BRAIN ADRENERGIC AND SEROTONERGIC RECEPTOR FUNCTION IN STREPTOZOTOCIN-DIABETIC RATS

THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
BIOTECHNOLOGY

by

PIUS STEPHEN PADAYATTI

DEPARTMENT OF BIOTECHNOLOGY

COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN - 682 022
KERALA, INDIA.

JULY 1996

CERTIFICATE

This is to certify that the thesis entitled " **Brain Adrenergic and Serotonergic Receptor Function in Streptozotocin-Diabetic Rats**", is a bonafide record of the research work carried out by Mr. **PIUS STEPHEN PADAYATTI** 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.

Dr.C. S.Paulose,
Lecturer,
Department of Biotechnology
Cochin University of Science and Technology

Dr. C. S. Paulose M.Sc., Ph.D., FIMSA.
Lecturer, Dept. of Biotechnology
Cochin University of Science & Technology
Cochin - 682 022 Kerala - India.

COCHIN-22
24/06/1996

DEPARTMENT OF BIOTECHNOLOGY
UNIVERSITY OF SCIENCE & TECHNOLOGY
COCHIN-
682 022
COCHIN

DECLARATION

I hereby declare that this thesis entitled " **Brain Adrenergic and Serotonergic Receptor Function in Streptozotocin-Diabetic Rats**", has not previously formed the basis for the award of any degree, diploma, associateship or other similar titles or recognition.

COCHIN-22
26/06/1996

PIUS STEPHEN PADAYATTI
(Ph.D. Reg. No.1422)

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my supervising guide Dr.C.S.Paulose, Lecturer, Department of Biotechnology, for his excellent guidance throughout this study. It is my pleasure to thank him for introducing me to the subject of Neurobiology. His patience and valuable suggestions will always be remembered with thanks.

I wish to thank Dr.G.S.Selvam, Head of the Department, Department of Biotechnology for providing me with facilities to undertake this work. Dr.M.Chandrasekaran, former Head of Centre for Biotechnology, for his help and encouragement in conducting this work. I wish to express my sincere thanks to Dr.Padma Nambissan.

I thank all my colleagues Asha, Sudha, Jackson, Biju and Ani for their kind help and encouragement throughout this study. I also wish to thank Dr K.G.Raghu,scientist CDRI. I wish to thank all of my lab mates for the nice time that we spent together.

I thank all the office staff of the Department of Biotechnology for taking care of my official things with utmost care.

I wish to thank Dr.Raghunath and Dr.Devadas, of Central Institute of Fisheries Technology, Cochin for making available facilities at their Institute.

I wish to express my deep thanks to Dr.Wahid, Mrs. Kamalamma, Radiation Officer of Kerala Agricultural University for making available the radiation facility during initial periods of my study. I thank them for being kind enough to patiently listen to our

difficulties and render timely help. Also I wish to thank Dr.Ramadasan Kuttan of Amala Cancer Research Centre, Trissur, for making available their radiation facilities.

I thank all people at the Small Animal Breeding Station of Veterinary College, Mannuthy for making available enough animals for all the experiments. I wish to express my special thanks to Mrs. Kamalamma, Animal care taker.

I wish to express my sincere thanks to Dr.P.Madhavan Pillai, Professor and Head, Department of Applied Chemistry, for his kind and caring nature. Also I thank him for making available the facilities of his Department. I thank all other faculty members of the same Department, especially Dr.Prathapan, Lecturer in Photochemistry for providing very useful chemicals. I thank Dr. P. A. Unnikrishnan, for his friendly nature and timely help during the whole period of my stay in the University.

Above all these, I wish to express my heartfelt thanks to my mother who suffered all the loneliness during the course of my Ph.D. I also like to thank all my brothers and sisters who patiently listened to my difficulties and guided me in my research career.

I wish to express my heartfelt thanks to my late father who would have been a very happy and proud person about my achievements. I dedicate my thesis and achievements to him.

I wish to remember all my teachers during my college and school education and all others who directly or indirectly contributed to my work.

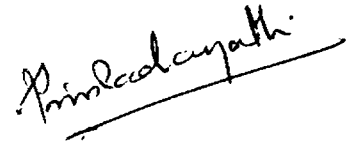
I would like to thank my friends Dr Wilson (MD) and Mr Maxon

I wish to express my thanks for providing fellowships by Department of Atomic Energy, BARC for a JRF, University Grants Commission for JRF (NET), and Lecturership and CSIR for an SRF.

I wish to express my respects to my uncle Mr.Joseph who was a constant encouragement during my whole period of Ph.D.

I wish to express my sincere gratitude to Mr.Sasi of CUSAT for typing this manuscript and for his unending co-operation.

I cannot forget the never-ending co-operation of all my colleagues in the Applied Chemistry Department. I take this opportunity to thank all of them.



ABBREVIATIONS USED IN THE TEXT

ACTH	Adrenocorticotrophic Hormone
Bmax	Binding Maximum
BS	Brain Stem
cAMP	cyclic Adenosine Mono Phosphate
CB	Cerebellum
CC	Cerebral Cortex
CNS	Central Nervous System
CS	Corpus Striatum
DA	Dopamine
DOPAC	Dihydroxy Phenyl Acetic Acid
EPI	Epinephrine
Gi	Inhibitory G-Protein
Gpp[NH]p	5'- Guanylyl- imidodiphosphate-tri sodium salt
GTP	Guanosine Triphosphate
HYPO	Hypothalamus
5-HIAA	5-Hydroxy Indole Acetic Acid
HPA	Hypothalmic Pituitary Adrenal axis
HPLC	High Performance Liquid Chromatography
5-HT	5-Hydroxytryptamine (Serotonin)

5-HTP	5-Hydroxytryptophan
HVA	Homovanillic Acid
Kd	Dissociation Constant
MHPG	3-methoxy-4-hydroxy-phenylglycol
NE	Norepinephrine
NPY	Neuropeptide Y
8-OH-DPAT	8-Hydroxy-n-dipropylamino tetralin
P	Level of significance
PAC	Para amino clonidine
PKA	Protein Kinase A
PKC	Protein Kinase C
PLC	Phospholipase C
PNMT	Phenyl N-methyl transferase
R	Receptor
R_H	High Affinity Receptors
R_L	Low Affinity Receptors
S.E.M	Standard Error Mean
STZ	Streptozotocin

CONTENTS

Introduction

INTRODUCTION

The central nervous system (CNS) neurotransmitters play an important role in the regulation of glucose homeostasis. The hypothalamic adrenergic and serotonergic neurons are the major components which play an important role in the release of releasing factors from the neurohormonal cells (Brownstein, 1977). The neurotransmitters are shown to help in restoring the glucose induced insulin and glycogen secretion in experimental diabetic animals (Ho, *et al.*, 1995). In turn, it is shown from *in vitro* experiments that glucose modulates the release of endogenous catecholamines (Jung *et al*,1993). The recent demonstration of CNS cell groups projecting into the pancreatic vagal motor neurons showed that they receive inputs from adrenergic, noradrenergic and serotonergic neurons of the lower brain stem and a dopaminergic input from paraventricular nucleus of hypothalamus (Lowey, *et al.*, 1994). This evidently showed the importance of CNS neurotransmitters in the pancreatic hormone secretion and their importance in the glucose homeostasis.

The metabolic disorder -diabetes mellitus- is associated with peripheral as well as central nervous system neuropathy (Satoshi *et al.*, 1993, Yagihashi, *et al.*, 1985). In contrast to many diabetes associated complications, the chronic diabetic complications of the CNS are subtle and remain unrecognized (Mooradian, *et al.*, 1988). The diabetic rats are reported to have altered hypothalamic growth hormone (GH) and leutinising hormone (LH) function (Martin, *et al.* 1992). The counter regulatory responses from the brain neuroregulatory centres through the hormone stimulus is also defective in diabetic state (Powell, *et al*, 1993). These studies reveal that diabetic CNS complications are themselves impaired and are not counter regulated effects of an altered hormonal status. Many pathogenic mechanisms have been suggested for the CNS dysfunction (Nowak, *et al.*, 1995; Karasu, *et al.*, 1995). Studies on the treatment of diabetic neuropathy with several

compounds not only helped in the treatment but also helped in understanding the pathologic mechanism of the neuropathy (Ido, *et al.,* 1994; Schmidt, *et al.,* 1989). Though considerable work has been done on diabetes related peripheral neuropathy as could be seen from the valuable contributions of Schmidt *et al.,* (1989, 1993), Nowak *et al.,* (1995), Maeda *et al.,* (1993), Stevens *et al.,* (1994), Sima *et al.,* (1993) and Schneider *et al.,* (1993), the available information on the diabetic central nervous system in relation to neurotransmitters is limited (Mooradian and Scarpace, 1988; 1988a, ; Bitar, *et al.,* 1987, 1992; Moratinos, *et al.,* 1975, 1988; Mans *et al.,* 1987; Garris, 1990, 1995; Xiang and McNeill, 1987, 1990; Satoshi, *et al.,* 1993). The strong evidences for a possible role for brain neurotransmitters and their receptors in glucose homeostasis have come from related studies. These findings emphasized more on the role of brain monoamines in glucose regulating function under normal conditions. All these findings show a requirement of carrying out such studies in diabetic state (Chaouloff *et al.,* 1987, Furman *et al.,* (1974, 1980), Gagliardino *et al.,* (1971), Smith , (1977), Smythi *et al.,* (1984, 1992), Iverson (1973), Hiyoshi *et al.,* (1995), Oda (1994), Sugimoto *et al.,* (1994), Yamada *et al.,* (1994), Hirose *et al,* (1993a,1993b). Another feature emerged from such studies is a close association of both adrenergic and serotonergic system in glucoregulatory function. This close association was studied using the drug 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (Chen and Reith 1995). The monoamine interactions measured after i.c.v.administration showed 8-OH-DPAT, though a $5H_1A$ agonist, produced releasing effects on noradrenergic and dopaminergic neurons. Also, activation of 5-HT receptors is reported to release [^{3}H]NE in rat limbic structure (Guillot *et al.,* 1995). A close association between adrenergic and serotonergic nerves of hypothalamic region suspected to be involved in glucoregulatory functions. The pharmacological studies have contributed greatly to the understanding of the receptor subtypes involved in this glucoregulatory function in CNS (James and Hodgson 1995, Hirose *et al.,* 1993; Jamnicky *et al.,* 1993;

Xiang *et al.*, 1990, Hirose, *et al.*, 1993a, Alvarez,*et al.*, 1993). The stress induced hypothalamic variations in neurotransmitters and their receptors have given more insight into a possible involvement of hypothalamic-adrenal axis in the glucose homeostasis (Takao, *et al.*, 1995 ; Yehuda *et al.*, 1984; Smythe *et al.*, 1983). The hypophagia and obesity research also pointed to a possible role of the hypothalamo-pituitary-adrenal (HPA) axis and its involvement in the diabetic state (Grignaschi, *et al.*, 1993; Levin,*et al.*, 1993). The research on the involvement of brain neurons in glucoregulatory function prompted us to take this problem. In the present study the neurotransmitters, its metabolites and receptors in streptozotocin (STZ) induced diabetes with special emphasis on α-$_2$ adrenergic and serotonin pathways were carried out.

Review of Literature

REVIEW OF THE LITERATURE

INSULIN SECRETION IS UNDER NERVOUS CONTROL

The role of central nervous system in the regulation of glucose homeostasis has been recognized for more than a century. Research in this regard till now strongly supported a close relation between catecholamines and glucose homeostasis. Recent studies done by many authors reveals several interesting aspects on regulation of pancreatic secretion by CNS. Ho *et al.*, (1995) demonstrated that reduction in acetylcholine (ACh) and norepinephrine (NE) potentiating effects, decreases glucose sensitivity of islet β and α cells in streptozotocin diabetic model. In another study by Hiyoshi *et al.*, (1995) by intracerebroventricular (i.c.v.) administration of neostigmine, caused an increase in plasma levels of catecholamines and glucose but not insulin. When atropine was co-administered with phentolamine, the phentolamine induced increase in insulin secretion, was inhibited. But neither phentolamine nor atropine affected plasma levels of catecholamine. At the same time,α-$_2$adrenoceptor antagonists Yohimbine and Idazoxan reversed adrenaline induced inhibition of insulin secretion and thus glucose levels (Hiyoshi *et al.*, 1995). The results suggest that inhibition of insulin release induced by adrenaline was reversed by antagonism of α-$_2$-adrenoceptors. The pancreatic function has been studied extensively in relation to CNS neuronal regulation by several others also. The enteropancreatic sympathetic nerve when analysed for its regulatory action through cholinergic and serotonergic neurons suggested that serotonergic enteropancreatic innervation inhibits pancreatic secretion via presynaptic receptors on cholinergic nerves (Kirchgessner *et al.*, 1995). The peripheral system adrenergic receptors and nerves involved in pancreatic glucagon and insulin secretion in rabbits revealed that β-adrenergic

receptor mechanism is an important component of adrenergic modulation of pancreatic glucagon secretion in conscious rabbits (Odo, 1995). The plasma insulin concentrations were suppressed by α-adrenergic stimulation more than α and β-adrenergic stimulation. The peripheral adrenergic receptor and regulation of glucose, insulin and amylin when analyzed using selective α-$_2$ adrenoreceptor agonist UK 14.304 produced a dose-dependent reduction in the normal rats in their content. It is suggested that insulin and amylin are both under inhibitory control via α-$_2$adrenoceptor though the responses may be differently regulated (Guillot, 1995). These recent observations on nervous regulation of blood glucose added to increasingly evident role and importance of studies on catecholamine involvement in glucose homeostasis in the metabolic disorder diabetes mellitus

DIABETIC NEUROPATHY IS ASSOCIATED WITH SEVERAL MEMBRANE ASSOCIATED CHANGES.

A common complication associated with diabetes mellitus is CNS and autonomous nervous system (ANS) neuropathy. Several authors have analysed this complication and extensive studies were carried out. These studies often revealed the impairment of certain vital mechanism in the nerves especially those associated with membranes. The impaired sodium-potassium-ATPase (Na^+-K^+ ATPase) activity is reported in diabetic peripheral nerves by several authors recently (Nowak,*et al.*, 1995, Maeda, *et al.*, 1993; Gurcharan and Sukhwinder 1994). Nowak *et al.*, (1995) found that this enzyme activity is impaired in the vagal nerves of streptozotocin (STZ) induced diabetic rats which has been invoked as being factorial in the genesis of diabetic peripheral neuropathy. Maeda *et al.*, (1993) analysed the same enzyme activity, which is important in the maintenance of Na^+/K^+ pump of the nerve membrane, and the improvement of this impairment using prostaglandin E_1 analogue OP 1206 α CD (OP). Their studies revealed that

all compounds OP, dibutyrl-cAMP (db cAMP) and aminophylline, which can elevate cAMP content of diabetic rat nerves, which is significantly reduced in the peripheral nerves could increase (Na^+K^+) ATPase activity dose dependently. Also those compounds which can inhibit Protein Kinase C (PKC) activity for example, Staurosporine, abolished the (Na^+K^+)ATPase activity which occurred within one minute. This showed that (Na^+K^+) ATPase activity may be modulated by the Protein Kinase A (PKA) pathway. Gurcharan and Sukhwinder,(1994) reported the same enzyme activity in discrete brain regions of rats with alloxan induced diabetes mellitus. They found a decrease in the activity of (Na^+K^+)ATPase in different brain regions including different hypothalamic nuclei.

Another aspect of diabetic neuropathy which attracted much attention for many workers is free radical induced damage and treatment with antioxidant on diabetic nerves. Karasu *et al.*, (1995) have studied the effect of antioxidants probucol or vitamin E on sciatic nerve dysfunction. Ohkuwa *et al.*, (1995) have studied the hydroxyl radical formation induced by streptozotocin in diabetic tissues. They found that the free hydroxyl radicals may be formed in heart, muscle and brain by enzymatic transformation which may account for some pathological processes in these tissues. Ido *et al.*, (1994) demonstrated a link between imbalances in carnitine metabolism and several metabolic and functional abnormalities associated with diabetic polyneuropathy and indicated that decreased sciatic nerve endoneurial ATPase activity in STZ model of diabetes is associated with decreased 1,2-diacyl-sn-glycerol (DAG).

The involvement of hyperglycemia in diabetic neuropathy is strongly supported by many workers like Schneider *et al.*, (1993), Pekinar *et al.*, (1993) and Hermenegildo *et al.*,(1993). Schneider argue that enhanced anaerobic glycolysis (due to hypoxia) produces resistance to hypoxia in hyperglycemic peripheral nerves

and that acidification may impair the function of peripheral axons when anaerobic glycolysis proceeds in a tissue with reduced buffering power. Pekinar analysed the actin brain neurons of diabetic animals. The brains from the diabetic animals contained an extra polypeptide that migrated close to actin and reacted with monoclonal antibody C4 against actin. But authors could not find any effect of glycation *in vitro* on the ability of muscle G. actin to form F-actin. Rosella *et al.*, (1985) report on the change in axon cross sectional area and slow transport in sciatic and primary visual systems of rats with STZ induced diabetes of 4-6 weeks duration. Nerve ischemia in diabetic rats is reported to be another reason for the nerve injury (Stevens *et al.*, (1994); Sutherland *et al.*, 1992). Several other authors have reported diabetes associated lipid alteration in the neurons (Park and John (1993), Suzuki *et al.*,(1991)). Mathew and Eichberg (1994) report on an alteration in a protein mediated phosphoinositidase C in solubilized rat peripheral nerve myelin. They predict a possibility of impaired cell signaling in experimental diabetic neuropathy.

The compounds like acetyl-L-carnitine sorbinil, gangliosides, antioxidants and acarbose is reported to have positive effects on deteriorating diabetic neuropathy(Ido *et.al.* (1994), Schmidt, *et.al.* (1989), Nowak *et.al.* (1995), Suzuki *et.al.*, 1991), Sima and Chakraborty (1993)). Sima and Chakraborty (1993) report a total prevention and partial recovery in other diabetic polyneuropathy syndrome by dietary acarbose treatment.The study on the diabetic neuropathy showed that several factors together seems to contribute to produce autonomous neuropathy. But interesting out of all these are diabetes associated membrane disorders which is associated with lipid metabolism and second messenger function in diabetic neurons

BRAIN MONOAMINES ARE ALTERED IN DIABETICS

The changes in the brain neurons during diabetes also attracted many workers (Bhattacharya and Saraswathi 1991, Garris,1990, Bitar *et al.*,1987, Lorden *et al.*,1975, Lackovic' *et al.*,1990, Sasaki *et al.*,1983). The changes in the brain monoamines during experimental diabetes have been reported by many authors. Lim *et al.*, (1995) have described the changes in the striatal region dopamine and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). The uptake affinity and velocity of dopamine in synaptosomes have been studied and found a significant decrease. Gurcharan *et al.*, (1994) have reported changes in the Na^+K^+ATPase activity from discrete areas of the rat brain to be decreased. Akira *et al.*, (1994) showed that 2-deoxyglucose induced hyperglycemia to have not only noradrenergic nerve component but also hypothalamic cholinergic system. Garris (1995) analyzed the developmental and regional changes in brain norepinephrine (NE) levels in diabetic C57BL/KSJ mice. Regional brain (i.e. amygdala, hypothalamus and medulla) NE levels were chronically elevated when compared to age matched controls (8 wk. of age). The brain NE elevation is proposed to be associated with an overt expression of the gene causing diabetes mellitus in this model. Also, he found that these changes may be modulated by ovarian steroid hormones. Kamei *et al.*, (1994) suggests altered dopamine turnover in the limbic forebrain to enhanced spontaneous locomotor activity. The increased dopamine neurotransmission in diabetic mice may also be due to the upregulation of δ opioid receptor mediated function because δ opioid receptor antagonist reduced the spontaneous locomotor activity in diabetic mice. Jung *et al.*, (1993) demonstrated through some simple experiments that glucose itself modulate release of endogenous catecholamines from hypothalamic fragments *in vitro*.

The effect of insulin treatment on catecholamine levels is reported in insulin treated normal, insulin treated diabetic and non treated control rats (Bellush

and Reid, 1994). The low and high doses of insulin did not show any variation on its effect on 5-hydroxy indole-3-acetic acid and 5-HT levels. The Insulin treated normal rats also showed induced hyperphagia and excessive weight gain. Another set of brain neurons called peptidergic neurons have also been studied in diabetic. The function of peptidergic nerves especially NeuroPeptide Yergic (NPYergic) nerves and feeding has been proposed. Frankish *et al.* (1993) have analyzed NPY receptor numbers in hypothalamus and found to be reduced. Diabetes as well as food deprivation markedly increased hypothlamic NPY and NPY mRNA levels. These changes were explained as an increased NPY release in hypothalamus and it may be mediating in food seeking behavior, hyperphagia and pituitary dysfunction observed in diabetic and food deprived animals.

The diabetes related changes in blood brain barrier have been proposed to play a role in CNS lesions. Mooradian and Scarpace (1992) has analyzed β-adrenergic receptor activity of cerebral micro vessels in STZ-diabetic rats. He found a reduced post receptor activation of adenylate cyclase in cerebral micro vessels while β-adrenergic density, affinity and receptor-cyclase coupling are not significantly altered. Lass and Krudsen (1990) had found a significantly reduced cerebral blood blow in response to propranolol in streptozotocin diabetic rats.

The dopaminergic function has been proposed to play a role in many behavioral changes. Heaton and Varrin (1993) have proposed a correlation between yawning behavior in diabetic animals and dopamine content. Chen (1992) reported an important aspect of monoamine changes in diabetic brain to duration of the disease. He reported a pattern of monoamine changes in the diabetic brain neurons.Bitar *et al.*, (1987) have analysed hypothalamic nuclei and the changes during diabetes-induced by STZ. The first mention of a neurotransmitter to a gene i.e., NE to a gene is proposed by Eleftheriou (1974). The genetically produced

diabetes is predicted to have genetic influence on hypothalamic NE level. Lorden *et al.*, (1975) have shown that in genetically diabetic mice there is an elevated NE level. Garris (1990) proposed adrenergic receptor changes along with increased NE level in genetically obese rats, which is suggested to be related to the expression of the obese genes. Lackovic *et al.*, (1990) analyzed human diabetic brain samples and compared with postmortem tissues equally taken from STZ and alloxan diabetic rats. They observed an increase in the serotonin content in the medial and lateral pallidus. The authors did not propose such changes in monoamines to be associated with any generalized metabolic disturbance. But the literature till now cited show a strong correlation between the central catecholamines and their role in glucose homeostasis.

MONOAMINES PLAY AN IMPORTANT ROLE IN GLUCOSE HOMEOSTASIS

Central nervous system and peripheral neurons have an important regulatory role in glucose homeostasis in diabetes mellitus. Ho *et al.*, (1995) showed that neurotransmitters especially ACh and NE partially restores glucose sensitive insulin and glycogen secretion in STZ-induced diabetic rats. They attribute these changes to a decline in acetylcholine esterase and monoamine oxidase activity in islet cells. Hiyoshi *et al.*, (1995) have i.c.v. injected imidazoline antagonists of α_2-adrenoceptors and analyzed endogenous adrenaline induced inhibition of insulin release in anaesthetized normal rats. The results suggested that inhibition of insulin release induced by adrenaline was reversed by antagonism of α_2-adrenoceptors (Yohimbine and Idazoxan). Potter *et al.*,(1977) have analyzed the greater hypoglycemic potency of epinephrine (EPI) as compared to isoproterenol in diabetic rats which is reported earlier (Moratinos *et al.*, 1975). They found that the catecholamines though did not directly act on liver, muscle and adipose tissue,

influence the responsiveness of the pancreatic islet cells which in turn, alters deposition of key metabolic substrates. In fasted rabbits it is shown that the hyperglycemic action of α_1-adrenoceptors is due to higher glycogenolysis while α_2-adrenoceptor effect on hyperglycemia is due to inhibition of insulin secretion and enhanced glycogenolysis (Reverte *et al.*, 1991). Gracia-Barrado *et al.*, (1992) have shown that Ca^{++} channel blockers play a role in insulin secretion mediated by catecholamines. Oda *et al.*, (1994) have shown that β-adrenergic receptor mechanism is an important component of adrenergic modulation of pancreatic glucagon secretion in conscious rabbits. They also conclude that hyperglycemia was induced by both α and β adrenergic stimulation but the extent was greater under β-adrenergic stimulation. Guillot *et al.*, (1995) have studied the α_2-adrenoceptor involvement in neonatal STZ-induced diabetic rats using the α_2-agonist UK 14.304. Their results suggest that insulin and amylin are both under inhibitory control via α2-adrenoceptor. It is also suggested that diabetes in the neonatal STZ-induced rat model is associated with a hypersensitivity of the pancreas to α_2-adrenoceptor stimulation. Ansari *et al.*, (1994) reported on the β-adrenergic modulation of rat brain insulin receptor activity in normal and hyperglycemic conditions. The insulin binding remained the same in hyperglycemic state but β-agonist treatment enhanced the receptor kinase activity. These authors claim to report for the first time for direct action of insulin on catecholamines. The recent observations on pancreatic innervation from CNS monoamine cell groups using the method of transneuronal labeling study showed that pancreatic vagal motor neurons receive inputs from adrenergic, noradrenergic and 5-HT neurons from the lower brain stem and from a potential dopaminergic input from paraventricular nucleus (PVN) (Loewy *et al.*,1994) Sugimoto *et al.*,(1994) studied the glycemic control in a hypoglycemia model induced by tolbutamide. He found that a 5-HT_1 and 5-HT_2 receptor antagonists strongly blocked the tolbutamide induced inhibitory effects. Tryptamine strongly inhibited tolbutamide effects and

induced hyperglycemia. This hyperglycemia was blocked by 5-HT_2 antagonist ketanserin. These results prompted the authors to predict involvement of 5-HT_2 receptors in tolbutamide induced hypoglycemia. This hyperglycemia was blocked by 5-HT_2 antagonist ketanserin. These results prompted the authors to predict involvement of 5-HT_2 receptors in tolbutamide induced hypoglycemia. They also predicted a possible role of tryptamine in glucagon release. This was proved true by another group of authors. Yamada *et al.*,(1994) showed peripheral S_2 receptor involvement in hyperglucagonemia. Kurose *et al.*, (1992) reported sympathetic neural activation and the sensitivity of β-cells enhancement in diabetic animal model. They analysed the glucagon, insulin and somatostatin secretion induced by electrical splanchnic nerve stimulation in STZ-induced neonates and STZ-induced diabetes in adults. Smythe *et al.* (1992) proposed noradrenergic intervention as a method of controlling hyperglycemia in diabetes mellitus. They examined whether a blockade of noradrenergic responses from hypothalamus to stress might suppress the associated hyperglycemia. Treatment of rats with 2-deoxy-D-glucose, Yohimbine or neostigmine increased both noradrenergic neuronal activity and serum glucose. When rats were additionally pretreated with pentobarbital, the noradrenergic neuronal activity and its effects were blocked. The data suggested and demonstrated that inhibition of central noradrenergic activity is also associated with an inhibition of hyperglycemia. All the above studies point to a possible involvement of CNS catecholamine changes to play a role in producing diabetic condition.

WHY DIABETIC HYPOTHALAMIC SEROTONERGIC AND ADRENERGIC ALTERATIONS ARE IMPORTANT

Similar studies on stress induced hormonal regulation by the hypothalamic noradrenergic and serotonergic nerves have helped to understand how

hyperglycemic state can be produced by changes in the hypothalamic nuclei. Moreover another feature which emerged from stress related hypothalamic studies is a close co-operation between adrenergic and serotonergic nerves. This is emerging as a new insight into mechanism of two different nerve types together regulating a physiological process. The drug called 8-hydroxy-2-(din-n-propylamino)tetralin (8-OH-DPAT) helped a lot in analyzing the hypothalamic monoamine interactions (Chen and Reith, 1995)). Their analysis using the technique of micro dialysis in the ventral tegmental area of rats treated systematically with 8-OH-DPAT showed that a low dose of the drug may act at presynaptic 5-HT_1A receptors to modulate 5-HT and DA release, while acting at post-synaptic 5-HT_1A receptors modulate NE release. But high dose of the drug may activate D_2 receptors to offset DA level increase and the locomotor stimulatory effect of 8-OH-DPAT might be mediated primarily by post synaptic 5-HT_1a receptors modulate NE system. Bluet *et al.*, (1995) in their experiments showed that PVN-lesioned animals showed a blunted ACTH response to 8-OH-DPAT. This indicate that major sites of 5-HT_1A interactions in PRL and ACTH regulation are located within the CNS and not in the pituitary. Tian *et al.*, (1993) showed a close intertalk between tryptaminergic, noradrenergic and dopaminergic neurons in hypothalamus (Arborelin *et al.*, 1993). Chaouloff and Jeanrenaud, (1987) analysed the receptor subtypes involved in the hyperglycemic and hypoinsulinemic effects of 8-OH-DPAT when applied i.c.v. into hypothalamus of conscious rats. They proposed that the drugs physiological manifestations are mediated through 5-HT_1a and α-$_2$ adrenergic receptors in the hypothalamus. The diabetic state in streptozotocin-induced rats reported to have altered anterior pituitary and hypothalamic functions (Bestetti *et al.*, 1995). The evidence from the adrenodemedullation (ADM) experiments revealed several interesting findings. Christine *et al.*, (1993) found that diabetogenic effect of streptozotocin reduced in the adrenodemedullated rats. Their results indicate that ADM rats appeared to be

more resistant to developing hyperglycemia after single injection of STZ than sham animals, and this being associated with a greater pancreatic insulin content. Social crowding stress was analysed by Bugajski *et al.*, (1993) for the changes it produced in pituitary-adrenocortical and hypothalamic histamine response to adrenergic stimulation. The results indicate that social stress of crowding considerably impairs the hypothalamic-pituitary-adrenocortical responsiveness to central β and α-$_2$ adrenergic receptor stimulation.

Although stress is suspected to play a role in the development of diabetes mellitus, no direct evidence for involvement of adrenal medulla in onset of the disease has yet been found. But Christine *et al.*, (1994) gave a good evidence for the importance of hypothalamo-pituitary-adrenal (HPA) axis in diabetes mellitus. Takahashi *et al.*, (1993) have shown how the sympathetic nervous activity and adrenal medulla synchronize the sympathetic tone in the body. They used adrenodemedullation and sympathectomy and found that some adrenergic nerves show functional compensation under conditions of depression of the adrenal medulla and that compensatory acceleration of the adrenal medullary function occurs under conditions of adrenergic dysfunction. But in diabetic state the hormonal feedback on sympathetic and parasympathetic nervous system is in fact impaired. Powell *et al.*, (1993) have studied counter regulation against hypoglycemia in IDDM in nondiabetic, spontaneously diabetic BB/wor rats using euglycemic/hypoglycemic clamping. Their data suggests that in IDDM iatrogenic hypoglycemia magnifies preexisting counter regulatory defects, thereby increasing the risk of severe hypoglycemia. The diabetic rats show abnormalities in growth hormone and prolactin secretion (Bestetti *et al.*, 1995, Locatelli *et al.*, 1985). This alterations is suspected to have an altered post synaptic function of NE system (Bitar *et al.*, 1987). Previous indications of an altered adrenergic activity in hypothalamus of diabetic rats point out an involvement of adrenergic nerves in an

altered HPA axis. So the literature thus far cited showed that there are strong indications of an adrenergic and serotonergic nervous system involvement in diabetic hypothalamus. But very few people (Garris 1990, Bitar *et.al.*, 1987, Mooradian *et.al.*, 1992 Lass *et.al.*, 1990) have analysed the receptor subtypes of both adrenergic and especially serotonergic receptor changes in the streptozotocin induced diabetic brain regions.

DIABETES DISRUPTS HORMONE SIGNALLING

The receptor changes analysed in the diabetic state also indicated large variations in the membrane associated second messenger system. Several authors have shown that diabetes is associated with an impaired membrane associated second messenger function. Diabetes can produce a state of hypothyroidism which is known to alter a adrenoceptor activity in rat hearts. Several authors have studied the altered adrenergic function in cardiac tissue (Stewart *et.al.*, 1994, Goyal .*et al.*, 1987, Bhimji and McNeill 1989, Ganguly *et.al.*, 1986,1987, Williams *et.al.*, 1983) The thyroid status in diabetic rats is suspected to be the cause of altered sympathetic activity and thus related complications of cardiomyopathy. Brain neurotransmitter, receptor studies in relation to thyroid function and hypertension have been established in pyridoxine deficient animal model (Paulose and Dakshinamurti 1984, 1985, 1988, Dakshinamurti and Paulose 1985, 1986, 1990, Viswanathan *et al.*,1990) Their studies clearly show the functional correlation between the neurotransmitters, receptors and the hormonal pathway in hypothyroidism and hypertension. Increased sympathetic stimulation is suggested to decrease insulin function. Diabetic heart was one of the organs in which several authors have reported changes in their messenger functioning. Gando (1994) have found a decrease in the functional responses to cAMP increasing agent like

β-adrenoceptor agonist. They found that this change is attributable to impaired phosphorylation of cardiac regulatory phosphoproteins including phospholamban. In streptozotocin induced diabetes GTP analogue mediated stimulation of phosphoinositidase C in peripheral nerve myelin and its alteration is studied (Mathew and Eichberg, 1994). In detergent solubilized myelin preparations from STZ-induced diabetic rats, a higher concentration of the guanine nucleotide analogue was required to acheive stimulation comparable to that obtained with corresponding preparations from normal animals. They predicted from the above studies, a possible impaired G-protein function in cell signalling in experimental diabetic neuropathy. Borghini *et al.*, (1994) studied different isoforms of PKC in sciatic nerves, but failed to observe any abnormality in PKC activity, in immunoreactive intensity or in the distribution of PKC isoforms. The authors concluded that the cause for altered Na^+K^+ ATPase activity may be due to a defective activation rather than an intrinsic activity. Finco *et al.*, (1992) studied adenosine 5'-diphosphate (ADP) ribosylation of G_i / G_o proteins in diabetic brain striatum. A marked decrease in pertussis toxin (PTX) catalyzed ADP-ribosylation is seen in diabetic animals. The diabetes is associated with a time related alteration of cerebral G_i/G_o proteins and this defect is an ongoing process and no change in G-protein content or mRNA level. Authors predict a modification of G-proteins structure or physiological status. Shindo *et al.*, (1993) have reported on cyclic AMP (cAMP) in sciatic nerve of rats made diabetic with streptozotocin. The cAMP content of sciatic nerves of diabetic rats was lower than in those of control rats. Administration of stable prostacyclin analog iloprost or dibutyrl cAMP (db cAMP) restored cAMP content in the sciatic nerves and motor nerve conduction velocity. The authors concluded that reduction in cAMP content is peripheral nerves may be involved in the pathogenesis of diabetic neuropathy and is caused by the impairment of adenylate cyclase activity in the diabetic state.

Similar changes are also reported by many other groups in body tissues of diabetic rats like heart and liver (Strassheim *et al.*, (1990), Xiang and McNeill (1992) Gawler *et al.*, 1987, 1988). Gawler *et al.*,(1987) showed a loss of expression of G_i protein in rat liver of streptozotocin induced diabetic rats. Xiang and McNeill (1992) report about an increased membrane bound protein kinase C activity which is proposed to be the reason for the diabetic cardiomyopathy. Strassheim *et al.*, (1990) , report about a reduced specific activity of adenylate cyclase in adipocyte membrane and enhanced stimulatory effect of isoprenaline. They suggested that diabetes bring selective changes in the functioning of Gi in adipocyte membranes which removes the tonic GTP dependent inhibitory function of this G-protein.

Materials and Methods

MATERIALS AND METHODS

BIOCHEMICALS AND THEIR SOURCES

Biochemicals used in the present study were purchased from SIGMA Chemical Co. All other re agents were of analytical grade purchased locally. HPLC solvents were of HPLC grade obtained from SRL, India.The following are the list of chemicals purchased from Sigma and used in this study.

Streptozotocin.

Neurotransmitter Standards

(±)Norepinephrine, (-) Norepinephrine-bitartrate salt

(±)Epinephrine, (-)-Epinephrine, 5-Hydroxytryptamine,

5-Hydroxytryptophan

5-Hydroxytyramine

5-Hydroxy Indole Aceticacid

Homovanillic acid

Buffer Constituents

Sodium octyl sulfonate

Ethylene Glycol-bis(β-aminoethyl ether)-EGTA

Ethylenediamine tetra acetic acid-EDTA

Benzamidine, Aprotinin (Trasylol),Phenylmethyl sulfonyl fluoride(PMSF)

Glycylglycine,HEPES(N-[2-Hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid]

The following are kind gifts from JANSSEN LABORATORIES, Belgium

Spiperone and Ketanserin

The following chemical is a gift from BOEHRINGER INGELHEIM

Clonidine

Neurotransmitter receptor antagonists purchased from SIGMA

Prazosin,

Yohimbine,

RADIOCHEMICALS PURCHASED FROM AMERSHAM ENGLAND

1-[7,8-^{3}H]Noradrenaline of specific activity 39.0 Ci/mmol.

[O-methyl-^{3}H]Yohimbine with specific activity 88 Ci/mmol.

5-Hydroxy (G-^{3}H]tryptamine creatinine sulphate of specific activity 18.4 Ci/mmol.

INDUCTION OF DIABETES MELLITUS IN ANIMALS

Adult Sprague Dawley male rats of 6-8 months of age and~200 g. weight were used for all experiments. The Streptozotocin was given as intrafemoral vein injections to the animals at a concentration of 45-50 mg/kilogram body weight by dissolving in citrate buffer pH 4.5. The rats were injected during the early hours of the day (10.00 a.m. to 11.00 a.m.). The control animals were given the vehicle alone. The rats were kept under constant lighting conditions (12 hr light/dark cycle) throughout the experiments. The food and water were given ad libitum. The food given contained mainly grams (*Cicer arietinum*) and standard food pellets (Hindustan Lever). The rats were kept under diabetic state for different periods (7, 14 and 28 days).

The diabetic rats were randomly divided into two groups. One group received no antidiabetic treatment while the other received subcutaneous injections of insulin daily for 12-15 days. The dosage varied from 2-6 units and a mixture of both plain and Lente insulin (commercially available) was given for a better control.(Sasaki and

Bunag 1983)). The daily injections were always given between 3.00 and 5.00 p.m. and blood samples were collected as per requirement. The initial doses of insulin (daily per rat) started with 2U and depending on the extent of glycosuria, adjusted upto 6U, the final injections were given the day before killing. The blood was collected by tail snipping method and glucose was estimated using glucose oxidase enzyme kits (Merck). The tap water was filled every other day after recording the volume ingested. Body weight was measured using a standard balance. The animals were killed between 10.00 and 11.30 hrs by decapitation. The brains were rapidly dissected into 5 different regions according to Glowinski and Iversen (1966). The dissection was carried out on a chilled glass plate into hypothalamus (Hypo) corpus striatum (CS), brain stem (BS), cerebellum (CB) and cerebral cortex (CC). These regions were immediately immersed into liquid nitrogen and stored at -70^{o} C for various experiments.

ASSAYS

Monoamine concentrations

Tissues from brains were homogenised in 0.4N ice cold perchloric acid. The homogenate was centrifuged and the clear supernatant was filtered and used for HPLC analysis. Norepinephrine (NE), Epinephrine (EPI), Dopamine (DA), 5-Hydroxy tryptamine (5-HT), 5-Hydroxytryptophan (5-HTP), 5- Hydroxy indole acetic acid (5-HIAA) and Homovanillic acid (HVA) were determined in high performance liquid chromatography with electrochemical detector (HPLC EC) (Shimadzu, Japan) fitted with CLC-ODS a reverse phase column of 5μm particle size, 4.6 mM internal diameter and 25 cm length. The mobile phase consisted of 75 mM sodium dihydrogen orthophosphate, 1 mM sodium octyl sulfonate, 50 μm EDTA and 8% acetonitrile pH adjusted to 3.2 with phosphoric acid, filtered through 0.4 μm filters (Millipore) and

deaerated. A Shimadzu model 10AS 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 0.8V, with the range set at 16 and a time constant of 1.5 seconds. Twenty µl aliquots of the acidified supernatant were injected into the system. The peaks were identified by relative retention times compared with standards and quantitatively estimated using an integrator interfaced with the detector.

The cholesterol estimation was done using Merck by Libermann Burchard method. Protein concentrations were quantified by the method of Lowry *et al.*, (1951) using bovine serum albumin as a standard.

NEUROTRANSMITTER RECEPTOR STUDIES USING [^{3}H] RADIOLIGANDS IN IN VITRO EXPERIMENTS

ADRENERGIC RECEPTORS

Brain Membrane preparations for [^{3}H] NE binding

The tissues used for assay include hypothalamus and brain stem. The membrane was prepared according to Geynet *et al.*, (1981) with modifications. The brain regions were homogenised in 50 volumes of the 50 mM Tris buffer containing 2 mM EGTA and 100µM PMSF pH 7.5, with ten up and down strokes in a tight fitting Teflon-glass homogeniser held in ice. After homogenisation, the suspension was centrifuged at 40000xg in a RP21 rotor in a Hitachi SCP 85 ultra centrifuge for 30' at 4^{o}C. The procedure was repeated again after resuspending the pellet.

SCATCHARD ANALYSIS USING [^{3}H] NE

The incubation mixture contained 50 mM Tris HCl, 10 mM $MgCl_2$, 1mM EGTA, 0.8 mM ascorbic acid, 3 mM catcehol pH was adjusted to 7.4. After the second centrifugation the pellet was resuspended in the incubation buffer for [^{3}H] NE. The assay mixture contained a protein concentration of 0.2-0.4 mg of protein and varied concentrations of radioligands from 25 to 100 nM. The mixture was incubated for 30 minute at 25^{o}C. The specific binding was defined as the difference between the radioligand binding in the absence and presence of 10 μM phentolamine. At the end of incubation, the reaction was stopped by filtering immediately through GF/B filters (Whatman) soaked previously in wash buffer (50 mM Tris buffer containing 10 mM $MgCl_2$ pH 7.4). The filters were rapidly washed in three 5 ml washes with ice cold wash buffer. The filters were dried overnight and counted with ten ml, Hisafe, liquid scintillation cocktail (Wallac), in a Wallac liquid scintillation counter model 1409 at an efficiency of 60%. The specific binding was defined as that binding which form 60% of the total binding.

Membrane preparation for [^{3}H] Yohimbine binding

[3H]Yohimbine binding studies were done according to Repaske *et al.*, (1987) with slight modifications.

Buffers used

Buffer 1) 5 mM Tris HCl, 5 mM EDTA, 2 mM EGTA, 10 mM benzamidine, 10 U/ml trasylol, pH 7.6.

Buffer 2) 5mM Tris HCL, 5 mM EGTA, 10 mM benzamidine 10 U/ml trasylol pH 7.6.

Buffer 3) 25 mM glycylglycine, 10 mM Hepes, 100 mM NaCl, 2 mM EGTA pH 7.6.

PREPARATION OF THE RAT BRAIN PARTICULATE FRACTION FOR α-2 RECEPTOR BINDING STUDIES

The brain tissues were homogenised in fifty volumes of buffer in a tight fitting teflon glass homogeniser by 10 up and down strokes with one interruptions on ice for 30 sec. The buffer contained 50 mM Tris HCl, 4 mM $MgCl_2$, 2 mM EGTA, pH 7.6, 10 mM benzamidine and 5 mM phenyl methyl sulfonyl fluoride (PMSF). The homogenate was centrifuged at 40,000xg in a RP 21 rotor in a Hitachi SCP 85 centrifuge for 10 minute at 4°C. The pellet was resuspended in the buffer number 1 and 2. The final pellet was resuspended in buffer number 3 and used for determination of [^{3}H]Yohimbine binding.

DETERMINATION OF [^{3}H] YOHIMBINE BINDING IN PARTICULATE PREPARATIONS

Membrane binding assays were performed in 0.5ml incubation containing 0.2-0.4 mg for brain stem protein and 0.1 to 0.2 mg of hypothalamus. The concentration of radioligand particulate preparation used varied from 3-10 nM [^{3}H]Yohimbine and incubated for 90 minutes at 15°C. The incubation was terminated by filtration through GF/C glass fiber filters (Whatman) in a Millipore vacuum manifold with three washes of 5 ml each of ice cold 25 mM glycylglycine pH 7.6. The filters were dried prior to counting.

ANALYSIS OF THE RECEPTOR BINDING DATA

The data were analysed according to Scatchard (1949) The data included both total and nonspecific binding at many concentrations of radioligand and the specific binding was calculated as the difference. Two parameters maximal binding (Bmax) and equilibrium dissociation constant (Kd) were found out by plotting the specifically bound radioligand on X axis and bound / free on y axis. This is called a Scatchard plot. The maximal binding (Bmax), which is a measure of the total number of receptors present in the tissue and the equilibrium dissociation constant (Kd) of the receptors for the radioligand. The Kd is inversely related to receptor affinity, i.e., the "strength" of binding, a low Kd means high-affinity binding, a high Kd means low affinity binding.

SEROTONIN RECEPTOR BINDING ANALYSIS

The membrane preparations were followed according to Paulose *et al.*,. (1985). In Short brain tissues were homogenised in 50 mM Tris-HCl buffer containing 1 mM PMSF and 1 µM pargyline (pH 8.3) The membrane preparation was exactly the same for other radioligands except that the homogenisation is done in the incubation buffer itself. The incubation was done at 37°C for 15'. The specific binding was determined by adding 10 µM of cold serotonin. After incubation the reaction was stopped by filtering through GF/B filters (Whatman). Three washes each of 5 ml ice cold 50 mM Tris-HCl pH 8.5 were done.

DISPLACEMENT CURVES

Both adrenergic and serotonergic binding were analysed using competing cold ligands.The competitive drugs used with [^{3}H]-Noradrenaline were Yohimbine, and prazosin. The competitive drugs used along with the [^{3}H] serotonin included ketanserin and spiperone.

The data is represented graphically with the log of concentration of the competing drug on x axis and % of total specific bound on y axis.

PHOSPHOLIPASE ASSAY

The enzyme was assayed according to Krug and Kent (1984.) with modifications. The brain tissue was homogenised in Tris-buffer.

Reagents.

Tris-HCl 0.4 M, pH 7.3 at 37^{o}C

Calcium chloride 50 mM

Bovine serum albumin 1 mg/ml

Phosphatidyl inositol 2.5 mM in absolute ethanol.

Ammonium sulfate 0.2 M

Alkaline phosphatase (*Escherichia coli,* Sigma Chemical Co., type III-S).

Sodium dodecyl sulfate 20%

Ascorbate-molybdate reagent; 1 part 10% ascorbic acid and 6 parts 0.42% ammonium molybdate in 1 N H_2SO_4.

Procedures:

Reagents were added in the following order and to the final concentrations indicated (final volume 80 µl): 50 mM Tris HCl; 6.3 mM calcium chloride, 0.13 mg/ml bovine serum albumin, 2.5 mM phosphatidyl inositol, 70 mM ammonium sulfate (including the amount of ammonium sulfate contributed by the suspension of alkaline phosphatase), 0.15 unit of alkaline phosphatase, and 0.1 to $1x10^{-3}$ unit of phospholipase C. Routinely, all the assay components except phospholipase C were pooled as one large suspension, and aliquots can be removed for each assay. The assay mixture was incubated at 37°C for 15 minutes in a shaking water bath. The reaction was terminated by addition of 80 µl of sodium dodecyl sulfate. Then 400µl of ascorbate-molybdate reagent was added followed by incubation at 45°C for 20 min or 37°C for 60 min. The absorbance was read in a 1 ml cuvette by adding ~~more~~ 560 µl of buffer at 820 nM. One nanomole of inorganic phosphate corresponds to a net absorbance of about 0.045 above a reaction mixture blank absorbance of 0.040. One unit of phospholipase C activity is defined as that which produces 1 µmol of inorganic phosphate per minute. The assay was linear with time to 30 min and enzyme activity to $1x10^{-3}$ unit.

STATISTICAL ANALYSIS

The data were analysed with Students' t-test and ANOVA (Campbell, 1987).

Results

RESULTS

GENERAL CHARACTERISTICS OF THE STREPTOZOTOCIN-INDUCED DIABETIC RATS

The blood glucose of streptozotocin diabetic rats significantly ($P<0.01$) increased (Fig.1). The body weight analysed showed a significant ($P<0.05$) reduction when compared to its initial weight and control animals. The water uptake significantly ($P<0.01$) increased in diabetic animals. But all these parameters which showed significant change in diabetes were reversed by insulin treatment (Table 1).

The protein was estimated in all brain regions and body regions during diabetes. All the regions examined except CC and CS showed a significant ($P<0.05$) reduction in protein content (Table 2).

The total cholesterol content estimated showed varied results in different tissues. Out of the brain regions analysed corpus striatum, hypothalamus, cerebellum and cerebral cortex showed a significant ($P<0.05$) decrease. Brain stem showed no significant change in the cholesterol content. The pancreas and kidney also showed variation in the total cholesterol content. Pancreas showed a significant ($P<0.05$) increase and kidney showed a significant ($P<0.05$) decrease (Table 3).

CHANGES IN THE CONTENT OF MONOAMINES AND THEIR METABOLITES

The different rat brain region were analysed for monoamines and their metabolites. The cerebral cortex (CC), which belonged to the upper brain region was analysed (Table 4). The results of changes in the content of monoamines in the cerebral cortex showed an increase in the activity of norepinephrine (NE) (+83%; $P<0.05$). Dopamine content significantly ($P<0.01$) increased (+322%). The other major neurotransmitter serotonin (5-HT) also increased significantly ($P<0.05$; + 55%).

The data of neurotransmitters and their metabolites were analysed according to the theory of Hery *et al.*, (1972) and Smythe *et al.*, (1983, ~~1983a~~). According to them the ratio of the concentration of the major metabolite of a brain monoamine neurotransmitter to brain monoamine concentration itself provides a good index of the overall turnover rate of the synaptic transmitter. The norepinephrine turnover to epinephrine when analysed showed no change in the content of epinephrine. But its turnover rate (Table 5) as calculated by EPI/NE ratio in diabetic CC compared to control CC was significantly lower ($P<0.05$; -59%). This may be indicating a lower turnover of NE to EPI which result in an increase in NE content in diabetic CC.

The serotonin (5-HT) precursor, 5-Hydroxy tryptophan when analysed, showed no change in its content while 5-HT content significantly increased ($P<0.05$; +55%). The turnover rate calculated remained same in both control and diabetic CC (Table 5). This shows no disturbance in serotonin synthesis. The turnover of 5-HT to 5-HIAA showed a significant decrease in CC of diabetic

brains. So in diabetic brain 5-HT increase which is observed may be due to a decreased turnover rate of 5-HT to 5- HIAA.

The DA content when analysed in a similar fashion with its major metabolite homovanillic acid showed a marked alteration in its metabolism. The HVA content showed a significant decrease in diabetic CC ($P < 0.05$; -77%). DA content showed an increase in CC. The turnover rate when analysed showed highly significant variation ($P<0.05$, -96%) (Table 4). This shows that DA turnover is very less in diabetic CC which is reflected as an increase in DA content and a decrease in the HVA content. The alteration in the dopaminergic nerves is highly significant ($P<0.05$) in diabetic CC.

The neurotransmitter content changes in the hypothalamus which is the centre of regulation of endocrine functions when analysed showed a significant increase ($P < 0.05$, +40%) in the noradrenergic activity (Table 6). The EPI content also increased significantly ($P < 0.05$). The turnover rate calculated for the conversion of NE into EPI showed an increase ($P< 0.05$) (Table 7). This increase in the turnover rate is reflected in the EPI content.

The serotonergic activity assessed in the diabetic hypothalamus by serotonin content showed a significant ($P<0.05$) decrease. This decrease is accompanied by an increase in the 5-HTP content in the diabetic state. The turnover rate for 5-HTP to 5-HT showed a significant increase ($P < 0.01$; +179%). Also, the turnover rate from 5-HT to 5-HIAA ~~and content of 5-HIAA~~ showed a highly significant increase (Table 7). This indicates a disturbance in both the metabolic pathway from 5-HTP to 5-HT and also 5-HT to 5-HIAA. The turn over rate calculated for a precursor to its neurotransmitter shows an increase which indicate an accumulation of the precursor. In this case 5-HTP accumulate in the

hypothalamus due to some block in the synthesis. The 5-HT to 5-HIAA turn over rate also increased which in effect resulted in a decreased 5-HT content.

The dopaminergic activity also showed a significant increase in the hypothalamus. But its metabolite HVA level in diabetic rats remained undetected (Table 6).

Of the lower brain regions analysed brain stem (BS) showed significant variations in both serotonergic and adrenergic activity. Epinephrine significantly increased. The turnover rate for the conversion of NE→EPI is significantly higher ($P < 0.05$; Table 8). This may be concluded as an increase in the adrenergic activity in thiss brain region also (Table 8). The serotonin level significantly increased ($P<0.01$) in BS. The turnover rate calculated for 5-HTP→5-HT showed a significant increase ($P<0.05$; +85%). But its breakdown metabolite 5-HIAA showed a significant decrease ($P<0.05$; -72%). The turnover rate calculated for this pathway showed a significant reduction ($P<0.05$). So the increased turnover rate for the 5-HTP→5-HT and a reduction in the turnover rate from 5-HT→5-HIAA may be resulting in an increase in 5-HT content.The dopaminergic activity in the BS also increased significantly ($P<0.05$). But the HVA content remained undetected in diabetic brain BS (Table 9).

The cerebellum when analysed for neurotransmitters and their metabolites showed no significant change in the norepinephrine content as well as epinephrine content. Dopaminergic activity decreased significantlly ($P<0.05$) during diabetes (Table 10) .Serotonin precursor showed a significant increase ($P<0.05$). 5-HIAA content also showed no significant change during diabetic state.

The turnover rate calculated for NE→EPI did not show any significant change. So generally it is interfered as a brain region which is least affected by diabetic state (Table 11).

The striatum showed significant changes in adrenergic, dopaminergic and serotonergic activities. The adrenergic activity showed a decrease in NE level associated with an increase in the EP1 content. The turnover rate calculated for the conversion of NE→EPI revealed that it is significantly higher ($P<0.01$) in diabetic state. This explains well the monoamine levels. The increased turnover may have resulted in an increase in EPI and a decrease in NE level (Table 12,13).The dopaminergic activity of the striatum showed marked variations in its level. The dopaminergic activity increased significantly ($P<0.001$; +6500%). This increase is accompanied by a decrease in the HVA content in diabetic rat striatum. The turnover rate data showed a highly significant ($P<0.001$) reduction in the breakdown pathway of the dopamine in diabetic rats. The serotonergic activity showed a marginal increase (28%) in activity with a very significant increase ($P<0.001$) in the 5-HTP content. The turnover rate for 5-HT→5-HTP showed a highly significant increase ($P<0.05$) (Table 13). The breakdown pathway analysed by 5-HIAA showed a significant increase ($P<0.01$) but no significant increase in the turnover data.

EFFECT OF INSULIN TREATMENT ON BRAIN MONOAMINE LEVELS

The insulin treatment to the diabetic animals showed recovery from the diabetic state. Some parameters seem to show recovery to a certain extent. But a complete reversal to a normal condition was not seen with the external insulin treatment. The reversal of blood glucose, body weight and water uptake is brought back to near normal level by insulin treatment.

The pattern of insulin treatment and recovery from the altered diabetic condition may be illustrated by taking few examples. The reversal is seen in the case of altered noradrenergic activity in hypothalamus of insulin treated rats (Table 6). But in the same region, increased dopaminergic content was brought to a decrease in insulin treated rats. Serotonin content showed a further decrease (Table 6). The data on insulin treated rats seem to be not reversed tocontrol levels in all the brain regions analysed (Table 4, 6, 8, 10, 12).

THE OBSERVED PATTERN IN MONOAMINE CONTENT REMAINED THE SAME IN DIFFERENT PERIODS OF STUDY

We compared the variations in the monoamine contents at the end of 7, 14 and 28 days in hypothalamus, to see whether the observed changes occurred later in the disease process or of early occurrence (Table 14). The major neurotransmitter norepinephrine showed a significant increase ($P<0.01$) in its content throughout the period. But this increase attenuated during the later stages of the disease ($P<0.05$). We were unable to detect epinephrine in diabetic brain during the early stages of the disease. The decrease in serotonin content remained the same during the period of 28 days. The reduction is maximum in the hypothalamus in the order of 14, 7 and 28 days respectively. This study indicated that when the neurotransmitter content is compared between different days of the disease there is a signifiicant difference.

NEUROTRANSMITTER RECEPTOR CHANGES IN STREPTOZOTOCIN DIABETIC RATS

CHANGES OF NORADRENERGIC RECEPTORS IN THE HYPOTHALAMUS

The Scatchard analysis of [^{3}H] Noradrenaline to synaptic membranes of hypothalamus showed that noradrenergic receptor number decreased significantly ($P<0.05$; Table 15; Fig. 2) in diabetic rats. The Figure 2 shows the Scatchard analysis of [^{3}H]NE in the hypothalamus of control and diabetic rats. The plot for control rats indicate a homogenous binding site with a dissociation constant (Kd) of 30.5 nM. The calculated maximal number of binding site (B_{max}) was 5584 fmoles/mg/nM. Also, in the diabetic rats the plot represented a homogenous binding site with a dissociation constant (Kd) of 30.4 nM. The calculated B_{max} for diabetic rats was 2584 fmoles/mg/nM.

DISPLACEMENT ANALYSIS OF NORADRENERGIC RECEPTORS USING ALPHA$_1$ AND ALPHA-$_2$ ADRENERGIC ANTAGONISTS

The displacement analysis was done in hypothalamic membrane preparations in order to characterise the involvement of α-adrenergic receptor subtypes among the adrenergic receptors identified by [^{3}H]NE. The competition curves for control hypothalamus in Figures 3 and 4 reveal that the adrenergic binding sites in control hypothalamus identified by [^{3}H]NE, is more of α-$_1$ adrenergic receptors than α-$_2$ receptors. A comparison of α-$_1$ antagonist prazosin displacement in control and diabetic hypothalamus showed that majority of [^{3}H]NE identified adrenergic sites in diabetic rats are more of α-$_1$ type The results for Yohimbine displacement in control&diabetic hypothalamus showed that Yohimbine did not bring out a

displacement as good as prazosin. Prazosin is more potent in displacing [^{3}H]NE in diabetic rats,(Figure 3). This indicate that the observed decrease in adrenergic receptor number during diabetes in hypothalamic tissue is in adrenergic binding sites ~~and~~ of α_1 binding sites. The saturation binding curves done for [^{3}H]NE and [^{3}H]Yohimbine in hypothalamus showed that [^{3}H]NE identifiable α-2 adrenergic receptors is only less than 15% of the total alpha adrenergic receptors identified by [^{3}H]NE. So the observed decrease in the [^{3}H]NE binding sites indicate that α-$_1$ adrenergic receptors of hypothalamus decreased during diabetes.

ALPHA-2 ADRENERGIC RECEPTORS IN HYPOTHALAMUS

The α-$_2$ adrenergic receptor kinetics was studied using [^{3}H]Yohimbine, a specific antagonist which is reported to identify α-$_2$ adrenergic receptors. The [^{3}H]Yohimbine binding to the hypothalamus of control rats under the conditions used in this study bound to a single class of binding sites (Figure 5) with a B_{max} of 529 ± 3 fmol/mg of membrane protein and the dissociation constant (Kd) 3.29 ± 0.15 nM. In the diabetic rats the same parameters, B_{max} and Kd were 588 ± 30 fmol/mg of membrane protein and 2.34 ± 0.18 nM. The B_{max} did not differ in diabetic hypothalamus, but Kd value decreased significantly. This shows that α-$_2$ receptor affinity increased in diabetic condition. The insulin treatment did not reverse the altered parameters. The insulin treated rats have a B_{max} of 617 ± 2 fmoles/mg membrane protein and a Kd of 2.02 ± 0.15 nM in the hypothalamus

NUCLEOTIDE AND SODIUM ION REGULATED RECEPTOR AFFINITY IN HYPOTHALAMUS

It is known that in platelet membranes and liver membranes (Hoffman *et al.*, 1980) guanine nucleotides reduced the agonist affinity at α-$_2$ adrenergic binding

sites. In porcine brain particulate and solubilized fractions the affinity of α-2 adrenergic binding sites identified by [^{3}H]Yohimbine also have been shown to decrease in response to both guanine nucleotides and sodium ions (Repaske *et al.*, 1987). In our study we analysed this affinity modulation in hypothalamic membrane fraction using [^{3}H]Yohimbine against (-)-epinephrine. (-)-Epinephrine is shown to have more potency for α-2 binding sites in platelets (Hoffman, 1979).

The guanine nucleotides bring out this reduction in affinity due to the abolishment of α-2_H state(high affinity), because agonist competion curves of [^{3}H]dihydroxyergocryptine (DHE) sites model to a single state of binding of low affinity sites (Tsai and Leftkowitz, 1979 and Hoffman *et al,* 1980). We used an antagonist concentration of [^{3}H]Yohimbine 10 nM against various agonist concentration of (-)-epinephrine. The curve pattern obtained in control rats indicate biphasic pattern (Figure 6 ●—●). Such patterns in previous reports for competition curves were interpreted as two site fits (Hoffman et al., 1980). The guanine nucleotides curves abolished this heterogeneous affinity state in control (Figure 6 ■—■).

Guanine nucleotides are not the sole compounds, which regulate the receptor affinity and their ability to mediate the inhibition of hormone mediated inhibition of adenylate cyclase. Sodium ions have been demonstrated to be required for at least enhance hormonal inhibition of adenylate cyclase in a number of systems (Ross *et al.*, 1987). In our studies the particulate membrane fractions were incubated with [^{3}H]Yohimbine and increasing concentrations of (-)-epinephrine in the absence or presence of 100 nM NaCl and both NaCl and Gpp[NH]p. In both Na+ containing and Na+-free incubations the monovalent cation ionic strength was kept constant by including in the buffer 100 mM NaCl. The results obtained indicate that in control hypothalamus the reduction in the affinity is observable (Fig 6 Δ—Δ)

Also, the curve "shifted" to the low affinity region. The two site curve is still observed. This shows that in the control rats the guanine nucleotide and sodium ion mediated affinity changes are intact. The curve obtained for both the Gpp[NH]p and NaCl (Figure 6 ∇—∇) moved to the low affinity region. In diabetic rat hypothalamus, the guanine nucleotides were not displaying any rightward shift. The curve obtained is almost same as the epinephrine curve (Figure 7 ■—■ against ●—●). Sodium ions were able to reduce the affinity states of the α-2 receptors to a certain extent (Figure 7 Δ—Δ). But in the case when both compounds were added a similar shift in the curve was not observed (In Figure 7 ∇—∇).

SEROTONERGIC RECEPTORS IN THE HYPOTHALAMUS OF DIABETIC RATS

The Scatchard analysis of [^{3}H]5-HT in hypothalamus of diabetic rats showed an increased number of receptors and no change in the affinity. In the control rats the Bmax value for high affinity S_1 receptors identified by [^{3}H]5-HT are 1081 ± 57 fmoles/mg of membrane protein and a Kd of 9.89 ± 1.2 nM. In diabetic rats the Bmax value and Kd values for the high affinity S_1 receptors are 2547 ± 60 fmoles/mg of membrane protein and 11.73 ± 2.0 nM (Figure 8, Table 15)

The competition curve for the diabetic hypothalamus showed that spiperone at high affinity concentration is able to displace the high affinity bound [^{3}H]5-HT to S1 receptors significantly ($P<0.05$; Figure 9). This shows that diabetic hypothalamus contain more of 5-HT receptors than their respective controls.

ADRENERGIC RECEPTORS OF BRAIN STEM

In brain stem the NE level remained unaltered EPI content increased significantly ($P<0.05$). The studies using [^{3}H]NE in hypothalamus revealed that the ligand is more specific for α-1 receptors than α-2 receptors. We used α-2 specific antagonist ligand [^{3}H]Yohimbine for studying α-2 adrenergic receptor changes. The B_{max} value of control rats in BS was 235 ± 10 fmoles/mg of membrane protein with Kd value of 5.26 ± 0.19 nM. The Bmax for diabetic rats was 240 ± 33 fmoles/mg of membrane protein and a Kd value of 7.67 ± 0.41 nM. In diabetic rats with insulin treatment Bmax 304 ± 4.0 fmoles/mg of protein and Kd was 5.35 ± 0.07 nM (Figure 10, Table 15). This shows that α-2 receptors of diabetic brain have a reduced receptor affinity for natural agonists.

EFFECT OF NUCLEOTIDES AND SODIUM IONS ON ALPHA-2 ADRENERGIC RECEPTOR AFFINITY IN DIABETIC BRAIN STEM

The effect of guanine nucleotides and sodium ions was assessed in control and diabetic brain stem to see whether the observed reduced receptor affinity is a result of altered nucleotide or ion mediated defective function. The control and diabetic displacement curves for (-)- epinephrine were almost identical (Figure 11 and 12 ●—●). The GTP analogue shifted the curve towards low affinity range in both control and diabetic groups, but the shift is more in diabetic rats (Figure 11 and 12 ■—■). Similarly the Na^+ ion induced shift of affinity is demonstrable in both the groups. But the shift is more in diabetic rats (Figure 11 and 12 Δ—Δ). The curve representing the added effect of both compounds in control and diabetic groups differed (Figure 11&12, ∇—∇). In control an intermediate effect is seen. But in the diabetic rats the shift is more towards low affinity region representing an added effect of both compounds.

SEROTONIN RECEPTORS IN THE BRAIN STEM

The serotonergic receptors in the brain stem was analysed using [^{3}H]5-HT. The high affinity receptors of S_1 type showed no change in the receptor numbers, but an increase in the Kd value representing a decrease in affinity (Figure 13; Table 15). The respective B_{max} values of control and diabetic rats were 197 ± 2 fmol/mg protein 222 ± 12 fmole/mg of protein. The Kd values observed were 1.24 ± 0.02 nM and 4.62 ± 0.12 nM. The diabetic rats treated with insulin showed a B_{max} (B_{max} 185 ±3 fmole/mg of protein) and no change in the Kd (1.31 ± 0.4nM).

CEREBRAL CORTEX AND SEROTONIN RECEPTORS

The upper brain region CC also showed a significant increase in the serotonin content. We assessed the serotonin receptors using [^{3}H]5-HT which is reported to have affinity for S1 receptors of high affinity (Figure 14). We observed a significant reduction in the serotonin receptors in this brain region of diabetic rats. The Bmax for control group was 260 ± 10 fmoles/mg protein and Kd was 2.5 ± 0.2 nM. In diabetic group Bmax observed was 122 ± 29 fmoles/mg protein and Kd was 1.9 ± 0.4 nM. The diabetic rats with insulin treatment showed that the B_{max} still reduced while Kd remained constant.

DISPLACEMENT ANALYSIS OF SEROTONIN RECEPTORS USING KETANSERIN IN CEREBRAL CORTEX

We observed a decrease in the high affinity S_1 sites using [^{3}H] 5-HT Scatchard in cerebral cortex. Our displacement analysis using S_2 antagonist, Ketanserin showed a significant displacement in diabetic CC compared to controls

(Figure 15). This indicate that in diabetic CC the observed decrease in [^{3}H] 5-HT identifiable sites may be due to a shift in the serotonin receptors to a receptor population containing more of S_2 type.

PHOSPHOLIPASE C ACTIVITY

The phospholipase C (PLC) activity was studied in hypothalamus and brain stem (Table 16). The membrane fraction of hypothalamus of diabetic rats showed no change while cytosolic activity of this enzyme was reduced by about 55% in diabetic rats. In the brain stem the PLC activity when analysed showed no change in cytosolic fraction while the membrane bound activity was not detected.

TABLE - 1

BODY WEIGHT AND WATER UPTAKE IN CONTROL, DIABETIC AND DIABETIC RAT TREATED WITH INSULIN

ANIMAL STATUS	BODY WEIGHT (in grams)	WATER UPTAKE (in ml)
CONTROL	Initial = 192 ± 19 Final = 182 ± 20	Initial = 25 ± 9 Final = 30 ± 5
DIABETIC	Initial = 200 ± 11 Final = 140 ± 5*	Initial = 30 ± 6 Final = 190 ± 22**
DIABETIC+ INSULIN	Initial = 200 ± 9 Final = 170 ± 8	Initial = 29 ± 4 Final = 100 ± 8**

*P<0.05 compared to control

**P<0.01 compared to control

(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

TABLE -2

PROTEIN CONTENT IN BRAIN REGIONS CS, HYPO,BS, CB, AND CC AND IN TISSUES- LIVER, PANCREAS, HEART AND KIDNEY OF CONTROL AND DIABETIC ADULT MALE RATS.

ANIMAL STATUS	CS	HYPO	BS	CB	CC	LIVER	HEART	PANCR-EAS	KIDNEY
CONTROL	123.26 ±13.9	164.04 ±14.4	164.9 ±16.7	179.98 ±13.6	202.03 ±2.0	262.21 ±14.9	265.21 ±14.9	248.4 ±18.3	248.99 ±19.99
DIABETIC	106.94 ±7.9	96.95* ±7.1	113.5* ±13.2	120.1* ±14.2	184.91 ±2.9	157.7* ±11.2	136.09* ±23.2	142.08* ±18.2	164.07* ±20.6

*P< 0.05 Compared to control

(VALUES ARE MEANS ± S.E.M. OF 4 - 6 SEPARATE DETERMINATIONS IN EACH GROUP)

TABLE - 3

CHOLESROL CONTENT (mg /gm wet weight) CS, HYPO, BS, CB, CC AND IN TISSUES LIVER, PANCREAS, HEART AND KIDNEY OF CONTROL AND DIABETIC ADULT MALE RATS.

ANIMAL STATUS	CS	HYPO	BS	CB	CC	LIVER	HEART	PANC-REAS	KIDNEY
CONTROL	14.80 ±2.90	14.38 ±3.70	24.47 ±1.38	18.59 ±2.30	20.89 ±0.40	12.32 ±2.40	6.08 ±1.50	3.75 ±0.30	13.13 ±2.30
DIABETIC	11.25* ±1.60	10.98* ±1.80	28.40 ±2.10	12.86* ±0.20	12.33* ±3.20	12.59 ±1.90	5.36 ±2.60	12.63* ±2.30	8.56* ±1.90

*P< 0.05 Compared to control

(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

NEUROTRANSMITTERS AND THEIR METABOLITES IN CEREBRAL CORTEX OF CONTROL, DIABETIC AND DIABETIC WITH INSULIN TREATED RATS

(28days, nanomoles/ gram wet weight)

ANIMAL STATUS	Norepinep-hrine	Epineph-rine	Dopamine	5-Hydroxy-tryptamine	5-Hydroxy-tryptophan	Homovan-illc acid	5-Hydroxy-indoleacetic acid
Control	0.86 ± 0.02	0.18 ± 0.05	0.27 ± 0.09	0.49 ± 0.19	0.68 ± 0.04	3.72 ± 0.30	3.84 ± 0.31
Diabetic	1.58* ± 0.07	0.19 ± 0.06	1.14 ± 0.15**	0.76 ± 0.07*	0.58 ± 0.01	0.86 ± 0.19*	4.67 ± 0.35
Diabetic+ insulin	2.15* ± 0.03	N.D	1.19 ± 0.35**	0.32 ± 0.03	N.D.	0.56 ± 0.06*	4.28 ± 0.23

*P<0.05 Compared to control
**P<0.01 Compared to control
(Values are mean ± S.E.M of 4 - 6 separate determinations)

TABLE–5

TURNOVER RATIO FOR NEUROTRANSMITTERS IN CEREBRAL CORTEX OF DIABETIC RATS
(28 Days, nanomoles/gram wet weight)

NEUROTR-ANSMITT-ERS	CONTROLS	DIABETIC	NEUROTR-ANSMITT-ERS	CONTROLS	DIABETIC
5-HIAA	3.84 ± 0.31	4.67 ± 0.35	EPI	0.18 ± 0.05	0.19 ± 0.06
5-HT	0.49 ± 0.19	0.76 ± 0.07*	NE	0.86 ± 0.02	1.58 ± 0.07*
5-HIAA/ 5-HT	11.17 ± 0.9	7.33 ± 0.46*	EPI/NE	0.19 ± 0.01	0.08 ± 0.01*
HVA	3.72 ± 0.3	0.86 ± 0.19	5-HTP	0.68 ± 0.04	0.58 ± 0.01
DA	0.27 ± 0.09	1.14 ± 0.15**	5-HT	0.49 ± 0.19	0.76 ± 0.01*
HVA/DA	17.3 ± 1.4	0.58 ± 0.09**	5-HTP/5-HT	1.78 ± 0.22	1.00 ± 0.02

*P< 0.05 Compared to controls
**P< 0.01 Compared to controls
(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

TABLE - 6

NEUROTRANSMITTERS AND THEIR METABOLITES IN HYPOTHALAMUS OF CONTROL, DIABETIC AND DIABETIC WITH INSULIN TREATED RATS

(28days, nanomoles/ gram wet weight)

Animal status	NE	EPI	DA	5-HT	5-HTP	HVA	5-HIAA
CONTROL	2.15 ± 0.33	0.34 ± 0.11	0.60 ± 0.10	1.19 ± 0.02	0.47 ± 0.19	2.11 ± 0.30	7.76 ± 0.56
DIABETIC	3.02 ± 0.5*	0.73 ± 0.20*	0.84 ± 0.09*	0.72 ± 0.08*	0.69 ± 0.02*	N.D.	8.33 ± 0.08
DIABETIC +INSULIN	2.30 ± 0.38	0.32 ± 0.05	0.35± 0.06*	0.32 ± 0.03*	2.85 ± 0.04*	0.63 ± 0.14*	7.98 ± 0.31

*$P<0.05$ compared to controls

(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

TABLE -7

TURNOVER RATIO OF NEUROTRANSMITTERS IN HYPOTHALAMUS

NEUROTRANSMITTERS	CONTROL	DIABETIC
EPINEPHRINE(EPI)	0.34 ± 0.11	0.73 ± 0.20*
NOREPINEPHRINE(NE)	2.15 ± 0.33	3.02 ± 0.57*
EPI/NE	0.12 ± 0.03	0.64 ± 0.20*
5- HYDROXY INDOLE ACETIC ACID(5-HIAA)	7.76 ± 0.56	8.33 ± 1.12
5- HYDROXYTRPTAMINE (5-HT)	1.19 ± 0.02	0.72 ± 0.08*
5-HIAA/5-HT	7.00± 0.72	9.45 ± 0.64*
5- HYDROXY TRYPTOPHAN (5-HTP)	0.47 ± 0.19	0.69 ± 0.02*
5-HTP/5-HT	0.29 ± 0.05	0.81 ± 0.08*

*P< 0.05 Compared to control

(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

TABLE - 8

NEUROTRANSMITTERS AND THEIR METABOLITES IN BRAIN STEM OF CONTROL, DIABETIC AND DIABETIC WITH INSULIN TREATED RATS

(28 Days, nanomoles / gram wet weight)

ANIMAL STATUS	NE	EPI	DA	5-HT	5-HTP	HVA	5-HIAA
CONTROL	2.84 ± 0.26	0.08 ± 0.01	0.32± 0.13	0.67 ± 0.13	0.37 ± 0.04	0.40 ± 0.11	3.64 ± 0.53
DIABETIC	3.45 ± 0.30	0.26 ± 0.07*	0.72 ± 0.12*	1.71 ± 0.11*	1.28 ± 0.15*	N.D.	1.49 ± 0.45*
DIABETIC +INSULIN	1.85 ± 0.15*	0.23 ± 0.07*	1.14 ± 0.34*	0.78 ± 0.15	0.19 ± 0.04*	N.D.	1.44 ± 0.40*

* P< 0.05 Compared to control

(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

TABLE-9

TURNOVER RATIO FOR NEUROTRANSMITTERS IN BRAIN STEM
(28 days,nanomoles/ gram wet weight)

NEUROTRANSMITTERS	CONTROL	DIABETIC
EPI	0.08 ± 0.01	0.26 ± 0.07
NE	2.84 ± 0.26	3.45 ± 0.3
EPI/ NE	0.18 ± 0.01	0.10 ± 0.04*
5-HTP	0.37 ± 0.04	1.28 ± 0.15
5-HT	0.67 ± 0.13	1.71 ± 0.11
5-HTP/5-HT	0.40 ± 0.01	0.74 ± 0.07*
5-HIAA	3.64 ± 0.53	1.49 ± 0.45
5-HIAA/5-HT	4.25 ± 0.75	1.18 ± 0.37*

*P< 0.05 Compared to Controls
(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

TABLE -10

NEUROTRANSMITTERS AND THEIR METABOLITES IN CEREBELLUM OF CONTROL, DIABETIC AND DIABETIC WITH INSULIN TREATED RATS
(28 DAYS, nanomoles/ gram wet weight)

ANIMAL STATUS	NE	EPI	DA	5-HT	5-HTP	HVA	5-HIAA
CONTROL	1.59 ± 0.29	0.28 ± 0.06	0.72 ± 0.21	N.D.	0.21 ± 0.18	0.32 ± 0.03	0.92 ± 0.33
DIABETIC	1.52 ± 0.22	0.33 ± 0.08	0.32 ± 0.11*	N.D.	0.43 ± 0.03*	N.D.	0.63 ± 0.17
DIABETIC+ INSULIN	0.85 ± 0.19*	0.17 ± 0.01*	N.D.	N.D.	0.08 ± 0.01*	N.D.	0.85 ± 0.02

* $P < 0.05$ Compared to controls
(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

TABLE -11

TURNOVER RATIO OF NEUROTRANSMITTERS IN CEREBELLUM

(28 Days,nanomoles/gram wet weight)

NEUROTRANSMITTERS	CONTROL	DIABETIC
NE	1.52 ± 0.22	1.59 ± 0.09
EPI	0.33 ± 0.11	0.28 ± 0.06
EPI/NE	0.22 ± 0.06	0.25 ± 0.04

(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

TABLE -12

NEUROTRANSMITTERS AND THEIR METABOLITES IN CORPUS STRIATUM OF CONTROL, DIABETIC AND DIABETIC WITH INSULIN TREATED RATS

(28 Days,nanomoles /gram wet weight)

ANIMAL STATUS	NE	EPI	DA	5-HT	5-HTP	HVA	5-HIAA
CONTROL	1.19 ± 0.09	1.03 ± 0.34	0.55±0.11	0.46± 0.08	0.60 ± 0.08	8.81± 1.03	6.82 ± 0.99
DIABETIC	0.72 ± 0.04*	2.46 ±0.07*	36.3 ± 4.18**	0.59±0.05*	13.2± 0.96*	3.42 ± 0.29*	10.39 ± 1.14*
DIABETIC+ INSULIN	1.15± 0.21	0.24 ± 0.13*	113.9 ± 4.5**	0.82± 0.05*	0.34± 0.05*	N.D.	3.09 ± 0.07*

*P<0.05 compared to control

**P<0.001 compared to control

(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

TABLE -13

TURNOVER RATIO OF NEUROTRANSMITTERS IN CORPUS STRIATUM
(28 Days,nanomoles/gram wet weight)

NEUROTRANSMITTERS	CONTROL	DIABETIC
EPI	1.03 ± 0.34	2.46 ± 0.07*
NE	1.19 ± 0.09	0.72 ± 0.04*
EPI/NE	1.11 ± 0.32	3.48 ± 0.28*
5-HTP	0.60 ± 0.08	13.2 ± 0.96
5-HT	0.46 ± 0.08	0.59 ± 0.05*
5-HTP/5- HT	1.23 ± 0.23	22.11 ± 2.45*
5- HIAA	6.82 ± 0.99	10.39 ± 1.14*
5- HIAA/5- HT	16.71 ± 2.25	18.44 ± 3.69*
HVA	8.81 ± 1.03	3.42 ± 0.29*
DA	0.55 ± 0.11	36.3 ± 4.18*
HVA/DA	14.9 ± 1.37	0.10 ± 0.02*

*P< 0.05 Compared to Controls
(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

TABLE-14

CHANGES IN THE CONTENT OF NEUROTRANSMITTERS IN THE HYPOTHALAMUS OF CONTROL AND DIABETIC RATS AT THE END OF 7, 14 AND 28 DAYS

(Nanomoles/gram wet weight)

ANMAL STATUS	NOREPINEPHRINE			EPINEPHRINE			SERTONIN		
	7 DAYS	14 DAYS	28 DAYS	7 DAYS	14 DAYS	28 DAYS	7 DAYS	14 DAYS	28 DAYS
CONTROL	2.40 ± 0.15	2.54 ± 0.20	3.34 ± 0.39	0.34 ± 0.22	0.30 ± 0.18	0.19 ± 0.02	1.19 ± 0.02	0.91 ± 0.02	1.14 ± 0.04
DIABETIC	37.84 ± 2.78*	25.14 ± 0.01*	14.45 ± 0.09*	N.D	N.D	0.73 ± 0.02*	0.35 ± 0.04*	0.092 ± 0.01*	0.72 ± 0.07*

*P< 0.05 Compared to Controls

(VALUES ARE MEAN± S.E.M OF 4-6 SEPARATE DETERMINATIONS)

TABLE-15

NEUROTRANSMITTER RECEPTOR BINDING PARAMETERS IN DIFFERENT BRAIN REGIONS OF CONTROL, DIABETIC AND DIABETIC+ INSULIN TREATED RATS

BRAIN REGION	HYPOTHALAMUS					
	[3H]NOREPINEPHRINE		[3H]YOHIMBINE		[3H]SEROTONIN	
ANIMAL STATUUS	Bmax	Kd	Bmax	Kd	Bmax	Kd
CONTROL	5584±200	30.5±5.0	529±03	3.29±0.15	1081±57	9.89±1.2
DIABETIC	2584±155*	30.4±7.0	588±30	2.34±0.18*	2547±60*	11.73 ±2.0
DIABETIC+INSULIN	—	—	617±2	2.02± 0.15*	—	—
BRAIN REGION	BRAIN STEM				CEREBRAL CORTEX	
	[3H] SEROTONIN		[3H]YOHIMBINE		[3H] SEROTONIN	
ANIMAL STATUS	Bmax	Kd	Bmax	Kd	Bmax	Kd
CONTROL	197± 2	1.24±0.02	235 ± 10	5.26 ± 0.19	260 ± 10	2.5± 0.2
DIABETIC	222± 12	4.62 ± 0.12*	240 ± 33	7.67 ± 0.41*	122 ±29*	1.9± 0.4*
DIABETIC+INSULIN	185 ± 3	1.31 ± 0.4	304 ± 40*	5.35 ± 0.07	—	—

*P< 0.05 compared to control. Bmax - Binding maximum, (fmoles / mg protein) .Kd - Dissociation constant(nM)

TABLE -16

PHOSPHOLIPASE C ACTIVITY IN BRAIN HYPOTHALAMUS AND BRAIN STEM OF STRPTOZOTOCIN DIABETIC RATS COMPARED TO CONTROLS

(Values are μ moles /minute/ mg of protein)

FRACTION	HYPOTHALAMOUS		BRAIN STEM	
	CONTROL	DIABETIC	CONTROL	DIABETIC
MEMBRANE FRACTION	8×10^{-3}	7×10^{-3}	NIL	NIL
CYTOSOLIC FRACTION	10.7×10^{-3}	1.33×10^{-3} *	4×10^{-3}	3×10^{-3}
TOTAL	18.7×10^{-3}	8.33×10^{-3}*	4×10^{-3}	3×10^{-3}

* 55% reduced activity compared to control

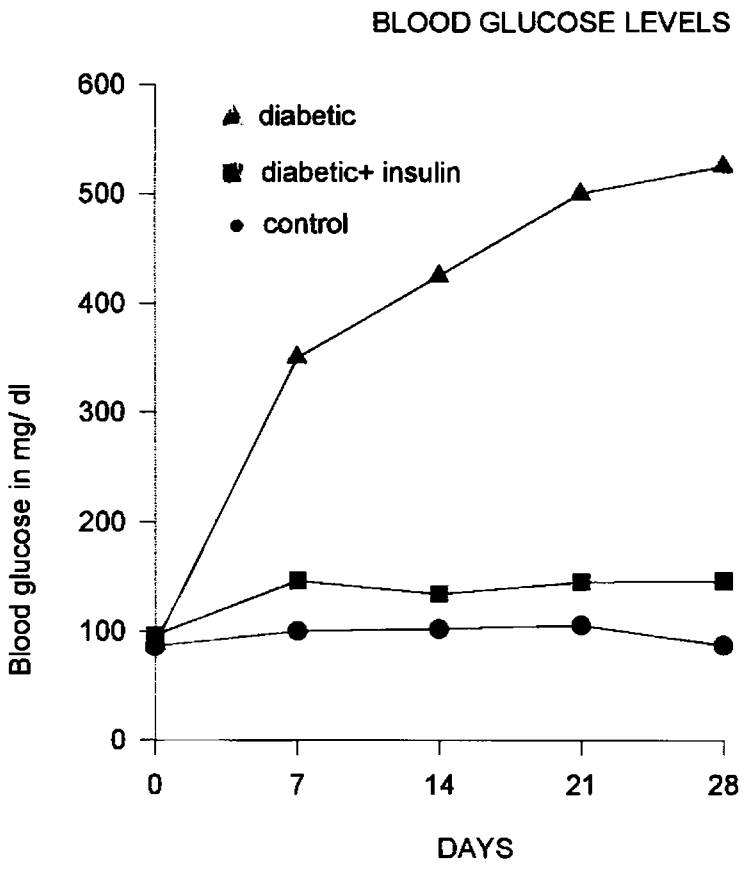

BLOOD GLUCOSE LEVELS
600
500
400
300
200
100
0
Blood glucose in mg/ dl
diabetic
diabetic+ insulin
control
0
7
14
21
28
DAYS

Figure 1

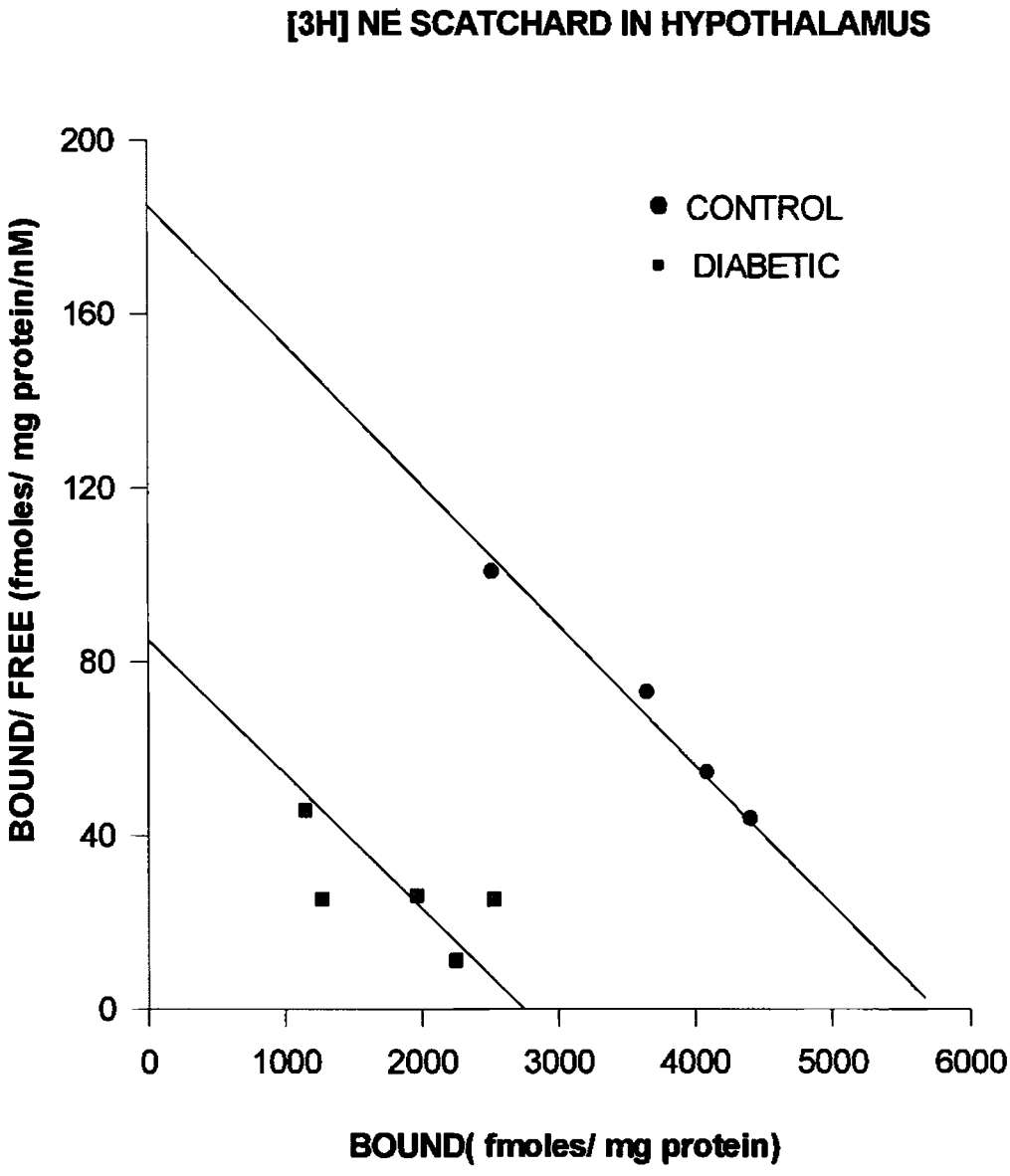

[3H] NE SCATCHARD IN HYPOTHALAMUS
CONTROL
DIABETIC
BOUND/ FREE (fmoles/ mg protein/nM)
200
160
120
80
40
0
0
1000
2000
3000
4000
5000
6000
BOUND(fmoles/ mg protein)

Figure 2

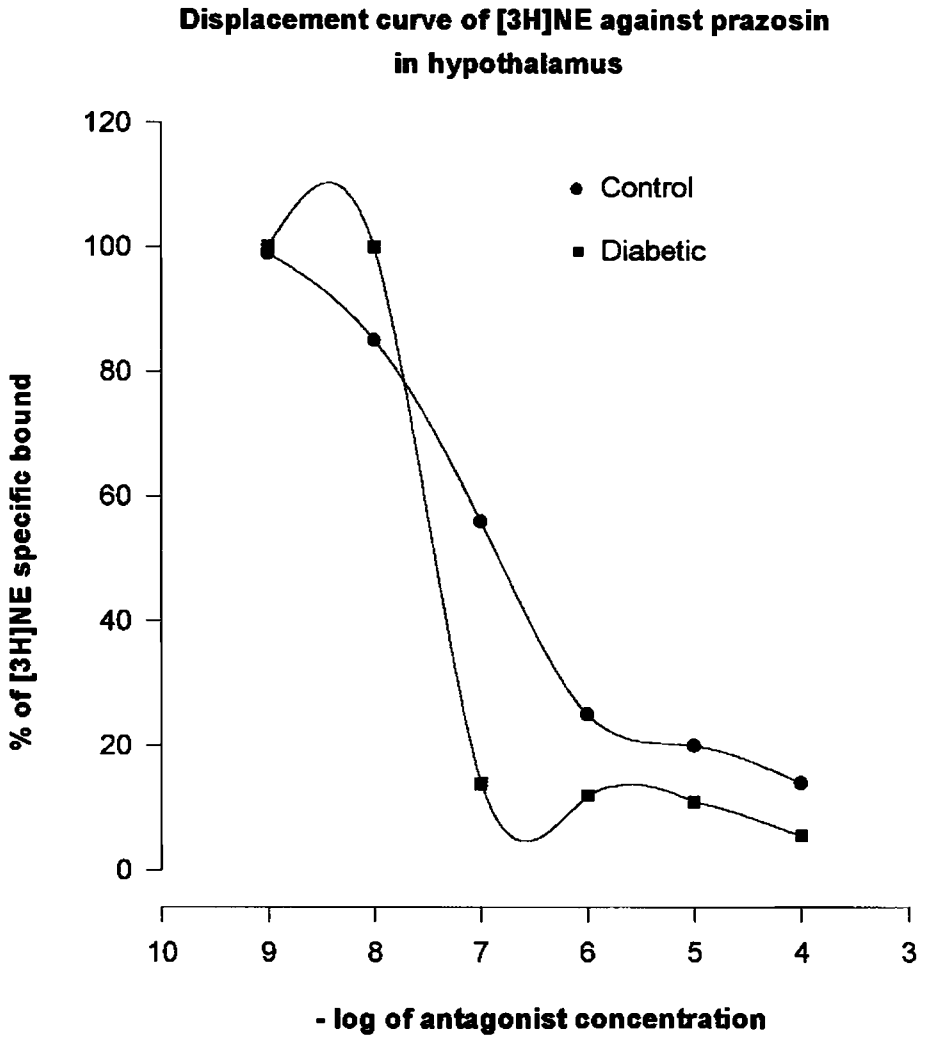

Displacement curve of [3H]NE against prazosin in hypothalamus
120
100
80
60
40
20
0
% of [3H]NE specific bound
Control
Diabetic
10
9
8
7
6
5
4
3
- log of antagonist concentration

Figure 3

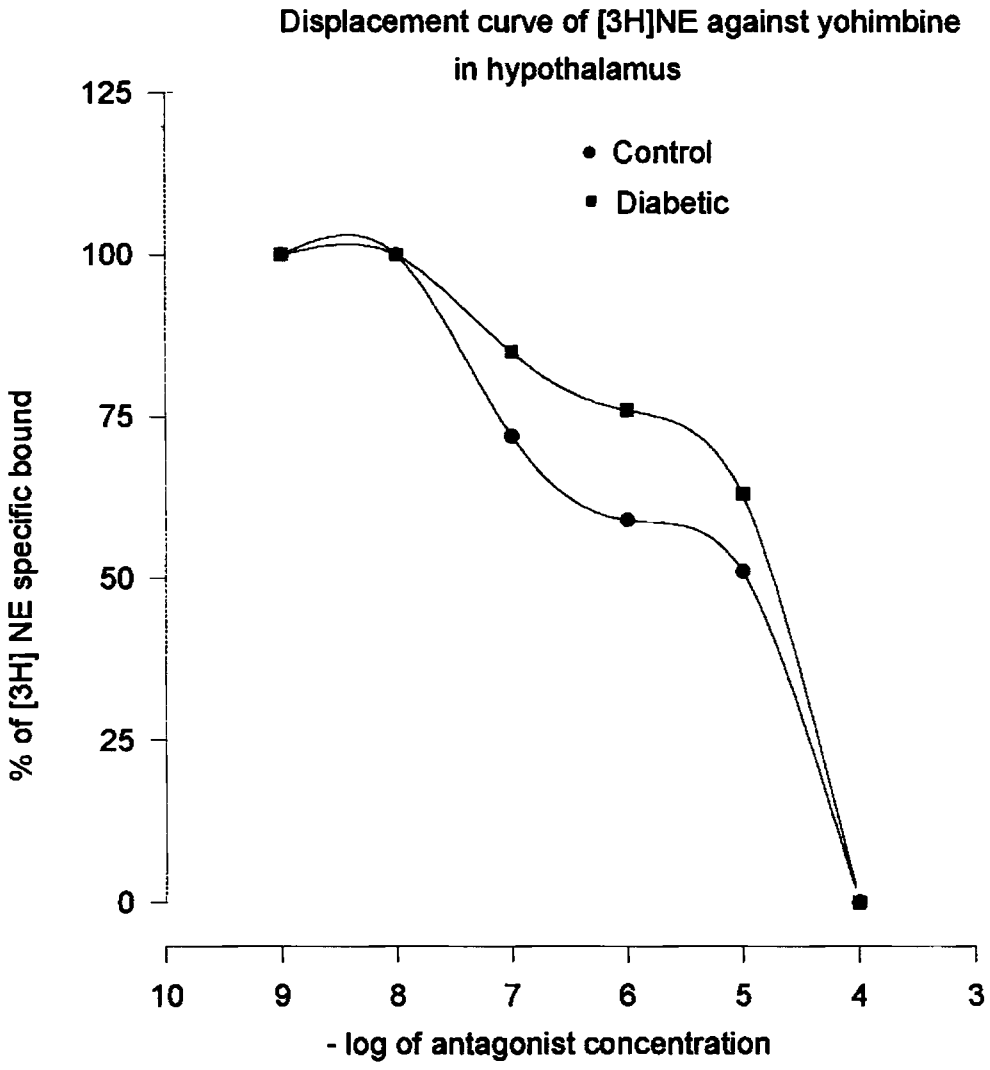

Displacement curve of [3H]NE against yohimbine in hypothalamus
Control
Diabetic
% of [3H] NE specific bound
125
100
75
50
25
0
10
9
8
7
6
5
4
3
- log of antagonist concentration

Figure 4

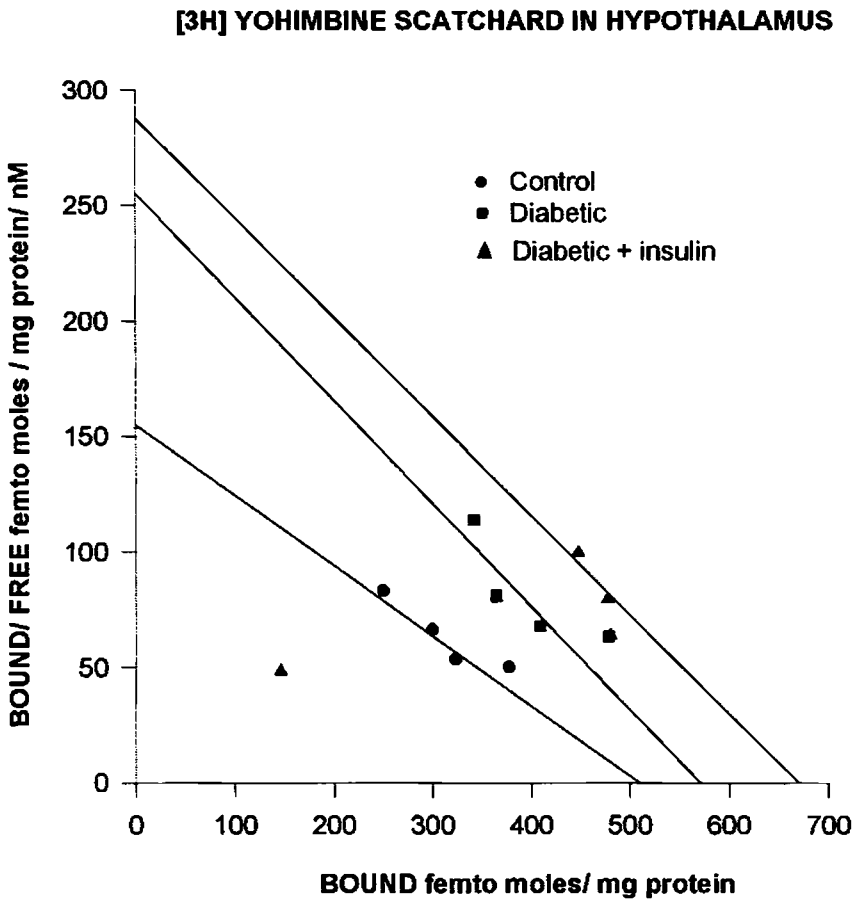

[3H] YOHIMBINE SCATCHARD IN HYPOTHALAMUS
BOUND/ FREE femto moles / mg protein/ nM
BOUND femto moles/ mg protein
Control
Diabetic
Diabetic + insulin
300
250
200
150
100
50
0
0
100
200
300
400
500
600
700

Figure 5

Displacement using (–) Epinephrine against [3H] yohimbine in hypothalamus and the effect of the GTP analogue Gpp[NH]p and sodium ions

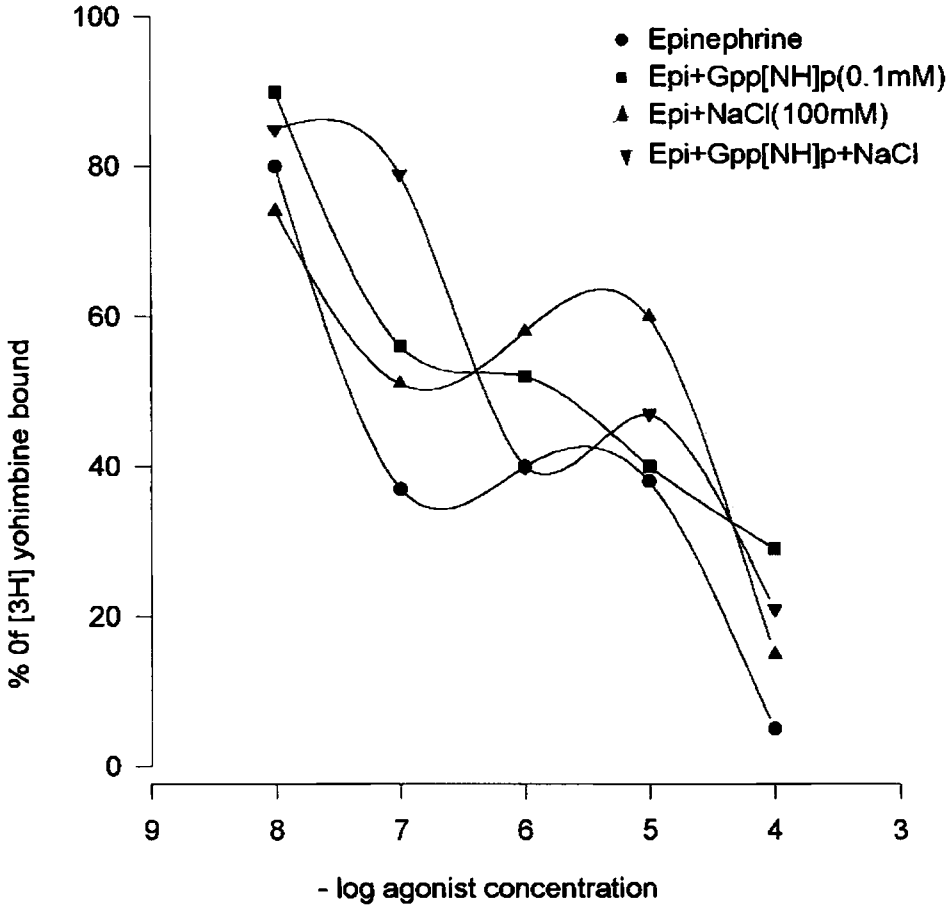


Figure 6

Control hypothalamus

Displacement using (–) Epinephrine against [3H] yohimbine in hypothalamus and the effect of the GTP analogue Gpp[NH]p and sodium ions

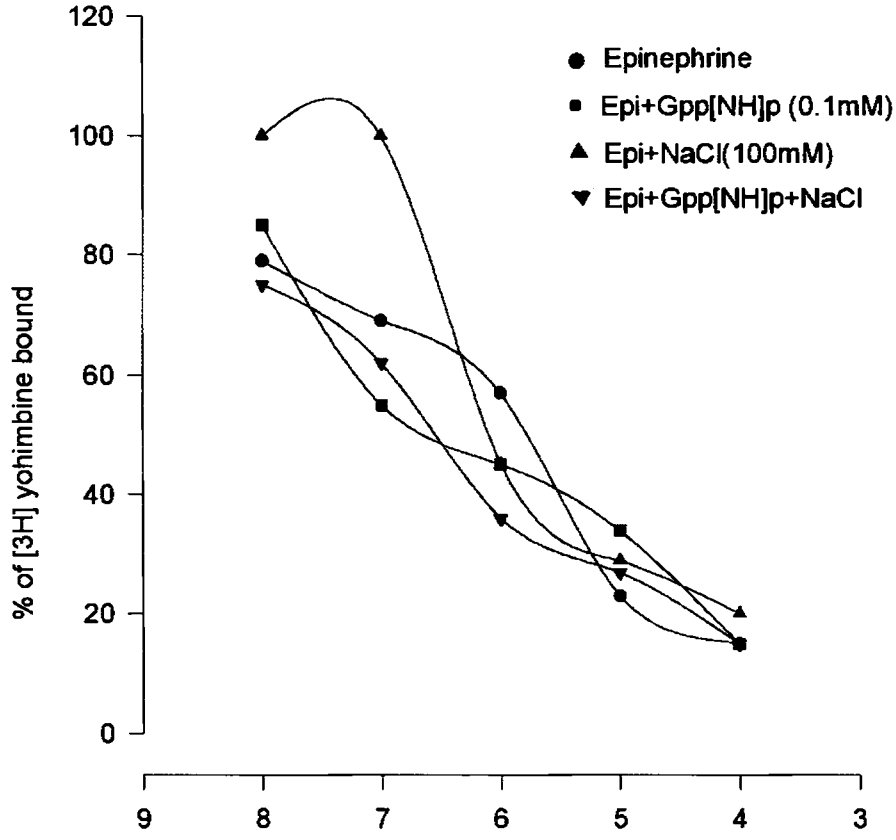


Figure 7

Diabetic hypothalamus

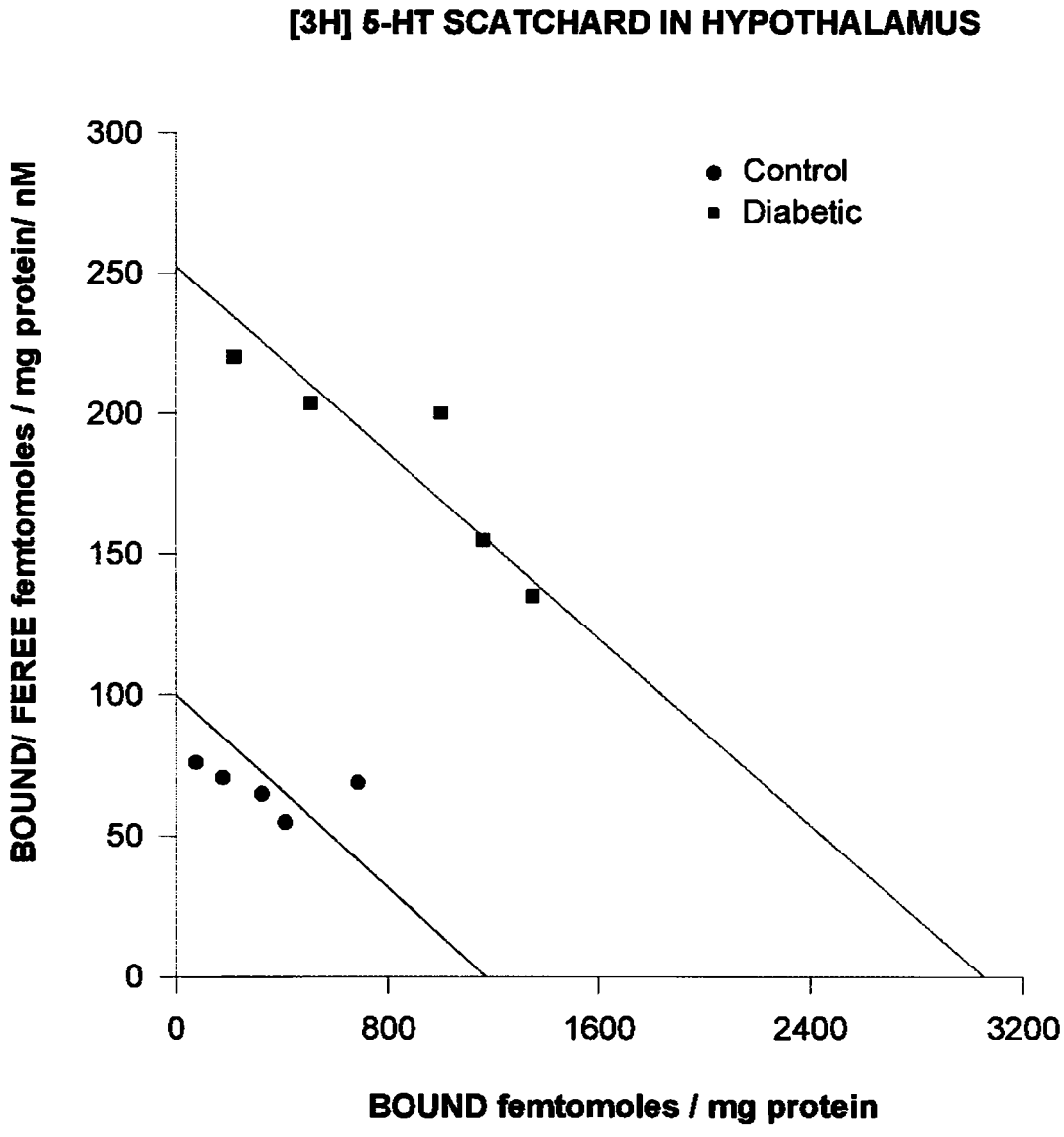

[3H] 5-HT SCATCHARD IN HYPOTHALAMUS
BOUND/ FEREE femtomoles / mg protein/ nM
300
250
200
150
100
50
0
Control
Diabetic
0
800
1600
2400
3200
BOUND femtomoles / mg protein

Figure 8

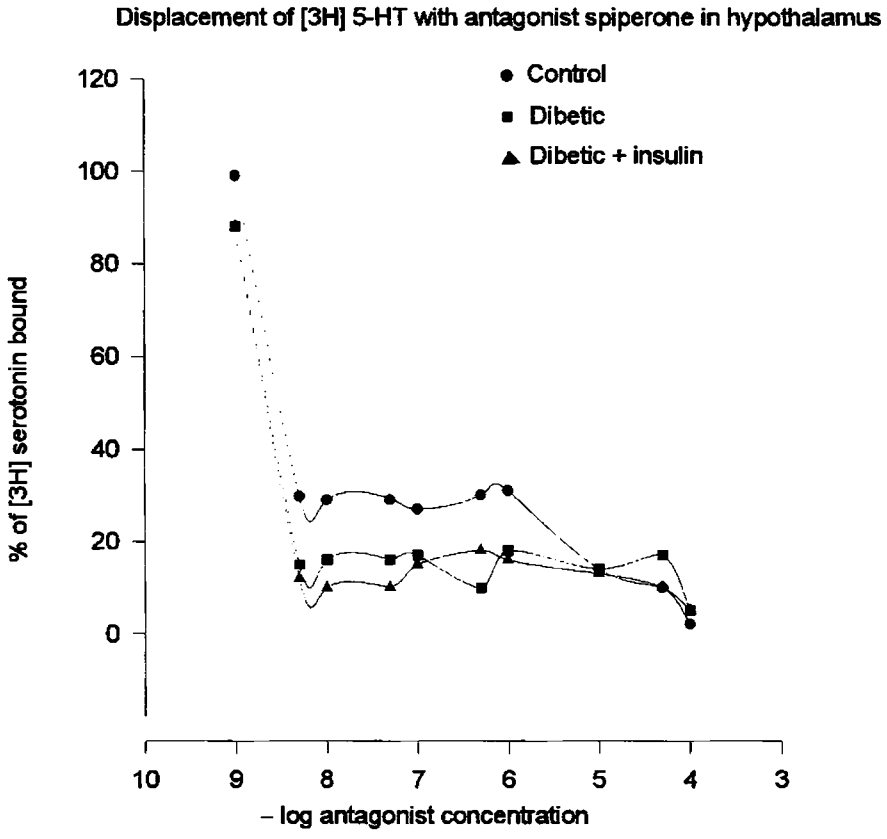

Displacement of [3H] 5-HT with antagonist spiperone in hypothalamus
Control
Dibetic
Dibetic + insulin
% of [3H] serotonin bound
120
100
80
60
40
20
0
10
9
8
7
6
5
4
3
– log antagonist concentration

Figure 9

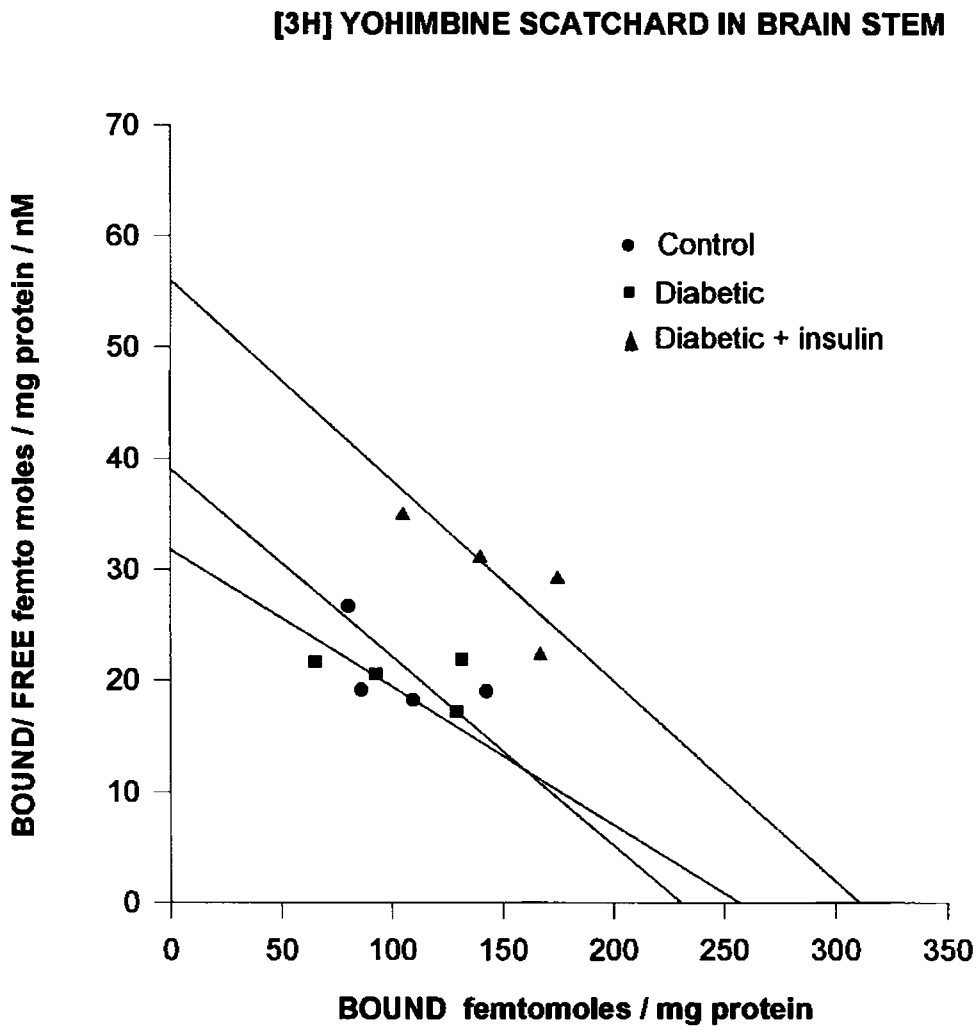

[3H] YOHIMBINE SCATCHARD IN BRAIN STEM
BOUND/ FREE femto moles / mg protein / nM
BOUND femtomoles / mg protein
Control
Diabetic
Diabetic + insulin
0
10
20
30
40
50
60
70
0
50
100
150
200
250
300
350

Figure 10

Displacement using (-) Epinephrine against [3H] yohimbine in brain stem and the effect of GTPanalogue Gpp[NH]p and sodium ions

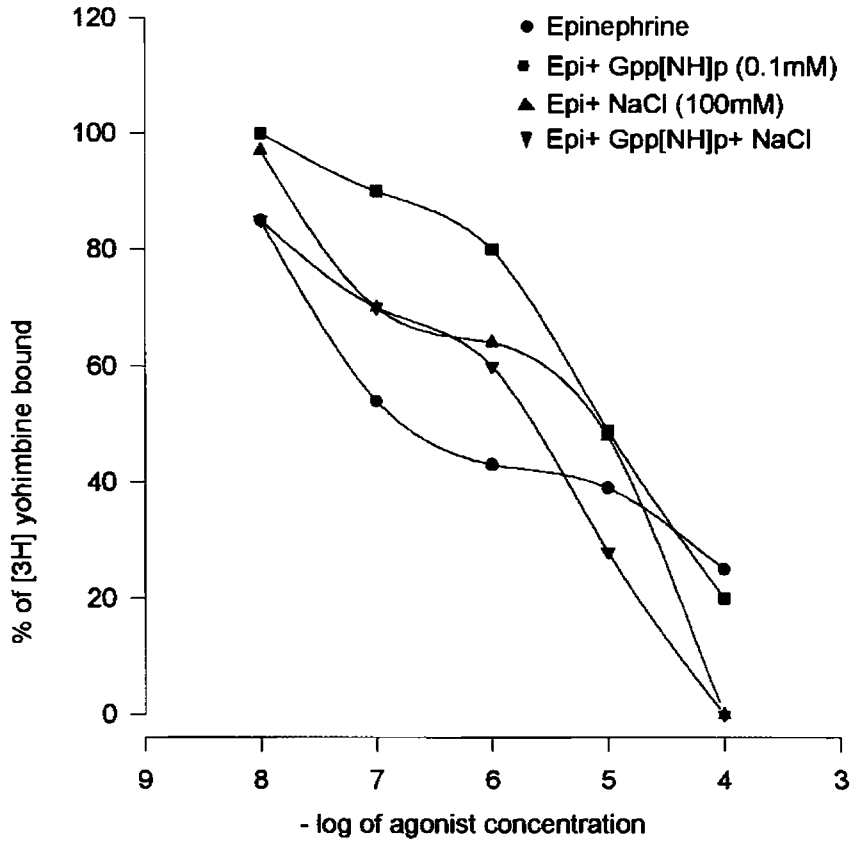


Figure 11

Control brain stem

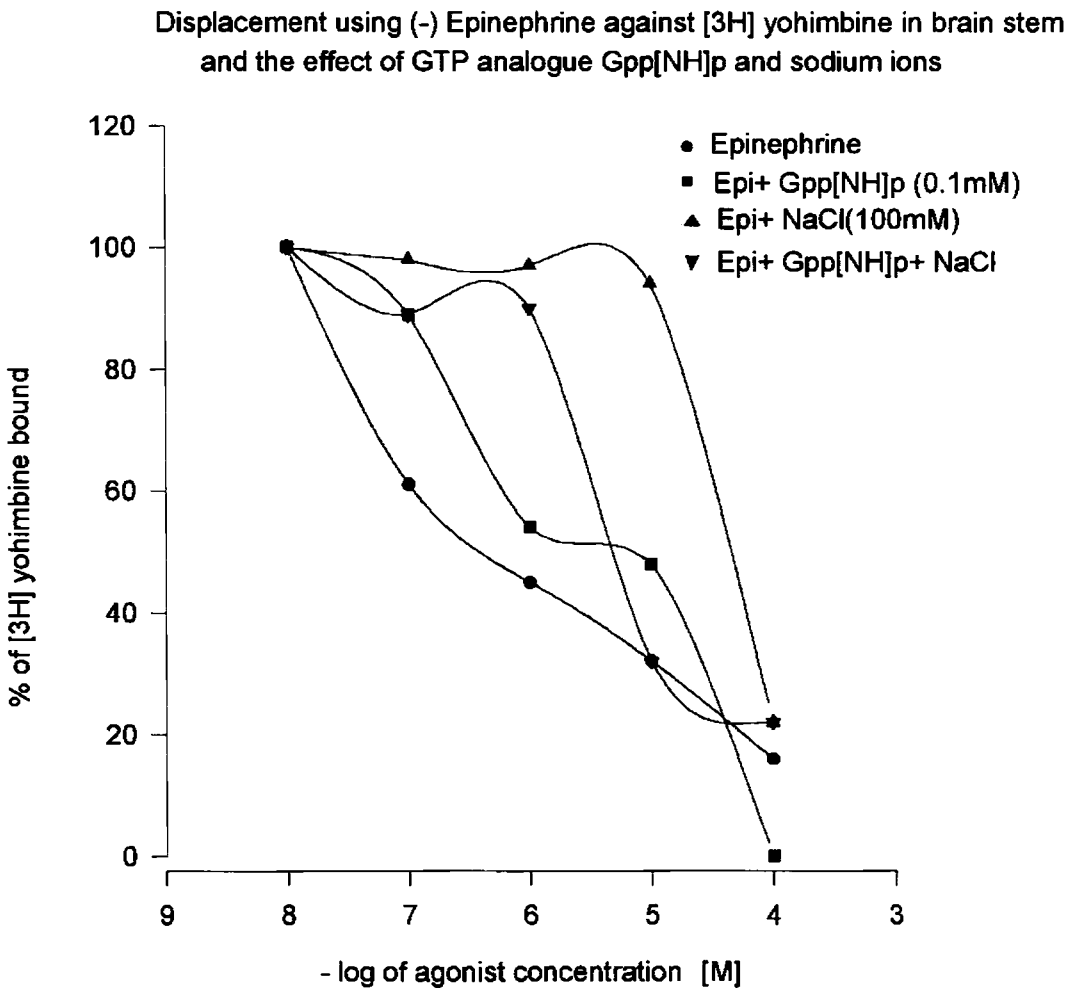


Figure 12

Diabetic brain stem

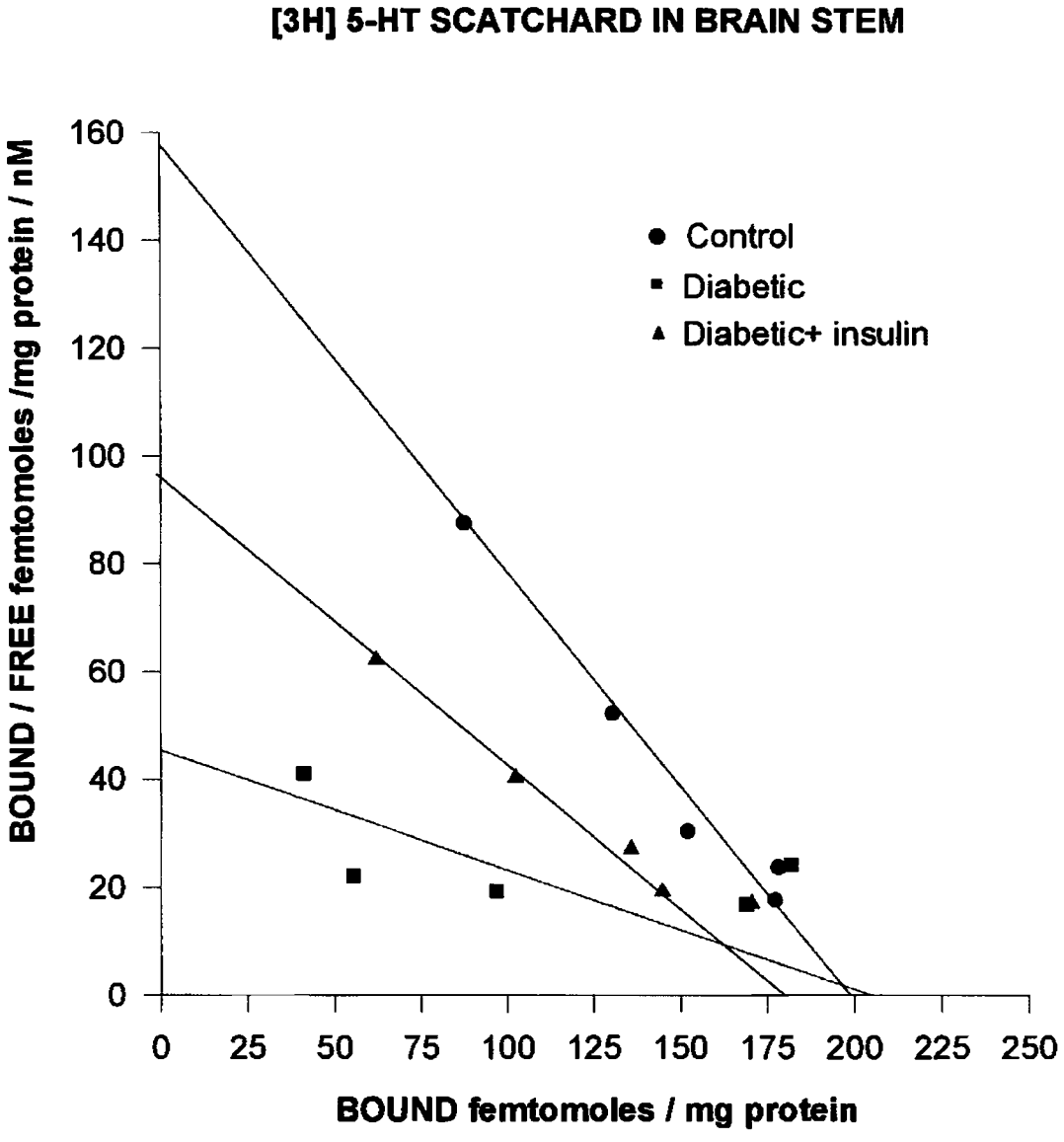

[3H] 5-HT SCATCHARD IN BRAIN STEM
BOUND / FREE femtomoles /mg protein / nM
160
140
120
100
80
60
40
20
0
0
25
50
75
100
125
150
175
200
225
250
BOUND femtomoles / mg protein
Control
Diabetic
Diabetic+ insulin

Figure 13

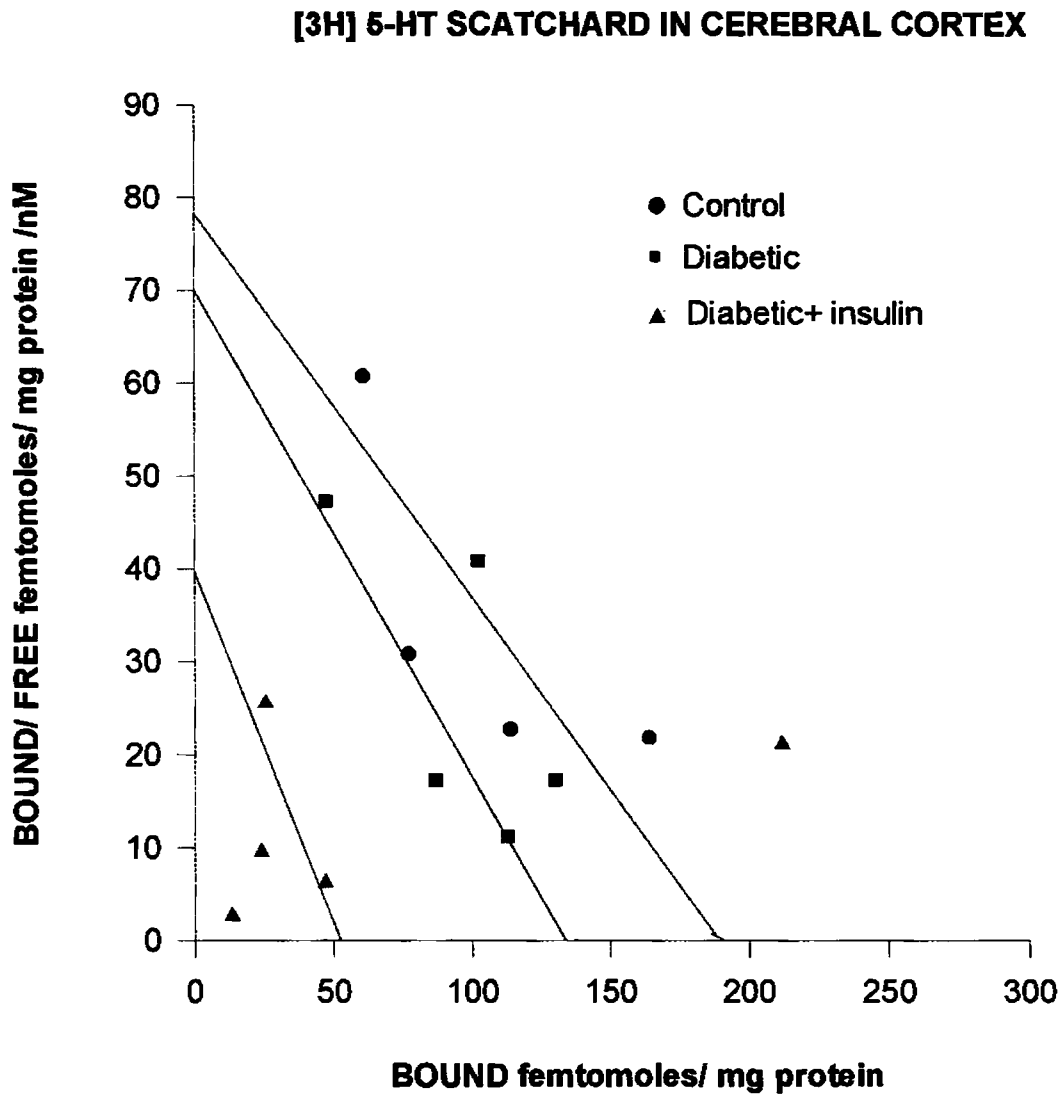

[3H] 5-HT SCATCHARD IN CEREBRAL CORTEX
BOUND/ FREE femtomoles/ mg protein /nM
BOUND femtomoles/ mg protein
Control
Diabetic
Diabetic+ insulin
0
10
20
30
40
50
60
70
80
90
0
50
100
150
200
250
300

Figure 14

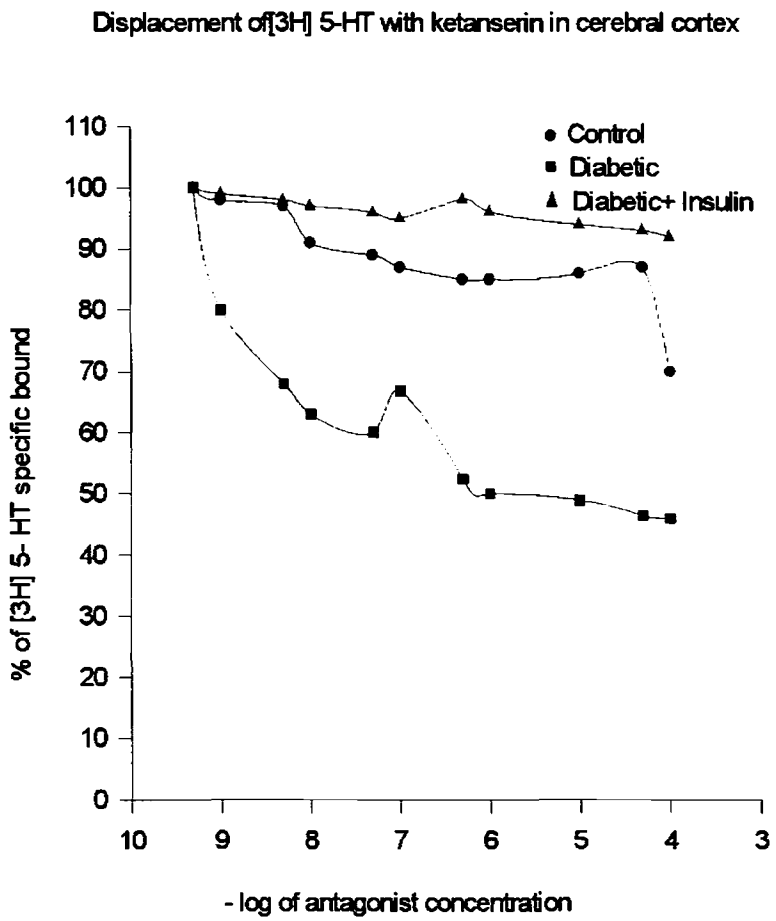

Displacement of[3H] 5-HT with ketanserin in cerebral cortex
Control
Diabetic
Diabetic+ Insulin
% of [3H] 5- HT specific bound
110
100
90
80
70
60
50
40
30
20
10
0
10
9
8
7
6
5
4
3
- log of antagonist concentration

Figure 15

Discussion

DISCUSSION

NEUROTRANSMITTER CHANGES AND THEIR SIGNIFICANCE IN STREPTOZOTOCIN DIABETES

The diabetic state induced by streptozotocin (STZ) produced an elevated blood glucose level, changed the protein and cholesterol content of brain as well as other body tissues. These gross changes can have profound effects on the cellular function. The streptozotocin induced diabetic brain when analysed with HPLC showed that the brain neurotransmitter content of diabetic rats changed in various brain regions differently . The norepinephrine content increased significantly in cerebral cortex (CC) and hypothalamus (Hypo), while in brain stem (BS) and cerebellum (CB) it remained unaffected. In BS, Hypo and CS the epinephrine content increased significantly. The corpus striatum showed a decreased norepinephrine content. The noradrenergic (NA) activity was assessed by measuring the level of NE and epinephrine (EPI). The models proposed for epinephrine formation by Mefford (1987) support that increased noradrenergic activity may be observed due to increased release, reuptake, blockade or inhibition of degradation. These changes can affect epinephrine pool in the nerves. The proposed model is based on the observation that both postsynaptic and presynaptic nerves of different brain regions contain the enzyme involved in the NE→EPI conversion, phenyl-N-methyl-transferase (PNMT). The enzyme localisation and its expression are important factors deciding the formation of epinephrine in the brain. The classical concept of epinephrine neurons is now discarded and it is considered as a unique metabolite of NE with important pharmacological actions and a receptor subtype in brain which monitors and regulates its formation (Mefford

1987). Epinephrine is recognized by the uptake system on noradrenergic terminals & so can compete for the storage in these noradrenergic neurons.

With the above modern concepts for epinephrine formation in the brain, we will try to understand this complicated NE→EPI conversion in diabetic brain regions (Table 5). The current view is consistent with the concept that the levels of 3-methoxy-4-hydroxy-phenylglycol (3-MHPG) and its sulfate ester reflect noradrenergic activity within CNS (Stewert, *et al.*,1994, Mefford, 1987). Previous data clearly documented that noradrenergic neurons within specific hypothalamic nuclei and other CNS regions are hyperactive in diabetes (Bitar *et al.*, 1987). In the cerebral cortex region the increase in NE is not accompanied by an increase in EPI and also a decreased turnover rate from NE→EPI. This decreased turnover may force us to think that NE→EPI turnover ratio decreased is the reason for increased NE level. But this may not be the actual scene. If a decrease in NE→EPI conversion is the prevailing condition in diabetic CC, we could expect an absence or decrease of EPI along with this. From the present condition of no change in the EPI content it seems that in the noradrenergic nerves there is an increased uptake of NE or a reduced release of NE occurs.

Similarly in the hypothalamus, the NE content along with EPI content increased while NE→EPI turnover rate also increased. This may be interpreted as a possible high noradrenergic activity and increased NE→EPI metabolic pathway in diabetic hypothalamus.

In the brain stem the NE content remained unchanged while EPI content increased. The turnover rate showed a decrease. A possible explanation for this situation is a changed activity or expression of the enzymes involved in the conversion of NE→EPI.

The striatal noradrenergic activity of a decrease in NE and an increase in both EPI and NE→EPI turnover rate explains a higher noradrenergic activity which resulted in an increased turnover rate and thus an increased formation of EPI.

The increased noradrenergic activity in the brain ventral tegmental area has been attributed to increased locomotor activity in the rats. In a recent observation a similarity between Schizophrenic and diabetic brain state was argued (Holden and Mooney, 1994). The brain changes observed in diabetics and Schizophrenics manifest a wide range of similar disturbing physiological symptoms, such as impaired sexual function, temperature control, low blood pressure, disrupted sleep patterns, excessive thirst, poor memory, insensitivity to pain and chronic unhappiness. Hyperthermia, hyperlocomotor activity, hyperphagia associated in diabetes attributed to changed noradrenergic nerve activity in the diabetic brain (Bitar, *et al.,* 1987).

The dopaminergic neuronal activity in diabetic brain regions showed an increase in its activity in all the brain regions examined. The most significant change of dopaminergic neurons observed is in striatum. The striatal dopamine and its metabolites in diabetic rats have been reported (Lim and Lee, 1995). This increase in the dopamine level of synaptosome is attributed to the decrease in the release of dopamine in hyperglycemic state. They also observed a decrease in the monoamine oxidase activity in striatal synaptosomes. In our study content of DA and HVA in striatal region of diabetic rats showed a significant increase and significant decrease respectively ($P<0.05$; -90%). The *in vitro* observations in striatal tissues showed a decrease in release of dopamine in 14 day old rats. The observed increase in dopamine content in our study indicate the synaptosomal

dopamine which accumulated due to an abnormality in the release. A decrease in HVA content may be due to the decreased release.

The overall increased dopaminergic activity may produce many physiological manifestations. The dopamine turnover in the limbic structures of diabetic rats were shown to increase significantly. Haloperiodol- a D_1 receptor antagonist reduced locomotor activity in diabetic mice (Kamei *et al.*, 1994). This finding bear importance since the observed dopamine increase in hypothalamic area may play a role in behavioral changes associated with diabetics. In another study the yawning behaviour was analysed in four week old STZ-induced diabetic Wistar rats (Heaton and Varrin, 1993). Dopamine changes were observed and an associated significantly lower rate of yawning was observed at 4 weeks of diabetes. The dopamine content thus may have effects on behaviour during diabetes.

In our study, the diabetic brain serotonergic activity was assessed by its ratios to both its precursor 5-Hydroxytryptophan (5-HTP) and its breakdown metabolite 5-HIAA. The turnover rate calculated for the precursor molecule to respective neurotransmitter showed an increase. It indicate an accumulation of the respective precursor. In hypothalamus 5-HTP gets accumulated due to some block in the synthesis. The hypothalamic turnover for 5-HT→5-HIAA was also higher. Thus both these can result in a decreased serotonin level in hypothalamus. An altered blood brain barrier (BBB) was observed in diabetic condition which result in a decreased content of aminoacids such as tryptophan, phenylalanine, tyrosine, methionine and lysine (Mans *et al.*, 1987). A lack of response to dietary carbohydrate or protein on brain tryptophan and serotonin in diabetic rats and an altered blood brain barrier may be an important factor bringing out the altered serotonergic function (Crandall and Fernstrom, 1980 &1983). In similar studies on serotonin content measured in diabetic rat hypothalamus revealed a reduced rate of

serotonin synthesis within the brain (Crandall and Fernstrom, 1983). In diabetes hypothalamus was reported to have a decreased serotonin content (Bitar *et al.*, 1987&1986). The significant finding from our study is that 5-HT→5-HIAA degradation pathway was increased which resulted in a decreased serotonin and an increased 5-HIAA content.

Hypothalamic serotonergic activity could produce altered pituitary hormone release. It is reported that in diabetes pituitary hormone secretion is altered (Locatelli *et al.*, 1985). Clonidine (0.15 mg/kg iv) failed to evoke a GH release in streptozotocin diabetic rats. This study suggests an impaired function of noradrenergic pathway controlling GH release in diabetic hypothalamus.

The studies carried out in other brain regions revealed that serotonergic neuronal alteration is not confined to hypothalamus alone. The CC content of 5-HT increased and 5-HIAA content remained unchanged and the turnover rate of 5-HT →5-HIAA decreased. This stand well as an explanation for the increased accumulation of 5-HT.

The brain stem results show an increase in serotonin content which is due to precursor and breakdown metabolite alteration. 5-HTP increased while 5-HIAA decreased. Turnover rates calculated for serotonin precursor and metabolite clearly showed that there is a defect in metabolic pathway of serotonin in diabetic BS region.

Cerebellum serotonin activity was not predictable due to the reason that 5-HT level was not detectable in all the samples analysed. Also that this region is more rich in GABAergic neurons (Dakshinamurti *et.al.*,1990)

The CS serotonergic synthetic pathway seems to be active as revealed by the high content of 5-HTP and the increased turnover rate. This region is considered to be the control centre of behavioral function in animals. So changes in serotonergic activity observed in the present study may be releated to behavioral changes associated with diabetes.

Thus, our study reveal that though previous studies generally conclude that in diabetic brain the rate of serotonin synthesis is reduced, but the way by which the different brain regions respond to this disturbance are different. It is known that serotonin precursor molecules and their entry across the BBB is impaired. Also, the changes in the brain level of each aminoacid paralleled the changes in the blood ratio of each aminoacid to the sum of the other aromatic and branched chain aminoacids. Crandall and Fernstrom, (1983) concluded from their observations of occasional decrease of tryptophan and tyrosine in blood of diabetic rats that the changes in the brain levels of aminoacids may influence the rates at which they are consumed in brain. It may be true that tryptophan and their availability in the brain is decreased during diabetes but the serotonergic pathways in different brain regions respond differently to this altered state. It is also possible that not only the altered state of serotonin metabolism is attributable to the availability of its precursor, but also, some other nerve impairment produced by hyperglycemic state.

WHY HYPOTHALAMIC AND BRAIN STEM REGION CHANGES IN NORADRENERGIC AND SEROTONERGIC NERVES ARE IMPORTANT?

Our study on neurotransmitters and their metabolite changes during diabetes revealed that noradrenergic and serotonergic pathways of the major neuroendocrine

system regulating region, hypothalamus as well as other brain regions are altered. Also, our study emphasized noradrenergic activity and the epinephrine pathway in brain regions.

The literature reviewed in the beginning of this study showed that there are evidences suggesting a clear cut role of adrenergic and serotonergic systems of hypothalamus in glucoregulatory function. The noradrenergic pathway in the brain stem analysed in the present study revealed alteration during diabetes. As we discussed earlier epinephrine and norepinephrine content are inter-related. The former is a major and unique metabolite of the latter and its formation is regulated by a unique adrenergic receptor subtype on the presynaptic membranes. In BS region we have observed a hike of epinephrine content while NE level remained same. This indicate a possible altered NE→ EPI pathway. In hypothalamus NE as well as EPI increased significantly. This indicate an increased noradrenergic release promoting the NE→ EPI formation. But in BS the NE→ EPI is not promoted. There is a unique way by which these neurotransmitters are regulated in the brain regions.

The post synaptic nerves as well as presynaptic nerves contain α_2 adrenergic receptors. These receptors which are present on the presynaptic nerves are autoreceptors and they regulate the release of norepinephrine from the presynaptic nerves into the synaptic cleft. The epinephrine level thus is regulated uniquely by these receptors. The increased epinephrine content despite a normal level of norepinephrine in BS may be indicating an altered presynaptic α-$_2$ receptors in the BS. In hypothalamus the norepinephrine content is still high even after an increase in the epinephrine content. This indicate an altered α-$_2$ receptor function in this region. Another important possible way by which these receptors and epinephrine content is regulated is through the PNMT gene and its expression. This

enzyme can also change the α-$_2$ receptor function. PNMT regulate the conversion of NE→EPI and this can regulate the autoreceptor expression in the nerves.

WHY NORADRENERGIC RECEPTORS ARE STUDIED ALONG WITH SEROTONIN RECEPTORS?

The availability of new tools and new emerging fields of molecular neurobiology have helped in understanding more how nerves in the discrete brain regions interact each other. In this regard the neuropharmacological drugs, new transgenic rat models and other applications of molecular techniques to neurobiology have helped in understanding the interactions of adrenergic and serotonergic neurons. The drug called 8-hydroxy di-n-propylamino tetralin, a 5-HT1a agonist in microdialysate of ventral tegmental area (VTA) revealed a dose dependent release on DA and NE (Chen and Reith, 1995).Mongeau *et al.*, (1994) demonstrated how an ion gated 5-HT3 receptors enhances the release of [^{3}H]NE, in preloaded slices of rat brain. Studies on pituitary-adrenocortical activation during stress showed that catecholamine and serotonin nerves together bring out this effect (Bugajaski *et al.*1993, Mefford, 1987, Debreceni, 1994, Smythe *et al.*, 1983). The evidence provided by Tian et al. (1993) clearly suggested a close association between the serotonergic and adrenergic neurons of hypothalamus. Their experiments revealed that 5-HT neurons tonically inhibit the activity of NE neurons terminating in the medial zona incerta (MZI) and dorsomedial nucleus (DMN) of the hypothalamus, but do not influence the activity in certain hypothalamic catecholaminergic neurons. 5-HT1a receptor interactions with dopamine were also revealed by several studies (Arborelino *et al.*, 1993).

WHY SEROTONIN AND NOREPINEPHRINE MEDIATED ADRENAL PATHWAY MAY BE IMPORTANT IN DIABETES?

Studies have shown that hypothalamic pituitary secretion in diabetes have been defective (Bestetti *et al.*, 1995, Locatelli *et al.*, 1985). Adrenalectomedullation on rats augmented NE release in the PVN neurons during stress (Mefford,1987). Also adrenodemedullation diminished the diabetogenic effect of streptozotocin in rats (Jean *et al.*,1994). This is attributed to a greater pancreatic insulin content. Finally it has been shown that α-$_2$ adrenoceptors modulate ingestive and autonomic functions in plasma glucose levels (Levin and Planas, 1993). [^{3}H]p-aminoclonidine ([^{3}H]-PAC) binding to α-$_2$ adrenoceptors in 5 of 17 brain areas (anterior, ventromedial and arcuate nucleus) assessed by autoradiography techniques; these studies revealed a significant positive correlation and a near significant correlation in the (PVN) and lateral hypothalamus respectively. In the coming sections we will discuss the altered adrenergic and serotonergic receptor function, with emphasis on α-$_2$ adrenergic receptors, STZ-diabetes and their physiological significance.

NEUROTRANSMITTER RECEPTORS AND THEIR REGULATION DURING DIABETES

In the previous part we discussed about neurotransmitter content changes in diabetes. The discussion revealed that noradrenergic and serotonergic neuronal function is altered during diabetes. A wide variety of radioligands, both agonists and antagonists, have been used to label α-adrenergic receptors in various tissues (Rapaske *et al.*, 1987, Hoffman *et al.*, 1979 and 1980, Tsai and Leftkowitz,1979). Hoffman *et al* have shown in their studies that radioligand properties should be taken into care while choosing the radioligand for binding tudies. In the present study we used (-) [^{3}H]NE which can identify more of α-1 adrenergic receptors than any other receptors (Geynet *et al.*,1981). The Scatchard analysis showed that noradrenergic receptors decreased in hypothalamus. The displacement analysis using [^{3}H]NE showed that [^{3}H]NE is better displaced by prazosin than yohimbine. This shows that [^{3}H]NE identifiable sites in hypothalamus contain more α-$_1$ sites. Previous studies in genetically diabetic mice showed an increased α_1 binding sites in diabetic brain (Garris,1990,1995). The saturation binding curves done showed that the α-$_2$ adrenergic receptors is representing less than 15% of [^{3}H]NE identified receptors over the same concentration. These results show that the observed decrease in [^{3}H]NE identifiable sites in diabetic hypothalamus could not directly indicate any appreciable change in the number of α-$_2$ adrenergic binding sites. Also,the displacement analysis show that α-$_1$ antagonist prazosin is a better displacing ligand than α-$_2$ antagonist yohimbine in control hypothalamus. So the observed shift or increased potency for prazosin in displacing [^{3}H]NE from diabetic hypothalamus can be inferred as an increase in α-$_1$ receptors. [^{3}H]NE Scatchard showed a decrease in adrenergic receptors. The observed decrease may be due to all other adrenergic receptors except α-$_1$ adrenergic receptors.

The neurotransmitter epinephrine that is closely related to α-$_2$ adrenergic receptors showed increased content in hypothalamus. But the α-$_2$ adrenergic receptors in this brain region of diabetic rats were not down regulated; but their affinity showed an increase. As we understood earlier the synaptic regulation of noradrenergic release is inhibited or controlled by autoreceptors of α-$_2$ type at the presynaptic membranes or on the cell body. These results may be interpreted as an abnormal regulation of α-$_2$ receptors on this neurotransmitter.

The altered receptor affinity regulation by guanine nucleotides observed in our study emphasize an increased affinity state for the α-$_2$ receptors observed in Scatchard analysis. The guanine nucleotide induced affinity change in diabetic hypothalamus is not demonstrable in the present study. The increased affinity observed in the scatchard analysis value showed that all receptors of α-$_2$ type may be present in α-$_2$ high affinity (α-$_2$H) state. The guanine nucleotides can abolish this state of receptor and bring all the receptors to α_2 low affinity state. The observed absence of this shift may be due to an altered Gi function in the brain hypothalamus. There are many similar reports about an altered Gi protein function (Gando,*et al.*,1995,Mathew *et al.*, 1994, Shindo *et al.*,1993, Gawler *et al.*,1987, Domino *et al.*,1992). It is Gi protein that give the capacity for receptor to remain in the high affinity state. GTP analogue which is a nonhydrolysable form of GTP, permanently disrupts the G protein from the receptor resulting in a decreased affinity state for the receptors. The detergent solubilisation of the α-$_2$ adrenergic receptors is reported to alter the properties of receptor-agonist interactions.

Taken together all our findings on α-$_2$ adrenergic receptors and their agonist in hypothalamus reveal a possible alteration in the G_i function.This is evidenced by an absence of GTP analogue induced steepening of the curve. This shows an impaired G protein function may keep the α-$_2$ receptors of hypothalamus

in an increased affinity state. As we have discussed in Chapter-1 these receptors can regulate the level of epinephrine formation. So we conclude that an observed increased epinephrine content in diabetic hypothalamus may be due to an altered Gi protein induced receptor affinity state leading to a disrupted epinephrine regulation.

Sodium ions and their ability to reduce the receptor affinity was demonstrated in the membrane preparations from diabetic rats. The sodium ions alone were able to demonstrate this shift. But both Gpp[NH]p and sodium ions were not able to demonstrate the shift towards low affinity side. This again may be indicating that interaction between the α-$_2$ receptors and G protein in diabetic hypothalamus may be altered.

Serotonergic activity assessed by serotonin content in diabetic hypothalamus is in agreement with an increased serotonin receptors. The serotonin receptors are identified using [^{3}H]5-HT. Many authors have referred the [^{3}H]serotonin as a selective S_1 receptor agonist in high affinity concentration (Paulose and Dakshinamurti, 1985). Our data showed a significant increase ($P<0.01$) in the serotonin receptors. This may be indicating an increase in S_1 class of receptors. The significance of this along with α-$_2$ receptor alteration may be an important change observed in our study. The 5-HT1a agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and its effect on hyperglycemic and hypoinsulinemic effects suggested that A type of 5-HT1 recognition sites and α_2 adrenoceptors mediate these changes. Our study clearly shows that hypothalamic α-$_2$ and 5-HT1 class receptors are altered during diabetes. These changes may be important in the disease state.

How 8-OH-DPAT and serotonin receptors interact?

The 5-HT receptors has been classified based on radioligand binding studies into major four classes of receptors 5-HT1, identifiable by trititated 5-HT, 5-HT2 by spiperone or ketanserin 5-HT3 an ion gated channel and 5-HT4 which is still to be pharmacologically demonstrated, but postulated to occur (Hoyer *et al.*, 1992). It was Pedigo *et al.*, (1981) who studied the multiple existing sites of 5-HT1 recognition sites. The "high affinity sites" for spiperone were called 5-HT1a, whereas low affinity sites were designated as 5-HT1b sites. The novel centrally active 5-HT agonist 8-OH-DPAT (Hjorth *et al.*, 1982) have a high affinity for 5-HT1a site, which exceeds by several orders of magnitude by its affinity for either 5-HT1b and 5-HT2 recognition sites. The studies on biochemical and behavioral effects of this drug are indicative of central post synaptic 5-HT receptors and occupancy on 5-HT cell bodies. Bluet *et al.*, (1995) showed that ACTH but not PRL control involves presynaptic interactions at 5-HT autoreceptors in raphe nuclei by 8-OH-DPAT. Chaouloff and Jeanrenaud (1987) found that α-$_2$ adrenoceptors antagonist Idazoxan and nonspecific 5-HT antagonist, methiotepin, prevented the hyperglycemic and hypoinsulinemic effects (Chaouloff 1987). This drug helped to reveal the exact serotonin receptors and adrenergic receptors mostly involved in glucose homeostasis signals from brain hypothalamus. In our study we were able to clearly show the involvement of 5-HT1 receptors in insulin function during diabetes.

The displacement analysis using a concentration of high affinity range of spiperone showed that spiperone is more potent in displacing the ligand in diabetic hypo than control hypo which shows an increased 5-HT1 receptors in the high affinity range.

The neurotransmitter epinephrine content is significantly higher in the lower brain stem region. The adrenergic receptors analysed in brain stem showed a decreased affinity for the antagonist [^{3}H] Yohimbine. Our analysis of receptor affinity may be a change in the G protein receptor association. It is not conclusive that G protein function is altered in this brain region also. The observed changes in the decreased affinity revealed by a shift to the low affinity when compared to control shows that, in diabetic state the G proteins induce α-$_2$ adrenergic receptors (R) that remain in a more in RLstate (low affinity) state than RH (High affinity) as an adaptation to the prevailing increased neurotransmitter content. The effect of sodium ions on the receptor affinity also underline the above finding. The shift to low affinity state is more evident in diabetic state than controls with sodium ions. The abnormal added effect of both compounds, in diabetic state is unexplained. The altered receptors affinity state and the complex interaction between the compounds Gpp[NH]p and Na^+ ions to a heterogeneous mixture of receptor population is attributed to such effects (Michel, 1980). In general, in diabetic state the receptor affinity of α-$_2$ receptors affected by guanine nucleotides are intact; but more shift to the low affinity state may be an adaptation to the altered neurotransmitter activity. The observed lowaffinity state of α-$_2$ receptors in brain stem can result in a reduced α-$_2$ receptor mediated responses from the brain stem.

The observation of serotonergic receptors of brain stem showed that tritiated serotonin identified sites of brain stem (S_1 type) showed a decreased affinity state. Generally the adrenergic and serotonergic responses from the diabetic brain stem decreased during diabetes. This may be due to down regulation brought about by the increased adrenergic and serotonergic out put observed within this region.

It has been shown that upper brain region cerebral cortex is well in communication with the lower brain regions (Paulose and Dakhinamurti, 1985). We had earlier discussed the noradrenergic and serotonergic nerve activity in cerebral cortex is increased during diabetes. Our results on the serotonergic receptors in cerebral cortex for high affinity S_1 receptors showed that S_1 high affinity receptors decreased in diabetic rats. The displacement data showed that in the high affinity regions ketanserin effectively displaced the ligand or it seem to be more potent displacing compound compared to controls. Ketanserin, an S_2 antagonist over the same range could not appreciably displace the tritiated serotonin in control rats. This indicate a heterogeneous population of receptors in diabetic cerebral cortex. The high affinity S_1 receptors estimated in Scatchard data may be indicating a decrease or switching over of serotonin receptors to a S_2 receptors containing population.

Phospholipase C (PLC) is an important enzyme having a function in many receptor mediated inositol pathways. The major receptors coupled to PLC pathway include α-$_1$ adrenergic, and 5-HT_2 & 5-HT_1c receptors and cholinergic receptors. The observed decrease in the PLC activity in hypothalamus may be attributed to an increased α-$_1$ adrenergic receptors. This is supported by our displacement analysis [^{3}H]NE binding using prazosin. But the observed change in the PLC activity may not be solely attributable to α-$_1$ adrenergic receptors alone. The S_2 class of receptors and cholinergic receptors may also be involved. The decreased total activity of PLC could be just an adaptation for the decreased receptor mediated signal transduction in diabetes or it may be possible that PLC, an enzyme with many forms, changed expression during diabetes (Casey and Gilman,1988). Our study clearly shows that phosphatidylinositol specific PLC activity is decreased in hypothalamus of diabetic rats. The first messengers of inositol phosphates and diacylglycerol pathway is PLC. The receptor coupling activate PLC and this lead to

a phosphatdylinositol break down mediated by PLC. The α-$_1$ adrenergic receptor changes of hypothalamus can also be due to a PLC affinity changes. The other major neurotransmitter involved in this pathway is acetylcholine (Berridge and Irvine, 1984). Diabetes is known to be associated with abnormalities of membranes. The membrane lipid constitution of peripheral nerves have been shown to be altered in diabetes. In our analysis of phospholipid content of diabetic brain hypothalamus using TLC the standard phosphtidylinositol comparable spot increased on visual observation. This support our data on enzyme activity showing decrease in activity. These results clearly argue that diabetic hypothalamus has large perturbations in the PLC mediated second messenger system.Thus our study of receptors and their second messengers associated with diabetic rat brain strongly support an altered signal transduction.

The insulin treatment and its effect on receptors

In hypothalamus the insulin treatment could not reverse the altered adrenoceptor parameters. In brain stem also α-$_2$ adrenoceptor parameter showed an increase in number compared to control (this parameter is not changed in diabetics) and reversal in the affinity is observed. These results indicate that α-$_2$ receptor affinity changes observed in both these regions are not reversed by insulin treatment. The insulin respond in a way that maximum compensating effects are produced. The insulin treatment data indicate an abnormal recovery, i.e. sometimes it bring out the reversal but otherwise with no effect. The insulin is a good hypoglycemic agent but external insulin and its ability to reverse the metabolic and receptor changes is reported to be defective (Katovich *et al.*,1993). Some authors found that insulin was able to partly normalize the altered β-adrenergic responsiveness associated with diabetic state. Our study also support this. The previous study from our lab on the effect of insulin on metabolic enzymes involved

in glucose metabolism in brain tissues showed that insulin reversal on the changed parameters of enzyme activity was not complete (Preetha *et al.*, in press.) A reversal trend is observed for the glutamate dehydrogenase activity in the brain with insulin treatment. In another set of studies an observation on ultrastructural changes in streptozotocin diabetic rats showed that insulin and other hypoglycemic agents were able to reverse the altered structural changes only partially (Das *et al.*, 1996; Seema *et al.*, 1996). In general, it may be concluded that although insulin is a good hypoglycemic agent, its ability to reverse the alteration at cellular level is partial. The possible reasons for this partial recovery may be either due to the insulin resistance or an alteration in the insulin receptors. Eventhough glucose levels are reversed to normal state by insulin treatment the metabolic disturbances and other brain changes are not reversed to near normal.

BRAIN NEUROTRANSMITTERS AND THEIR RECEPTORS HAVE A ROLE TO PLAY IN DIABETES

One of the major findings emerged from the present study was that norepinephrine conversion to epinephrine metabolic pathway is important indicator of any disturbance in the α-$_2$ receptor mediated pathway. Since this receptor is an inhibitory one at presynaptic sites, their regulation can affect the content of epinephrine. This is well demonstrated in our study.

The neurotransmitter changes studied over a period of time showed that their content varied over the same period. So for a comparison neurotransmitter contents of different days of analysis should take care of the day at which the animals were killed. Our study on neurotransmitter changes of serotonin content showed a pattern of decrease. The norepinephrine also showed an attenuated pattern of changes over a period of 7, 14 and 28 days. The neurotransmitter content estimated at the end of different periods can differ.

The noradrenergic activity increased and serotonergic activity decreased in the diabetic brain. The diabetic hypothalamus was reported to have altered responses in the release of pituitary hormones. Both adrenergic and serotonergic nerves are shown to be involved in this response. The studies using adrenodemedullation, stress and its effect on these two nervous system suggested that the noradrenergic neurons regulate the ACTH release positively while serotonergic neurons regulate negatively. In the diabetic state we observed changed affinity of receptors for agonists. These changes are due to an altered G protein regulation of α-$_2$L receptor state to α-$_2$H state. The serotonin receptors of hypothalamus increased without any change in the affinity. The displacement analysis data showed that serotonin receptors of diabetic hypothalamus are more of

S_1 class of receptors. The hypothalamic i.c.v. injection of, 8-OH-DPAT, a 5-HT1a receptor agonist mediated hypoinsulinemia and hyperglycemia possibly through the adrenal pathway (Bitar *et al.*, 1987). This effect was abolished by α-$_2$ specific antagonist (Chaouloff *et al.*, 1987). Our results show that in streptozotocin induced diabetic rats the α-$_2$ adrenergic and 5-HT1 serotonergic class of receptors are altered. This altered receptor function can have profound effects by changing the autonomic nervous activity and releasing hormones of hypothalamic neurohormonal cells. This may control the insulin function. A defective neuroendocrine system might bring about diabetic state.

The brain stem of diabetic rats showed an altered α-$_2$ adrenergic function. The receptor affinity was reduced in both α-$_2$ adrenergic and serotonergic receptors. Several autonomic nerves have their regulatory centres in the brain stem. The observed receptor affinity reduction can alter the autonomic function in diabetic rats.

The other important finding of our study was that cerebral cortex of diabetic rats contain more of S_2 receptors, which may lead to the altered hypothalamic function which in turn might affect the insulin function. The decrease in α-$_1$ adrenergic receptors of diabetic hypothalamus may be related to an altered phospholipase C activity in diabetes.

The receptor studies and neurotransmitter changes in the diabetic stage caught up a momentum when it was shown in genetically obese diabetic mice noradrenergic activity is altered in the brain regions (Garris 1990,1995). This is implicated as manifestations associated with the expression of obese gene. Also, obesity research and hyperglycemia research pointed to more involvement of genetic influence on hitherto far away processes like eating habits. The

hypothalamic lesions and its effect on hyperphagia and blood glucose regulation also have helped in understanding the role of brain in a diabetic context. The future research on this aspect should be directed more to the second messengers associated with these receptors and their physiological manifestations.

Let us conclude our discussion with a note on the hope that next century will see a place where diabetes related manifestations are better managed where neuronal control will be emphasized at the molecular level.

SUMMARY

1) The neurotransmitter content observed in hypothalamus of diabetic rats showed an increased adrenergic activity and a decreased serotonergic activity. The brain stem adrenergic activity remained the same except epinephrine content was increased. Serotonergic activity also was increased. The cerebral cortex of diabetic rats showed an increased adrenergic and serotonergic activity. The dopaminergic activity of corpus striatum showed an increased dopamine content. Serotonin and other amines of cerebellum remained unaffected by diabetes.

2) The α-$_2$ adrenergic receptors of hypothalamus showed an increased affinity. Serotonergic receptors of hypothalamus switched to more of S_1 receptors in diabetic rats.

3) The brain stem α-$_2$ adrenergic receptors and serotonergic receptors showed decreased affinity for their agonists during diabetes.

4) The cerebral cortex of diabetic rats showed more of S_2 receptors than their controls.

5) The guanine nucleotide regulated affinity changes in diabetic hypothalamus is impaired. This keep the diabetic hypothalamus α_2 adrenergic receptors in high affinity receptor state. An altered Gi function is proposed in diabetic rats.

6) The hypothalamic α-$_1$ adrenergic receptors increased in number and a decreased phospholipase C activity coincided with this change. These two changes may be related.

7) In the brain stem, the guanine nucleotide and sodium ion mediated affinity changes are intact but their effect on the α-$_2$ receptors to keep all the receptors in α-2Lstate may be an adaptation for the increased epinephrine content.

Together all the changes in the α-$_2$ receptor affinity with 5-HT$_1$ receptor increase in hypothalamus indicate a possible role for hypothalamic neurons in producing streptozotocin-induced diabetic state. But it is yet to clearly demonstrate that it was these changes that produce diabetes. It is also concluded that treatment with insulin did not reverse the neurotransmitters and their receptor changes. This clearly indicate that in diabetics the glucose level may reverse to normal with treatment of insulin, but the metabolic disturbances and behavioral changes affected is not reversed. A better cure is awaited in the coming years where brain neurotransmitter receptor-hormonal network will be given more importance.

REFERENCES

Akira, T., Hirohisa, I., Yasushi, I., Eiko, Ki and Yujui, M. Hypothalamic cholinergic and noradrenergic neurons in hyperglycemia induced by 2-deoxyglucose, Brain Research, 1994, 665(1), 13-17

Alvarez C., Blade, C. V., Cartana J., α-2 adrenergic blockade prevents hyperglycemia and hepaticglutathione depletion in nickel injected rats.,Toxicol. Appl.Pharmacol.,1993, 121,112-17.

Ansari, M.A., Gupta, G. and Baquer, N.I., β-adrenergic modulation of rat brain insulin receptor activity in normal and hyperglycemic conditions, Indian J. Exp. Biol. 1994, 32(2), 92-4.

Arborelino, L., Nomikos, G.G., Hacksell, U. and Svenson, T.H. (R)-8-OH-DPAT preferentially increases dopamine release in rat medial prefrontal cortex, Acta. Physiol. Scand. 1993, 148(4), 465-66.

Berridge,M. J., and Irvene R. F., Inositol Inositol Triphosphate, a novel second messenger in celluar signal transduction-review,Nature, 1984,312, 315-321.

Bestetti, G.E., Abramo, F., Locatelli, V. and Rossi, G.L. The reduction of circulating growth hormone and prolactin in streptozotocin-induced diabetic male rats is possibly caused by hypothalamic rather than pituitary changes, J. Endocrinol. 1995, 145(1), 1926.

Bellush, L.L., and Reid, S.G., Metabolic and neurochemical profiles in insulin-treated diabetic rats, Am. J. Physiol. 1994, 266(1 Pt.2) R87-R94.

Bhattacharya. S.K., and Saraswathi, M., Effect of intracerebroventricularly administered insulin on brain monoamines and acetylcholine in euglycemic and alloxan-induced hyperglycemic rats. Indian J. Exp. Biol. 1991, 29, 1095-100.

Bhimji, S., and McNeill, J.H. Isoproterenol-induced ultrastructural alterations in hearts of alloxan-diabetic rabbits. Gen. Pharmac. 1989, 20(4) 479-85.

Bitar, M.S., Koulu, M., and Linnoila, M. Diabetes-induced changes in monoamine concentrations of rat hypothalamic nuclei. Brain Research, 1987, 409(2), 236-42.

Bitar, M., Koulu, M., Rapoport, S.I., Linnoila, M. Diabetes-induced alteration in brain monoamine metabolism in rats. J. Pharmacol. Exp. Ther. 1986, 236,432-40

Bluet, P., Marie, T., Mounier, F., di Sciullo, A., Schmidt, B. and Kordon, C. Differential sites of action of 8-OH-DPAT, a 5HT1A agonist on ACTH and PRL secretion in the rat, Neuroendocrinology, 1995, 61(2), 159-66.

Borghini, I., Ania-Lahuerta, A., Regazzi, R., Ferrari, G., Gjinovci, A., Wolheim, C.B. and Pralong, W.F. α, βI, βII, δ and ε protein kinase C isoforms and compound activity in the sciatic nerve of normal and diabetic rats. J.Neurochem. 1994, 62(2), 686-96.

Brownstein, M., Neurotransmitters and hypothalamic hormones in the central nervous system. Fed. Proc.1977, 36, 1960-63 .

Bugajski, J., Gadek-Michalska and J.Borycz, Social crowding stress diminishes the pituitary adrenocortical and hypothalamic histamine response to adrenergic stimulation. J.Physiol. Pharmacol. 1993, 44(4), 447-56.

Campbell, J., in "Statistics for biologists."1987, Cambridge university press.

Casey P. J. and Gilman A.G., G Protein involvement in receptor - effector coupling. minireview., J. Biol. Chem., 1988, 263, 2577-80.

Chaouloff, F. and Jeanrenaud, B. 5HT1A and α_2 adrenergic receptors mediate the hyperglycemic and hypoinsulinemic effects of 8-hydroxy-2-(di-n-propylamino)tetralin in the conscious rats. J.Pharmacol. Exp. Ther. 1987, 243(3), 1159-1166.

Christine, J., Tancrede, G. and Nadeau,A. F., Effect of adrenaldemedullation and or physical training on glucose tolerance. Can. J. Physol. Pharmacol., 1993, 71, 667-73.

Christine J., Diminished diabetogenic effect of streptozotocin in adrenodemedullatted rats, Can. J. Physol. Pharmacol., 1994, 72, 992-8.

Chen, C.C. Effect of the duration of streptozotocin-induced diabetes on turnover of central biogenic amines in mice. Neuroendocrinology, 1992, 56(5), 629-32.

Chen, N.H. and Reith, M.E.A. Monoamine interactions measured by microdialysis in the ventral fegmental area of rats treated systematically with ± 8-OH-DPAT. J.Neurochem. 1995, 64(4), 1585-97.

Coscina, D.V. and DeRooy, E.C.H. Lack of synergistic feeding enhancement by systemic clonidine and 8-OH-DPAT. Pharmacol. Biochem. Behav., 1993, 44(4), 777-81.

Crandall, E.A. and Fernstrom, J.D. Acute changes in brain tryptophan and serotonin after carbohydrate or protein ingestion by diabetic rats. Diabetes, 1980, 29(6), 460-466.

Crandall, E.A. and Fernstrom, J.D. Effect of experimental diabetes on the levels of aromatic and branched-chain aminoacids in rat blood and brain. Diabetes, 1983, 32(3), 222-30.

Currie, P. J. and Wilson L. M., Yohimbine attenuates clonidine- induced feeding and macronutrient selection in genetically obese (ob/ob) mice. Pharmacol. Biochem. Behav., 1992, 43, 1039-46.

Dakshinamurti K., Paulose C.S., Viswanathan M., Siow, Y. L. and Vriend, J.,Thyroid function in pyrodoxine -deficient young rats.Ann. N. Y. Acad. Sci., 1990, 585, 128-44.

Dakshinamurti, K., Paulose C. S., Thilveris J. A. and Vriend, J. Thyroid function in pyrodoxine deficient young rats. J. Endocr. 1985,104, 339-44.

Dakshinamurti, K., Paulose, C. S., and Vriend, J. Hypothyroidism of hypothalamic origin in pyrodoxine deficient rats. J. Endocr., 1986, 109, 345-49.

Das, A. V., Padayatti, P. S., and Paulose, C. S., Effect of leaf extract of Aegle marmelose (L.) Correa ex Roxb. on histological and ultrastructural changes in

tissues of streptozotocin induced diabetic rats, Indian J. Exp. Biol., 1996,34, 341-45.

Debreceni, L., Hartmann, G. and Debreceni, B. Effect of morphine on hypothalamic catecholamine and serotonin level in relation to the stress-induced pituitary-adrenocortical activation in the rat. Exp. Clin. endocrinol. 1994, 102(4), 307-12.

Domino, S.E., Repaske, M.G., Bonner, C.A., Kennedy, M., Wilson, A.L., Brandon, S and Limbird, L.E. Synthesis of a yohimbine-agarose matrix useful for large-scale and micropurification of multiple α_2 receptor subtypes. in Meth. Enzymol. 1992, 215, Academic Press.

Eleftheriou, B.E., A gene influencing hypothalamic norepinephrine levels in mice, Brain Research, 1974, 70, 538-40.

Feldman, R. and Limbird,L. E., Biochemical characterisation of human adrenergic receptors. in "Adrenergic receptors in man" edt. by Insel, P. A., Marcell Dekker Inc., NewYork and Basel, 1987, 161-260.

Finco, C., Abbracchio, M.P., Malosio, M.L., Cattabeni, F., DiGiulio, A.M., Paternieri, B., Mantegazza, P. and Gorio, A. Diabetes-induced alterations of central nervous system G-proteins: ADP-ribosylation, immunoreactivity, and gene expression studies in rat striatum. Mol. Chem. Neuropathol. 1992, 17(3), 259-72.

Furman, B. L., The hypoglycemic effect of 5-hydroxytryptophan. Br. J. Pharmacol., 1974, 50, 575-80.

Furman, B. L. and Wilson, G. A., Further studies on the effects of 5-hydroxytryptophan on plasma glucose and insulin in the mouse. Diabetologia,1980, 19, 386-90.

Frankish, H.M., McCarthy, H., David, D.S., Kilpatrick, A. and Williams, G. Neuropeptide Y receptor numbers are reduced in the hypothalamus of streptozotocin-induced and food-deprived rats: further evidence of increased activity of hypothalamic NPY-containing pathways. Peptides (Pergamon), 1993, 14(5), 941-48.

Gando, S., Pharmacological studies an alterations in myocardial β-adrenoceptors and their intracellular signal transductions in experimental diabetic rats. Hokkaido Igaku-Zasshi, 1994, 69(5), 1140-53 (Japan).

Ganguly, P.K., Beamish, R.E., Dhalla, K.S., Innes, I.R. and Dhalla, N.S. Norepinephrine storage, distribution and release in diabetic cardiomyopathy, Am. J. Physiol. 1987, 252 (Endocrinol. Metab. 15): E734-E739.

Ganguly, P.K., Dhalla, K.S., Innes, I.R., Beamish, R.E. and Dhalla, N.S. Altered norepinephrine turnover and metabolism in diabetic cardiomyopathy, Circ. Res. 1986, 59(6) 684-93.

Garcia-Barrado, M.J., Reverte, M. and Moratinos, J. Role of Ca^{2+} channel blockers in insulin secretion resulting from α_1- and β-adrenoceptor stimulation in the rabbit. European J. Pharmacol. 1992, 219, 461-464.

Garris, D.R., Developmental and regional changes in brain norepinephrine (NE) levels in diabetic C57BL/K_SJ mice; effects of estradiol and progesterone. Dev. Brain. Res., 1995, 89(2), 314-19.

Garris. D. R., Age and diabetes-associated alterations in regional brain norepinephrine concentrations and adrenergic receptor populations in C57BL/K_SJ mice. Dev. Brain Res., 1990, 51, 161-166.

Gagliardino,J. J., Zieher,L. M., Iturriza F. C., Hernandez R. E.and Rodrigurz, R.R., Insulin release and glucose changes induced by serotonin. Horm. Metab.Res.,1971, 3, 145-50.

Gawler, D., Milligen, G., and Houslay, M. D., Treatment of streptozotocin-diabetic rats with metformin restors the ability of insulin to inhibit adenylate cyclase activity and demonstates that insulin does not exert this action through the inhibitory guaninie nucleotide regulatory protein Gi Biochem. J. 1988, 249, 537-42.

Gawler,D., Milligen G., Spiegel, A. M., Unson, C. G., and Houslay, M. D., Abolishan of the expression of inhibitory guanine nucleotide regulatory protein Gi activity in diabetes.,1987, 327, 229-32.

Geynet, P., Ferry, N., Borsodi, A. and Honoune, J. Two distinct α_1 adrenergic receptor sites in rat liver: differential binding of (-)-[^{3}H]norepinephrine, [^{3}H]Prazosin and [^{3}H]dihydroeneryocryptine: effects of guanine nucleotides and proteolysis; implication for a two site model of α-receptor regulation. Euro. J. Pharmacol. 1981, 30(12), 1665-1675.

Glowinski, J. and Iversen, L.L. Regional studies of catecholamines in the rat brain. The disposition of [^{3}H]norepinephrine [^{3}H]dopamine and [^{3}H]dopa in various regions of the brain. J. Neurochem. 1966, 13, 655-669.

Goyal, R.K., Rodrigues, B. and McNeill, J.H. Effect of Tri-iodothyronine on cardiac responses to adrenergic-agonists in STZ-induced diabetic rats. Gen. Pharmac. 1987, 18(4), 357-62.

Grignashi, G., Mantelli, B. and Samanin, R., The hypophagic effect of restraint sterss in rats in rats can be mediated by 5-HT2 receptors in the paraventricular nucleus of the hypothalamus. Neurosci. Lett., 1993, 152, 103-6.

Guillot, E., Coste, A., and Angel, I., α_2-adrenoceptors and the regulation of glucose, insulin and amylin levels in diabetic rats. Life Sci. 1995, 57(33), 2081-90.

Gurcharan, K. and Sukhwinder, L.S. Effect of alloxan-induced diabetes on Na^+/K^+-ATPase activity from discrete areas of the rat brain. Biochem. Mol. Biol. Int.1994, 34(4), 781-88.

Heaton, J.P.W. and Varrin, S.J. Effects of streptozotocin induced diabetes on dopaminegic functioning in the rat: Analysis of yawning behaviour Pharmacol., Biochem. Behav. 1993,44(3), 601-4.

Hermenegildo, C., Raya, A., Roma, J. and Romero, F.J. Decreased glutathione peroxidase activity in sciatic nerve of alloxan-induced diabetic mice and its correlation with blood glucose levels. Neurochem. Res. 1993, 18(8), 893-6.

Hery, F., Rouer, E., Glowinski, J. Daily variation of serotonin metabolism in the rat brain. Brain Res. 1972, 43, 445-55.

Hirose, H., Role of adrenergic receptors in the regulation of pancreatic hormone secretion, Keio Igaku, 1993, 70, 325-36.

Hirose, H., Maruyama,H., Ito,K., Kido,K., Koyama,K. and Saruta,T., Effects of α2 and β-adrenergic agonism on glucagon secretion from perfused pancreata of normal and streptozotocin -induced diabetic rats,Metab. Clin. Exp., 1993, 42, 1072-8

Hirose, H., Maruyama, H., Ito, K., Koyama,K., Kido,K. and Saruta, J., Glucose - induced insulin secreation and α2- adrenergic receptor subtypes. J. Lab. Clin. Med. 1993b, 121, 32-7.

Hjorth, S., Coulson, A., Limberg, P., Sanchez,D., Wikstrom,H., Devidson, L. E., Hacksell,V., and Nileson, J. L. G., 8-hydroxy-2-(di-n-propylamino) tetralin,8-OH-DPAT, a potent and selective simplified ergot congenes with central 5-HT receptor stimulatory activity. J. Neural. Transm., 1982, 55, 169-88.

Ho, K., Hirose, H., Maruyama, H., Fukumachi, S., Tashiro, Y. and Saruta, T., Neurotransmitters partially restore glucose sensitivity of insulin and glucagon secretion from perfused pancreas in streptozotocin-induced diabetic rats. Diabetologia,1995, 38(11), 1276-84.

Hiyoshi, Y., Miura, H., Uemura, K., Endo, H., Ozawa, K., Maeda, N., Tamagawa, T. and Iguchi, A. Effects of imidazoline antagonists of α_2 adrenoceptors on

endogenous adrenaline-induced inhibition of insulin release. Eur. J. Pharmacol. 1995, 294(1), 117-23.

Hoffman, B.B., Michel, T., Kilpatrick, D.M., Lefkowitz, R.J., Tolbert, M.E.M., Gilman, H. and Fain, J.N. Agonist versus antagonist binding to α-adrenergic receptors, Proc. Nat. Acad. Sci. USA, 1980, 7(8), 4569-4573.

Hoffman, B.B., Kilpatrick, D.M and Lefkowitz, R.J., Desensitisation of β-adrenergic stimulated adenylate cyclase in turky erythrocytes. J.Cyclic nucleotide Res.1979,5(5),355-66.

Holden, R.J. and Mooney, P.A. Schizophrenia is a diabetic brain state: an elucidation of impaired neurometabolism. Med. Hypotheses, 1994, 43(6), 420-35.

Hoyer, D., Boddeke, M. and Schoeffter, P. Second messenger in the definition of 5-HT receptors in "Molecular Biology, Receptors and Functional effects ed. by Fozard, J. R. and Saxena P. R. 1992, Birkhausen Verlag bBasel / Switzerland.

Ido, Y., Orlfalian, Z., Kilo, C., Corr, P.B. and Williamson, J.R. Neuronal dysfunction and metabolic imbalances in diabetic rats: prevention by acety-L-carnitine, Diabetes, 1994, 43(12), 1469-77.

Iverson,J., Adrenergic receptors and the secretion of glucagon and insulin from perfused canine pancreas, Eur. J. Pharmacol., 1986, 129, 347-52.

Jakobs, K. H., Inhibition of adenylate cyclase by hormone and neurotransmitters, Mol. Cell. Endocrinol.1979, 16,147-156.

James, G. M. and Hodgson, W. C., Attenuated 5-HT2 receptor mediated responses in hind quarters of diabetic rats. Eur. J. Pharmacol., 1995, 294, 109-15.

Jamnicky, B., Muck-Seler, D. and Slijepcevic, M. Favourable effects of tryptophan / insulin treatment on serotnergic imbalance in alloxan diabetic rat. Comp. Biochem. Physiol., A Comp. Physiol. 1993, 105, 267-73.

Jean, C., Roy, D. and Nadeau, A. Diminished diabetogenic effect of streptozotocin in adrenodemedullated rats. Can. J. Physiol.Pharmacol. 1994, 72(9),992-8.

Jung, J.S., Hwangs,H.S., Myung,B., Song, D.K., Kim, Y.S. and Kin, Y.H. glucose modulation of release of endogeneous catecholamines from hypothalamic fragments in vitro,Korean J. Pharmacol.1993, 29(2),183-8.

Kamei, J., Saitoh, A., Iwamotor, Y., Funada, M., Suzuki, J., Misawa, M., Nagase, H., and Kasuga, Y. Effects of diabetes on spontaneous locomotor activity in mice. Neuro Sci. Lett., 1994, 178(1), 69-72.

Karasu, C., Dewhurst, M., Stevens, E.J., and Tomlinson, D.R. Effects of antioxidant treatment on sciatic nerve dysfunction in streptozotocin-diabetic rats, comparison with essential fattyacids. Diabetologia,1995, 38(2), 129-34.

Katovich, M. J., Marks, K. S. and Sninsky,C. A. Effect of insulin on the altered thyroid function and adrenergic responses in the diabetic rat. Can. J. Physiol. Pharmacol. 1993, 71, 568-75.

Kirchgessner, A.L. and Gershon, M.D., Presynaptic inhibition by serotonin of nerve mediated secretion of pancreatic amylase. Am. J. Physiol., 1995, 268(23 Pt.1), G-339-G-345.

Kurose, T., Tsuda, K., Ishida, H., Tsuji, K., Okamoto, Y., Tsuura,Y., Kato, S., Usami, M., Imura, H. and Seino, Y. Glucagon, insulin and somatostatin secretion in response to sympathetic neural activation in streptozotocin-induced diabetic rats. A study with isolated perfused rat pancreas in vitro, Diabetologia, 1992, 35(11), 1035-41.

Krug, E. L., and Kent,C., Assay for Phospholipase C in Methods in Enzymology, 1984, 72, 347-51.

Lackovic, Z., Salkovic, M., Kuci, Z. and Relja, M. Effect of long-lasting diabetes mellitus on rat and human brain monoamines, J. Neurochem. (1990), 54, 143-47.

Lass, P. and Krudsen, G. Cerebral blood flow response to propranolol in steptozotocin diabetic rats. Neuro Report, 1990, 1(3-4), 232-34.

Levin, B.E. and Planas, B. Defective glucoregulation of brain α_2 adrenoceptors in obesity-prone rats. Am. J. Physiol. 1993, 264 (2 Pt.2) R305-R311.

Lim, P.K. and Lee, K.M. Changes in the distribution of dopamine and its metabolite in streptozotocin-induced diabetic rat striatum. Arch. Pharmacol. Res. 1995, 18(4), 271-76.

Locatelli, V., Miyoshi, H., Bestetti, G., Rossi, G.L. and Muller, E.E. Effect of growth hormone-releasing stimuli in streptozotocin diabetic rats, Brain Research, 1985, 341(1), 35-40.

Loewy, A.D., Franklin, M.F. and Haxhiu, M.A. Central nervous system monoamine cell groups projecting to pancreatic vagal motor neurons: a transneuronal labelling study using pseudorabies virus. Brain Res.1994, 638 (1-2), 248-60.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, J. Protein measurement with Folin Phenol reagent. J. Biol. Chem. 1951, 193, 265-75.

Lorden, F.L., Oltmans, G.A. and Margules, D.L. Central catecholamine levels in genetically obese mice (Obob and dbdb), Brain Research, 1975, 96, 390-94.

Maeda, K., Yasuda, H., and Shigeta, Y. Modulation of diabetic Na^+/K^+-ATPase activity independent of myo-inositol: possible role of cAMP in regulation of nerve Na^+/K^+ATPase activity. Tonyobyo (Tokyo) 1993, 36(1), 25-32 (Japan) Chem. Abstr. Vol.122(3) January 16, 1995.

Mans, A.M., Regina de Joseph, M., Davis, D.W. and Hawkins, R.A. Regional amino acid transport into brain during diabetes: effect of plasma amino acids. Am. J. Physiol.1987, 253 (Endocrinol. Metab. 16), E575-E583.

Martin, F. J., Miguez, J. M., Aldegunde, M. and Atienza, A., Effects of streptozotocin-induced experimental diabetes and insulin therapy on platet serotonin uptake and volume, Biog. Amine., 1995, 11, 77-85.

Martin, F. J., Miguez, J. M., Aldegunde, M. and Atienza,G., Effect of streptozotocin-induced diabetes mellitus on serotonin measures of peripheral tissues in rats, Life Sci., 1995,56, 51-9.

Martin, P., Sonia, C., William, V.T., Rjajat, B., Michael, T., Wylie, V., Jean, R. and Robert, S.S. Pituitary response to growth hormone releasing hormone in IDDM; Abnormal response to insulin and hyperglycemia, Diabetes,1992, 41,17-21.

Mathew J. and Eichberg, J., Guanosine-5'-(3-O-thio)triphosphate mediated stimulation of phosphoinositidase C in solubilized rat peripheral nerve myelin and its alteration in streptozotocin induced diabetes. J. Neurosci. Res. 1994, 37(1), 83-91.

Mefford, I.N. Are there epinephrine neurons in rat brain? Brain Res.,1987, 12, 383-395.

Michel,T.M.,Hoffman,B.B.,and Leftkowitz, R.J. Differintial regulation of the α_2-adrenergic receptor by Na^+ and guaninenucleotides. Nature.1980, 288, 709-11.

Mongeau, R., DeMontigny, C. and Blier, P., Activation of $5HT_3$ receptors enhances the electrically evoked release of [^{3}H]NE in rat brain limbic structures.Eur. J. Pharmacol. 1994, 256(3), 269-79.

Mooradian, A.D. Diabetic complications of the central nervous system. Endocr. Rev.1988, 9(3), 346-56.

Mooradian, A.D. and Scarpace, P.J. β-adrenergic receptor activity of cerebral microvessels in experimental diabetes mellitus. Brain Res. 1992, 583, 155-160.

Moratinos, J., Potter, D.E. and Ellis, S. The influence of propranolol on catecholamine-induced changes in carbohydrate metabolism in the rabbit. European J. Pharmacol. 1975, 32, 186-94.

Nowak, T.V., Castelaz, C., Ramaswamy, K., and Weisbruch, J.P., Impaired rodent vagal nerve sodium-potassium-ATPase activity in streptozotocin diabetic rats. J.Lab. Clin. Med., 1995,125(2), 182-86.

Oda, S., Tsuda, T., and Sasaki, Y. Adrenergic effects on pancreatic glucagon and insulin secretions in rabbits, Tohoku, J. Agric. Res. 1994, 45(1-2), 29-35.

Ohukuwa,T., Sat, Y., and Naoi, M., Hydroxyl radical formation in diabetic rats induced by streptozotocin. Life sci., 1995, 566, 1789-98.

Pacak, K., Kvestnansky, R., Palkovits, M., Fukuhara, K., Yadid, G., Kopin, I. and Goldstein, D.S. Adrenalectomy augments in vivo release of norepinephrine in the paraventricular neurons during immobilisation stress. Endocrinology, 1993,133(3), 1404-1410.

Paulose, C. S., Dakshinamurti, K., Packer, S. and Stephens, N. L. Sympathetic stimulation and hypertension in pyridoxine deficient adult rat. Hypertension, 1988, 11, 387-91.

Paulose C. S. and Dakshinamurti,K., Enhancement of high affinity γ- aminobutyric acid receptor binding in cerebellum of pyridoxine deficient rats. Neurosci. Lett. 1984. 48, 311-16.

Paulose, C. S. and Dakshinamurti, K. Effect of pyridoxine deficiency in young rats on high- affinity serotonin and dopamine receptors. J. Neurosci. Res., 1985, 14, 263-70.

Paulose, C.S. and Dakshinamurti, K. Effect of pyridoxine deficiency in young rats on high- affinity serotonin and dopamine receptors, J. Neurosci. Res. 1985, 14, 263-270.

Park, E.J. and Jhon, G.J. Ganglioside analysis in alloxan-induced diabetic rat brain, Korea Biochem. J. 1993, 26(6), 340-46.

Preetha, N., Padayatti, P. S., Asha, A. Sudha, B., Raghu, K. G. and Paulose,C.S., Glutamatedehyrogenase induction in the brain of streptozotocin-diabetic rats. Indian J. Biochem. Biophys. (1996, in press).

Pedigo, N.W., Yamamura, H.I. and Nelson, D.L. Discrimination of multiple [^{3}H]-5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain, J. Neurochem. 1981, 36, 220-226.

Pekinar, Can, Culum, N.A., Hughes, J.N., Hargreaves, A.J., Mabon, J., Cason, I.F. and Machean, W.G. Glycation of brain actin in experimental diabetes. J. Neurochem. 1993, 61(2), 436-42.

Potter, D.E., Moratinos, J. and Ellis, S. Metabolic responses to isoproterenol and epinephrine in the rabbit. Influence of state of nourishment, alloxan diabetes and pretreatment with propranolol. Biochem. Pharmac. 1977, 26, 1065-69.

Powell, A.M., Sherwin, R.S. and Shulman, G.I. Impaired hormonal responses to hypoglycemia in spontaneously diabetic and recurrently hypoglycemic rats. Reversibility and stimulus specificity of the deficits. J. Clin. Invest.1993, 92(6), 2667-74.

Repaske, M.G., Nunnari, J.M. and Limbird, L.E. Purification of the α_2 adrenergic receptor from porcine brain using a yohimbine-Agarose affinity matrix. J. Biol. Chem. 1987, 262(25) 12381-12386.

Reverte, M., Gracia-Barrado and Moratinos, J. Metabolic effects derived from α_1 and α_2 adrenoceptor stimulation. Adrenoceptors; structure, mechanisms, function in Advances in Pharmacological sciences, 1991, Birkhauser, Verlag Basel.

Rosella, M., Lucila, A.G., Salvatore, M. and Pierluigi, G. Experimental diabetic neuropathy: Impairment of slow transport with changes in axon cross-sectional area. Proc. Natl. Acad. Sci. USA,1985, 82, 7716-20.

Rothwell, N.J. and Stock, M.J., A role for insulin in the diet-induced thermogensis of cafeteria-fed rats. Metabolism,1981, 30, 673-678.

Ross,F. and Limbird,L.E., Biochemical characterisation of human adrenergic receptors in "Adrenergic receptors in man" Edted. by Insel, P. A., Marceldecker Inc., Newyork and Basel, 1987, 161-99.

Routh, V.H., Hamilton, J.S., Stern, J.S. and Horwitz, B.A. Litter size, adrenalectomy and high fat diet alter hypothalamic monoamines in genetically lean (Fa/Fa) Zucker rats. J. Nutr. 1993, 123(1) 74-84.

Saphier, D. Adrenoceptor regulation of paraventricular nucleus neuronal activity as related to hypothalamo-pituitary-adrenocortical responses. Stress proc. Int. Symp. Catecholamines other neurotransm. Stress, 5th 1991 (Pub. 1992), 1, 481-88. Edited by Kvetnansky, R., Mccarty, R. and Axelrod, J. gordon Breach, Philadelphia, Pa.

Sasaki, S. and Bunag, R.D. Insulin reverses hypertension and hypothalamic depression in streptozotocin diabetic rats, Hypertension, 1983, L5, 34-40.

Satoshi, G. Ynichi, H. and Moriso, K. Altered cardiac adrenergic neurotransmission in streptozotocin-induced diabetic rats. Br. J. Pharmacol. 1993, 109(4), 1276-81.

Scatchard, G., The attraction of proteins for small molecules and ions. Ann. N. Y. Acad. Sci., 1949, 51, 660-72.

Schimdt, Robert, E., Plurad, Santiago, B., Sherman, William, R., Williamson, Joseph, R., Tilton, Ronald, G. Effects of aldose reductase inhibitor sorbinil on neuroaxonal dystrophy and levels of myo-inositol and sorbitol in sympathetic autonomic ganglia of streptozocin-induced diabetic rats. Diabetes 1989, 38(5), 569-79 (Eng.).

Schmidt, R.E., Dorsey, D.A., McDaniel, M.L. and Corbett, J.A. Characterisation and NADPH diaphorase activity in rat sympathetic autonomic ganglia-effect of diabetes and aging. Brain Res.,1993, 617(2), 343-48.

Schneider, U., Niedermeier, W. and Grafe, P. The paradox between resistance to hypoxia and liability of hypoxic damage in hyperglycemic peripheral nerves, evidence for glycolysis involvement. Diabetes,1993, 42(7), 981-87.

Seema, P. V., Sudha, B., Padayatti, P. S., Asha, A., Raghu, K. G. and Paulose, C. S., Kinetic parameters of purified malate dehydrogenase in liver of streptozotocin-diabetic rats and the effect of leaf extract of *Aegle marmelose* (L.) correa ex Roxb., Indian J. Exp. Biol. (July, 1996, in press).

Shindo, H., Tawata, M. and Onaya, T. Reduction of cyclic AMP in the sciatic nerve of rats made diabetic with streptozotocin and mechanism involved. J. Endocrinol. 1993, 136(3), 431-38.

Shindo, H., Tawata, M. and Onaya, T. Cyclic adenosine 3',5'-monophosphate enhances sodium, potassium-adenosine triphosphatase activity in the sciatic nerve of streptozotocin-induced diabetic rats. Endocrinology (Baltimore), 1993, 132(2), 510-16.

Sima, A.A.F. and Chakrabarti, S., Acarbose slows the progression of neuropathy in the diabetic BB/wor rat. Drugs Dev. 1993, 1(α-glucosidase inhibition: potential use in diabetes), 219-25.

Smythe, G.A. and Edwards, S.R. Suppression of central noradrenergic neuronal activity inhibits hyperglycemia. Am. J. Physiol. 1992, 263 (5 pt.1), E823-E827.

Smythe, G.A., Bradshaw, J.E. and Vining, R.F. Hypothalamic monoamine control of stress-induced adrenocorticotropin release in the rat. Endocrinology, 1983, 113, 1062-1071.

Smith, S. A. and Pogson, C. I., Tryptophan and the control of plasma glucose concenterations in the rat, Biochem. J., 1977, 168, 495-506.

Stevens, E.J., Carrington, A.L. and Tomlinson, D.R. Nerve ischemia in diabetic rats: time course and development, effect of insulin treatment plus comparison of streptozotocin and BB models. Diabetologia, 1994, 37(1) 43-48.

Strassheim, D., Milligan, G. and Houslay, M.D. Diabetes abolishes the GTP-dependent, but not the receptor-dependent inhibitory function of the inhibitory gnanine-nucleotide-binding regulatory protein (Gi) on adipocyte admylate cyclase activity. Biochem. J. 1990, 266, 521-526.

Stewart, J.K., Campbell, T.G., and Gbadebo, T.D., Narasimhachari, N. and Manning, J.W. Cardiovascular responses and central catecholamines in streptozotocin diabetic rats. Neurochem. Int. 1994, 24(2), 183-89.

Sugimoto, Y., Yamada, J., Kimura, I., Watanabe, Y., and Horisaka, K. Inhibitory effects of tryptamine on tolbutamide-induced hypoglycemia in mice: mediation by 5-HT receptors. Neurochem.Res. 1994, 19(1), 19-22.

Sutherland, R.G., James, P, Sutherland, E., Tyson, R., Pai, F., Kozlowski, P. and Saunders, J.K. Forebrain Ischemia in diabetic and nondiabetic BB rats studied with ^{31}P magnetic resonance spectroscopy, Diabetes, 1992, 41, 1328-34.

Suzuki, K., Matsumoto, M., Toyota, T. and Goto, Y. Effect of gangliosides on the peripheral nerve impairment in diabetic G.K.rat Tonyobyo Dobutsu, 1991, 5, 181-86.

Takao, K., Nagatani,T., Kitamura,Y., Kawasaki,K., Hugakawa, H. and Yamawaki, S., Chronic forced swim stress of rats increases frontal cortical 5-HT2 receptors and

wet dog shakes they mediate but not frontal cortical β-adrenoceptors. Eur. J. Pharmacol., 1995, 294, 721-6.

Takahashi, A., Ikarashi, Y., Ishimaru, H. and Maruyama, Y. Compensation between sympathetic nerves and adrenal medullary activity: effects of adrenode-medullation and chemical sympathectomy on catecholamine turnover. Life Sci. 1993, 53(20) 1567-72.

Tian, Y., Eaton, M.J., Goudrean, J.L., Lookingland, K.J. and Moore, K.E. Neurochemical evidence that 5-Hydroxytryptaminergic neurons tonically inhibit noradrenergic neurons terminating in the hypothalamus. Brain Res. 1993, 607(1-2), 215-21.

Tsai, B.S. and Leftkowitz, R.J.Agonist-specific effects of guanine nucleotides on α-adrenergic receptors in human platets. Mol.Pharmacol. 1979, 16, 61-68.

Viswanathan, M., Paulose,C. S., Lal, K. J., Sharma, S. K. and Dakshinamurti, K., Alterations in brain stem α2-adrenoreceptor activity in pyrodoxine-deficient rat model of hypertension.,Neurosci. Lett., 1990, 111, 201-5.

Williams, R.S., Schaible, T.F., Scheuer, J. and Kennedy, R. Effects of experimental diabetes on adrenergic and cholinergic receptors of rat myocardium. Diabetes, 1983, 32: 881-86.

Xiang, H. and McNeill, J. H. Protein kinase C activity is altered in diabetic rat hearts. Biochem. Biophys. Res. Commun., 1992, 187, 703-10.

Yagihashi, S. and Sima, A.A.F. Diabetic autonomic neuropathy, the distribution of structural changes in sympathetic nerves of BB rat. Am. J. Pathol. 1985, 121, 138-147.

Yamada, J., Sugimoto, Y., Ikuho, K., Yoshiko, W. and Horsaka, K. Effects of tryptamine on plasma glucagon levels in mice, Neurochem. Res., 1994, 19(1), 15-18.