BRAIN ADRENERGIC AND SEROTONERGIC RECEPTOR FUNCTION IN STREPTOZOTOCIN-DIABETIC RATS

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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.

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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.

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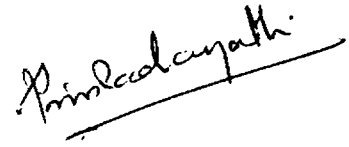

ABBREVIATIONS USED IN THE TEXT

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