into the pancreatic islets by NE is as a result of its increased uptake by neuronal and extraneuronal tissue which causes the inhibition of insulin secretion. The following points are inferred from our uptake studies

1) Dopamine transport into the islets requires glucose and high concentrations of DA prevent glucose transport into the pancreatic islets.
2) Dopamine in the secretory granules of the pancreatic islets could be one of the possible elements that operate at physiological glucose concentrations. It acts in synergy with signals that integrate messages originating from hypothalamic neurons and pancreas and damage to this could be a possible cause of the inhibition of insulin secretion during diabetes.

Effect of dopamine on glucose induced insulin secretion *in vitro*

Dopamine in the presence of glucose had a dose dependent effect on insulin secretion. `We observed that low concentrations of DA increased glucose (20mM) induced insulin secretion while high concentration caused the maximum inhibition. Dopamine iat high concentration s reported to inhibit insulin secretion from the islets (Carpinelli, *et al.*, 1994). Also, high concentrations of norepinephrine, dopamine, and serotonin in the pancreatic islets are reported to decrease glucose-stimulated insulin secretion (Feldman, *et al.*, 1980).

We observed that butaclamol, antagonist for dopamine receptors blocked the inhibitory and the stimulatory effect of DA in the pancreatic islets mediated. The addition of sulpiride a potent dopamine D_2 receptor antagonist to the pancreatic islets effectively blocked the dopaminergic action on insulin secretion. In previous studies from our laboratory reported that addition of forskolin an activator of cAMP resulted in overcoming the effect of DA on insulin secretion (Abraham, 1998).

Dopamine D_2 receptors agonists bromocriptine (BRC) and 7-OH-DPAT were used to study their effect on glucose induced insulin secretion in the pancreatic islets *in vitro*. Bromocriptine a potent dopamine D_2 agonist at low concentrations stimulated glucose induced insulin secretion in the presence of 20mM glucose while in high

concentrations had an inhibitory effect. The stimulatión by BRC at its low concentration was not as effective in the presence of 4mM glucose. It has been reported previously that BRC treatment in hyperglycemic state had a strong stimulatory response to insulin secretion (Oliveira, *et al.*, 1998). The agonists of dopamine by acting through the neuroendocrine system improves peripheral energy metabolism and impaired islet function. (Lang, *et al.*, 1998). 7-OH DPAT showed an inhibitory effect on glucose induced insulin secretion. Previous reports suggest that 7-OH DPAT induced hyperglycemia decreased insulin secretion (Hillegaart, *et al.*, 1996). *In vitro* studies confirmed the stimulatory role of dopamine D_2 receptors on insulin secretion.

Norepinephrine is reported to have an antagonist effect on insulin secretion in the pancreatic islets (Porte, *et al.*, 1967). Low concentration of NE did not affect the stimulatory effect of DA on insulin secretion while high concentrations of NE was found to be inhibitory. It has been previously reported that high concentrations of NE inhibited the glucose induced insulin secretion (Zren, *et al.*, 1980). During diabetes there is an increased neuronal and extra neuronal uptake of NE that increases the sympathetic stimulation (Eisenhofer, *et al.*, 1992). This blocks the insulin secretion as the increased sympathetic tone elevates peripheral insulin resistance and hyperglycemia. Thus, our *in vitro* results show that increased concentrations of NE blocked the stimulatory effect of low concentrations DA.

Our *in vitro* studies show that low concentration of dopamine is necessary in the stimulation of insulin by glucose and this is mediated through the dopamine D_2 receptors in the pancreas. We report a decrease in the metabolism of DA with differential alterations in the dopamine DA and D_2 receptors in the brain and pancreas during diabetes. The decreased dopaminergic tone with a high turnover to NE and EPI results in an increased sympathetic stimulation decreasing the β-cell responsiveness to parasympathetic stimulation to secrete insulin. The increased NE not only blocks the uptake of DA but also inhibits its stimulatory effect on insulin secretion. Dopaminergic dysfunction is an important factor during diabetes which not only affects the central functions but also is a cause for the decreased insulin secretion from the pancreatic islets.

CONCLUSION

We conclude from our studies that dopaminergic system can regulate insulin secretion from pancreatic islets. Diabetes caused a marked increase in blood glucose levels and decreased the body weight. Histological studies revealed destruction in the pancreas as a result of diabetes. During diabetes a decrease in insulin secretion secretion resulted in the accumulation of glycogen granules in the brain regions of corpus striatum, cerebral cortex and hypothalamus which were reversed by insulin therapy. The changes in dopamine and DA D_2 receptor function in the corpus striatum, cerebral cortex, hypothalamus, brain stem and pancreas during diabetes causes an alteration in dopamine mediated functions. Though stimulation of sympathetic nervous system during diabetes is suggested to be an important factor in decreasing the insulin secretion, neurotransmitters their receptors and their regulatory functions have not been emphasized. We have observed an increase in the DA content in corpus striatum, cerebral cortex and decrease in hypothalamus and brain stem during diabetes. The functional significance of these changes was further explored by studying the DA and DA D_2 receptors in the brain. In the corpus striatum total dopamine receptors decreased during diabetes while the dopamine D_2 receptors increased. In the hypothalamus the dopamine DA receptor showed a decrease in affinity while the dopamine D_2 receptor number decreased with an increase in affinity. The receptor functional changes in the cerebral cortex showed that the DA receptors increased with a decrease in affinity while the dopamine D_2 receptors also increased with no change in affinity. In the brain stem the dopamine D_2 receptors there was a decrease in the receptor number with a decrease in affinity during diabetes. The expression pattern of dopamine D_2 receptors in the brain regions were in concordance with the receptor alterations. These alterations in the brain dopaminergic system result in an increased sympathetic stimulation during diabetes which inhibits insulin secretion. An increased sympathetic activity as a result of increased NE and EPI content directly inhibits the pancreatic insulin secretion. In the pancreatic islets an increased uptake of high concentrations of DA inhibits while a low concentration stimulates the glucose induced insulin secretion. During diabetes

the content of DA in the pancreas decreased with an increase in dopamine DA receptors. The dopamine D_2 receptors decreased in the islets with an increase in its affinity. Pancreatic islets dopamine receptors are involved in the regulation of insulin secretion and impairment during diabetes affects its functional nature. Dopamine in the secretory granules of the pancreatic islets could be one of the possible elements that operate at physiological glucose concentrations controlling insulin secretion. Dopamine concentrations remain low in the pancreatic islets when there is high glucose inducing insulin secretion and this ceases as glucose concentration decreases.

Thus, we conclude that dopaminergic system is impaired during diabetes. diabetes. The dopamine D_2 receptors increased in the corpus striatum and cerebral cortex but decreased in the hypothalamus and brain stem indicating their involvement in regulating insulin secretion. Dopamine D_2 receptor which has a stimulatory effect on insulin secretion decreased in the pancreatic islets during diabetes. Our *in vitro* studies confirmed the stimulatory role of dopamine D_2 receptors in stimulation of glucose induced insulin secretion. A detailed study at the molecular level on the mechanisms involved in the role of dopamine in insulin secretion, its functional modification could lead to therapeutic interventions that will have immense clinical importance.

SUMMARY

1. Streptozotocin induced diabetic rats were used as model to study the role of dopamine and its receptors in insulin secretion.

2. The dopamine content increased in the striatum of rats injected with different doses of STZ with a decrease in the HVA and HVA/DA ratio. Dopamine content began to increase 12hrs after the injection of STZ in the striatum showing significant increase when the rats became diabetic. HVA content began decreasing 3hrs after the injection of STZ and remained low when the rats turned diabetic.

3. The dopamine content increased in the corpus striatum, cerebral cortex and decreased in the brain stem and hypothalamus of diabetic rats showing an overall decrease in the metabolism of DA.

4. The homovanillic acid content was decreased in the striatum, brain stem and hypothalamus of diabetic rats as the turnover from DA to HVA decreased proportionally.

5. The dopamine receptors in the corpus striatum decreased with no change in affinity while it increased in the cerebral cortex with a decrease in affinity. In the hypothalamus there was a decrease in affinity. Thus, the alterations in the dopamine receptors in the different brain regions had a differential effect during diabetes.

6. The B_{max} of dopamine D_2 receptors in the corpus striatum and cerebral cortex increased during diabetes with no change in affinity. In the hypothalamus there was a decrease in B_{max} with an increase in affinity while in the brain stem both B_{max} and affinity decreased during diabetes.

7. During diabetes the expression of dopamine D_2 receptor mRNA was found to be increased in the striatum and cerebral cortex. In the hypothalamus and the brain stem dopamine D_2 receptor mRNA expression decreased during diabetes.

8. The differential alteration of dopamine DA and dopamine D_2 receptors in the brain could lead to the increased sympathetic activity that decreases insulin secretion in the pancreatic islets.

9. Plasma and adrenal dopamine and homovanillic acid content decreased while there was an increase in NE and EPI content during diabetes.

10. Pancreatic DA and HVA content decreased in diabetic rats. The dopamine receptors in the pancreatic islets increased with a decrease in affinity while the dopamine D_2 receptors decreased in number with an increase in affinity.

11. *In vitro* studies using [^{3}H] DA in the pancreatic islets showed an increased uptake of DA at high concentrations in the presence of glucose.

12. High concentrations of norepinephrine blocked the uptake of [^{3}H] DA into the pancreatic islets.

13. Dopamine in high concentration (10^{-4} M) inhibited the glucose induced pancreatic insulin in the pancreatic islets.

14. Dopamine in low concentration (10^{-8} M) in the presence of 20mM glucose concentration stimulated significantly insulin secretion in the pancreatic islets.

15. Norepinephrine at high concentrations inhibited the stimulatory role of DA in glucose induced insulin secretions in presence of 20mM glucose concentration.

16. Bromocriptine a potent dopamine D_2 receptor agonist at low concentrations stimulated glucose induced insulin secretion while 7-OH DPAT an agonist of dopamine D_3 like receptors inhibited insulin secretion.

17. Dopamine in the pancreas mediated its action through its receptors and dopamine D_2 receptors are involved in the stimulation of insulin secretion

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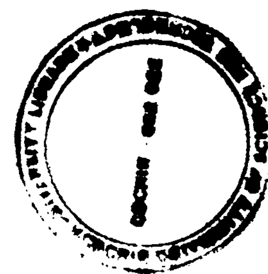

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