ADRENERGIC RECEPTORS AND MONOAMINES IN THE BRAIN AND PANCREATIC ISLETS OF STREPTOZOTOCIN DIABETIC RATS: ROLE IN INSULIN SECRETION AS A FUNCTION OF AGE

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By

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CERTIFICATE

This is to certify that the thesis entitled **"Adrenergic receptors and monoamines in the brain and pancreatic islets of streptozotocin diabetic rats: Role in insulin secretion as a function of age"** is a bonafide record of the research work carried out by Ms. **ASHA ABRAHAM** 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.

Kochi-22
30/03/1998

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DECLARATION

I hereby declare that this thesis entitled **"Adrenergic receptors and monoamines in the brain and pancreatic islets of streptozotocin diabetic rats: Role in insulin secretion as a function of age"** has not previously formed the basis of any degree, diploma, associateship or other similar titles or recognition.

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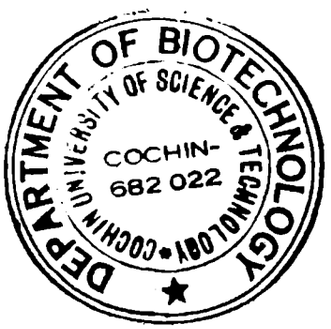

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(ASHA ABRAHAM)

ABBREVIATIONS USED IN THE TEXT

AChE	Acetyl Choline Esterase
ACTH	Adrenocorticotropic Hormone
ADM	Adrenomedullation
ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
B_{max}	Binding Maximum
BS	Brain Stem
cAMP	Cyclic Adenosine Mono Phosphate
CB	Cerebellum
CC	Cerebral Cortex
CNS	Central Nervous System
CRF	Corticotrophic Releasing Factor
CRH	Corticotrophic Releasing Hormone
CS	Corpus Striatum
DA	Dopamine
EPI	Epinephrine
G-protein	Guanine Nucleotide Binding Protein
GABA	Gamma Amino Butyric Acid
GAD	Glutamic Acid Decarboxylase
GTP	Guanosine Triphosphate
5-HIAA	5-Hydroxy Indole Acetic Acid
HPLC	High Performance Liquid Chromatography
5-HT	5-Hydroxy Tryptamine
5-HTP	5-Hydroxy Tryptophan
HVA	Homovanillic Acid
IDDM	Insulin Dependant Diabetes Mellitus
K_d	Dissociation Constant

MIF	Macrophage Migration Inhibiting Factor
mRNA	Messenger Ribonucleic Acid
MSH	Melanocyte Stimulating Hormone
NADH	Nicotinamide Adenine Dinucleotide, Reduced Form
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
NE	Norepinephrine
NIDDM	Non-Insulin Dependant Diabetes Mellitus
NMN	Normetanephrine
p	Level Of Significance
Pi	Inorganic Phosphate
PKC	Protein Kinase C
PLC	Phosph olipase C
POMC	Pro-Opio-Melanocorticotropin
S.E.M.	Standard Error Of Mean
STZ	Streptozotocin

CONTENTS

Introduction

INTRODUCTION

Ageing is characterised by progressive impairment of bodily activities. Normal human ageing is associated with a progressive impairment of glucose tolerance (Davidson 1979). Total glucose stimulated insulin secretion has been described as being unchanged (Draznin *et al.*, 1985), suppressed (Molina *et al.*, 1985) or increased (Curry *et al.*, 1984) as an animal ages. Recently it was demonstrated in Wistar rats that ageing is indeed associated with progressive decline in beta cell number, the pancreatic insulin content, amount of insulin secreted and insulin mRNA levels (Perfetti *et al.*, 1995). Impairment of insulin action as a function of age has also been reported. There is an impairment of insulin induced glucose disposal in old compared with young subjects (Haruo *et al.*, 1988). Few laboratories have attributed the alterations in glucose stimulated insulin secretion with age to changes in diet rather than ageing, *per se* (Hara *et al.*, 1992).

Insulin secretion by pancreatic β-cells is influenced by a number of humoral factors such as metabolic substrates, together with systemically or locally released hormones and neural factors. Basal insulin release is partially regulated by the sympatho-adrenal system and species difference exists (Ahren *et al.*, 1981). Noradrenaline and adrenaline inhibit the secretion of insulin when administered *in vivo* (Porte, 1967), via the activation of α-and β-adrenergic receptors (Loubatieres *et al.*, 1977). The inhibition of insulin release is mediated mainly through α_{2A}-adrenergic receptors (Ullrich & Wollheim, 1985; Niddam *et al.*, 1990; Oda *et al* .,1991; Lacey *et al.*, 1993) present in the pancreatic islets, which are linked to adenylate cyclase, resulting in reduction in the content of cAMP (Yamazaki *et al.*,1982). Stimulation of insulin release is mainly affected through β_2-adrenergic receptors (Lacey *et al* .,1996) and β_3-adrenoceptors (Atef *et al.*, 1996). Monoamines like serotonin and dopamine were also found to inhibit glucose induced insulin secretion and $^{45}Ca^{2+}$ net uptake in islets (Lindstrom *et al.*, 1983; Zern *et al.*, 1980).

Central nervous system neurotransmitters and their receptors play an important role in the pancreatic hormone secretion and glucose homeostasis. Plasma glucose levels

appears to be under separate serotonergic and dopaminergic control exerted via 5-HT_{1A} and DA-D_3 receptors respectively (Alster & Hillegaart, 1996). Serotonin induced increase in insulin levels appear to be modulated by 5-HT_{1C} and 5-HT_2 receptors (Sugimoto *et al.*, 1996; Chaouloff *et al.*, 1990). CNS via noradrenergic pathways can cause hyperglycaemia by the activation of sympatho-adrenal system (McCaleb & Myers, 1982).

There is progressive increase in adrenal medullary hormones (Veith *et al.*, 1986) and glucocorticoids with age (Slotkin, *et al.*, 1996). The activity of tyrosine hydroxylase, which is a key enzyme in catecholamine biosynthesis, increases during stress conditions, resulting in increase in catecholamine levels (Thoenen, 1970). Central catecholamines play a stimulatory role on the secretion of adrenocorticotrophic hormone (ACTH) through an action at the hypothalamic level, particularly via the release of CRF (Giullaume *et al.*, 1987). It has been demonstrated that adrenaline, acting via α_1- and β-adrenergic receptors or noradrenaline acting via α_1-receptors induce stress like ACTH surges in rats (Szafarczyk *et al.*, 1987) and the effect may be mediated via CRH-41 (Plotsky, 1987) and vasopressin neurones (Hiwatari & Johnston, 1985; Al-Damluji *et al.*, 1990). ACTH from the brain influences the adrenal cortex to secrete corticosteroids. Glucocorticoids are known to increase insulin resistance in type II diabetes (Brindley & Nang, 1996). It has also been reported that glucocorticoids up-regulate α_{2R}-receptor expression and signalling in pancreatic β-cells (Hamamdzic *et al.*, 1995). Neurotransmitters and their receptors undergo age related changes. An increase in α_2-receptors in the platelets of elderly subjects have been reported (Davis & Silski, 1987). Adult pancreatic islets of hamsters exhibited higher number of α_2-adrenoceptors than young while, the dissociation constant (the affinity) did not change (Lacombe *et al.*, 1993). There is also a suggestion that adrenergic system may play a greater role in the regulation of insulin release from neonatal rats than from adult rats (Gembal & Wojcikowski, 1993). In the adipocytes of Fisher 344 rats the mRNA of β_1, β_2 and β_3-adrenergic receptors declined as a function of age (Gettys *et al.*, 1995). The levels of β-adrenergic receptors were also reported to decline in various brain regions during the ageing process (Paulose *et al.*, 1982). Misra *et al.*, (1980) have

reported that in the cerebellum, β_1-adrenergic receptors increased and β_2-adrenergic receptors decreased in older animals. These factors could contribute to the progressive decline in pancreatic function, which may represent the biological feature of age-dependent risk for the development of diabetes mellitus. Studies conducted in ageing humans subjects show that alterations of pancreatic beta-cell function may be a predisposing factor to the development of impaired glucose tolerance or NIDDM in elderly subjects (Shimizu *et al.*, 1996).

Significant increase in the activities of the metabolising enzymes and levels of catecholamines during experimental diabetes have been reported (Gupta, *et al.*, 1992). STZ-induced diabetes produced significant alteration in brain beta-adrenoceptor subtypes. The densities of β_1-but not β_2-adrenoceptors, were increased in hypothalamus, thalamus and amygdala (Bitar & De'Souza, 1990). Studies conducted in C57BL/KsJ mice revealed that all of the α_1-and α_2-adrenergic receptor population were elevated in the regional brain samples of diabetic compared with controls. However, β-adrenergic receptor populations were depressed in diabetes compared with age matched controls (Garris, 1990). Studies from our lab, have shown that hypothalamus α_1-adrenergic receptors increased in number and α_2-adrenoceptors expressed altered affinity in hypothalamus and brain stem of STZ-diabetes rats. (Pius, 1996). Studies using isolated pancreatic islet β-cells from STZ-treated rats revealed that they are more sensitive to α_2-adrenoceptor agonism than controls (Ostenson *et al.*, 1989).

The evidence from the adrenodemedullation (ADM) experiments revealed several interesting findings. Results indicated that adrenodemedullated rats appeared more resistant to developing hyperglycaemia after single injection of streptozotocin than sham animals and this being associated with a greater pancreatic insulin content (Christine *et al.*, 1993). Adrenalectomy and peripheral sympathectomy have been shown to have attenuated the streptozotocin induced hyperglycaemia or derangement of islet morphology (Yang & Lin, 1995). All these, suggests the significance of adrenal medullary and cortical hormones in the pathophysiology of diabetes mellitus.

The reports so far stated did not attempt a comparative study of the adrenergic-receptor functional correlation in the brain and pancreatic islets as a function of age in STZ-induced diabetes mellitus. Also, no detailed study on the effect of adrenal hormones on neurotransmitter alterations in the brain and pancreas in diabetic state as a function of age have been carried out. We have therefore, investigated the effect of adrenal hormones on the alterations of monoamines in the brain and pancreas in order to understand its functional correlation in the pathogenesis of diabetes mellitus as a function of age.

MAJOR OBJECTIVES

1) To study the changes in the content of brain monoamines in streptozotocin-induced diabetes as a function of age and to find the role of adrenal hormones in diabetic state.

2) To assess the adrenergic receptor function in the brain stem of streptozotocin-induced diabetic rats of different ages.

3) To study the changes in the basal levels of second messenger cAMP in the brain stem of streptozotocin-induced diabetic rats as a function of age.

4) To study the changes occurring in the content of monoamines and their metabolites in whole pancreas and isolated pancreatic islets of streptozotocin-diabetic rats as a function of age and the effect of adrenal hormones.

5) To study the adrenergic receptors and basal levels of cAMP in isolated pancreatic islets in young and old streptozotocin-diabetic rats.

6) The *in vitro* study of cAMP content in pancreatic islets of young and old rats and its effect on glucose induced insulin secretion.

7) The *in vitro* study on the involvement of dopamine and corticosteroids in glucose induced insulin secretion in pancreatic islets as a function of age.

Review Of Literature

REVIEW OF LITERATURE

REGULATION OF INSULIN RELEASE

Insulin release from pancreatic islet β-cells appears to be regulated by the co-ordinated interaction of the availability of nutrients and substrates with hormonal and central nervous system settings (Albert : Renold *et al.*, 1978)

Substrates and nutrients

Glucose

D-Glucose is the major physiological stimulus for insulin secretion (Ashcroft, 1980). Glucose oxidation is an essential step in glucose induced insulin release. Glucokinase is thought to act as a glucose sensor, with phosphorylation of glucose to glucose-6-phosphate serving as the rate limiting step in glucose oxidation (Schuit, 1996). Glucokinase is also linked to the phosphate potential, [ATP[/([ADP] [Pi]) (Sweet *et al.*, 1996). An increased ATP/ADP ratio is believed to close K^+-ATP channel at the plasma membrane, resulting in decreased K^+ efflux and subsequent depolarisation of the beta cell (Dunne & Peterson, 1991). Depolarisation, activates voltage-dependent Ca^{2+} channels, causing an influx of extracellular Ca^{2+} (Liu *et al.*, 1996). Although intracellular Ca^{2+} activates protein kinases such as Ca^{2+} and calmodulin dependent protein kinase (Breen *et al.*, 1997), it remains unclear how increases in intracellular Ca^{2+} leads to insulin release. Intracellular Ca^{2+} stores appears to regulate a novel plasma membrane current [Ca^{2+} release activated non-selective cation current I_{CRAN}], whose activity may control glucose activated secretion. Lesions in these pathways leads to the pathogenesis of diabetes mellitus (Dukes *et al.*, 1997). Glucose induced insulin secretion is also partly dependent upon the activation of typical isoforms of protein kinase C (PKC) within the β-cell (Harris, *et al.*, 1996). It is suggested that PKC may be tonically active and effective in the

maintenance of the phosphorylation state of the voltage-gated L-type Ca^{2+} channel, enabling an appropriate function of this channel in the insulin secretory process (Arkhammar *et al.*, 1994).

Amino acids

Amino acids are potent stimulators of insulin release. L-arginine causes insulin release from pancreatic β-cells. Several *in vitro* studies have suggested that production of nitric oxides from islet nitric oxide system may have a negative regulatory influence on the L-arginine induced secretion of insulin and glucagon in mice (Akesson *et al.*, 1996).

Fatty acids

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

Substrates derived from nutrients

Substrates derived from nutrients alter insulin release both by substrate specific and less specific mechanisms. The latter may involve indirect reflex stimulation triggered by food intake or local islet stimulation through the production of a metabolite common to several substrates like pyruvate (Lisa *et al.*, 1994), citrate, ATP (Renold, 1970; Tahani, 1979), NADH and NADPH (Iain *et al.*, 1994). Adenosine diphosphate acts as an intracellular regulator of insulin secretion. Mg-ADP is required for the stimulation of K^+-ATP channels in intact β-cells. Other intracellular factors such as arachidonate, guanine nucleotides, small monomeric GTP-binding proteins such as rab 3A (Regazzi *et al.*, 1996) and the heterotrimeric GTP-binding protein $G_{\alpha i}$ are involved in regulating glucose induced insulin release (Konrad *et al.*, 1995). GTP analogues are also important regulators of

insulin secretion (Lucia *et al.*, 1987). Glucose induced insulin secretion in accompanied by an increase in the islet content of cAMP (Rabinovitch *et al.*, 1976).

Substrate specific stimulators

Substrate specific stimulation is more likely to result either from metabolic intermediates, such as those arising from the direct oxidation of glucose-6-phosphate (Montague & Taylor, 1968), mannose (Coore & Randle, 1964), ribose (Steinberg *et al.*, 1967) and xylitol (Kuzuya *et al* , 1966) stimulates insulin secretion.

Hormones and agents other than nutrients

Glucagon

Glucagon secreted by pancreatic α-cells have been shown to stimulate insulin release in the absence of glucose (Sevi & Lillian, 1966). The presence of specific glucagon receptors on isolated rat pancreatic β-cells as well as a subpopulation of α- and δ-cells shows the relevance of glucagon on regulation of insulin secretion (Kieffer, 1996). Intra islet glucagon appears to be a paracrine regulator of cAMP *in vitro* (Schuit *et al.*, 1996). Glucagon stimulates insulin release by elevating cAMP (Rasmussin *et al.*, 1990). cAMP through activation of protein kinase A, increases Ca^{2+} influx through voltage dependent L-type Ca^{2+} channels, thereby elevating $[Ca^{2+}]i$ and accelerating exocytosis (Carina *et al.*, 1993). Protein phosphorylation by Ca^{2+}/Calmodulin and cAMP dependent protein kinase play a positive role in insulin granule movement which results in potentiation of insulin release from the pancreatic β-cell (Hisatomi *et al.*, 1996).

Somatostatin

This hormone is secreted by the pancreatic D-cells of the islets of Langerhans. Somatostatin inhibits insulin release (Ahren *et al.*, 1981). Its action is dependent on the

activation of G-proteins but not associated with the inhibition of the voltage dependent Ca^{2+} currents or adenylate cyclase activity (Renstrom *et al.*, 1996).

Adrenaline and noradrenaline

Adrenaline and noradrenaline inhibit insulin secretion, both *in vivo* and *in vitro* (Renstrom *et al.*, 1996; Porte, 1967). Epinephrine (adrenaline) exerts opposite effects on peripheral glucose disposal and glucose stimulated insulin secretion (Avogaro *et al.*, 1996).

Adrenergic receptors

The effects of adrenaline and noradrenaline are mediated by a class of membrane bound receptors termed as adrenergic receptors. Broadly there are two types of adrenergic receptors-alpha adrenergic and beta adrenergic receptors. These are again sub divided into several types based on pharmacological properties and functional characteristics. Alpha adrenergic receptors are designated as α_1-adrenergic receptors and α_2-adrenergic receptors. Alpha$_1$-adrenergic receptors are coupled to the phospholipase C signal transduction pathway (Dean *et al.*, 1997) and α_2 - adrenergic receptors mediate their action by inhibiting adenylate cyclase (Howell & Montague, 1973) and lowers the level of cAMP (Turtle & Kipnis, 1976). α_1-adrenergic receptors consists of $\alpha_{1A/D}$, α_{1B} and α_{1C} subtypes in humans (Price *et al.*, 1994a) and α_2-adrenergic receptors include $\alpha_{2A/D}$, α_{2B} and α_{2C} (Hamamdzic *et al.*, 1995). Alpha$_{2C}$ again has many types. α_{2C-2}, α_{2-C4} and α_{2-C10} (Price *et al.*, 1994b). Three types of β-adrenergic receptors have been identified, they are β_1, β_2 and β_3 respectively. Beta adrenergic receptors are positively coupled to adenylate cyclase (Dohlman *et al.*, 1991).

Expression of adrenergic receptors on pancreatic islets

α_1 and α_2-adrenoceptors are expressed in insulin secreting cell line RINm5F (Niddam *et al.*, 1990). Post synaptic α_2-adrenergic receptors are present in rat pancreatic islets (Nakaki *et al*, 1981). $\alpha_{2A/D}$ is the adrenoceptor expressed in pancreatic β-cell lines

HIT-T15 and RIN-5AH (Hamamdzic *et al.*, 1995). Alpha$_{2C-1}$ and α_{2C-10} adrenergic receptors have been reported in human pancreas (Price *et al.*, 1994b). β_2-adrenoceptors have been reported to be present in islets β-cells and not in the exocrine cells (Lacey *et al.*, 1996). β_3-adrenoceptors also are expressed in pancreas (Atef *et al.*, 1996)

Mechanism of action

Insulin secretion *in vivo* is modulated by a functional balance between α- and β-adrenoceptor activity. (Loubatieres, *et al.*, 1977). Thus α-adrenoceptor stimulation suppresses insulin secretion while, β-adrenoceptor stimulation enhances insulin release (Gagliardion *et al.*, 1970). Rat islet cell membrane is equipped with α_2-adrenoceptors (Filipponi *et al.*, 1986) which are linked to adenylate cyclase, which inhibits insulin secretion (Lacey *et al.*, 1993) by reducing the cellular content of cAMP (Yamazaki *et al.*, 1982). However, in pigs, it is the α_1-adrenoceptors which are involved in inhibiting insulin secretion (Gregersen *et al.*, 1991). In rats and human pancreatic islets, insulin secretion is enhanced via mediations of β_2-adrenergic receptors (Lacey *et al.*, 1993). β_3-adrenoceptor stimulation also results in enhanced insulin secretion (Atef *et al.*, 1996). In mice, insulin secretion is promoted mainly via β_3-receptors and partially via β_2-receptors on pancreatic islet β-cells (Yoshida *et al.*, 1991).

Isolated human islets respond to adrenaline in a complex manner. At higher concentration of adrenaline the α_2-inhibitory response is entirely predominant and there is no evidence for any β_2-stimulation of insulin secretion. At lower adrenaline concentration the initial response is stimulatory but this is overcome by net inhibition of secretion (Lacey, *et al.*, 1993). Epinephrine appears to inhibit glucose induced insulin secretion by a dual α-adrenergic mechanism: a cAMP independent effect at low concentration of epinephrine and a cAMP-dependent action at higher concentrations. Both effects of epinephrine may be Ca^{2+} dependent. Action of epinephrine which is independent of the adenylate cyclase-cAMP system may be the usual physiologically operating one (Rabinovitch *et al.*, 1978).

The molecular mechanism of the inhibition of exocytosis by catecholamines remains obscure. In different cell types, adenylate cyclase inhibitors like norepinephrine, somatostatin and opiates block Ca^{2+} channels through the mediation of G-proteins in a cAMP-independent manner. The activation of G_{ei} blocks the function of putative pore thought to span the plasma and granule membranes during exocytosis. Epinephrine inhibits Ca^{2+} currents and blocks adenylate cyclase and exocytosis via coupling to a unique or more likely to different G-proteins. The same G-proteins may therefore act both on Ca^{2+} channels and the fusion pore (Ullrich & Wollheim, 1988).

There is also a report which suggests that α_2-adrenoceptor stimulation does not result from suppression of L-type Ca^{2+}-current (Bolevist *et al.*, 1991). The primary mechanism by which noradrenaline inhibits insulin secretion is independent of alterations in islet-cell cyclic AMP levels and Ca^{2+} influx rates, and must result from changes that occur at an intracellular site which lies distal to both these events (Morgan & Montague, 1985). Actin micro filaments are implicated in regulating the access of secretory granules to the plasma membrane prior to exocytosis. $Alpha_2$-adrenoceptors are coupled to the control of actin polymerisation in HIT-T15 cells. Regulation of F-actin formation could be a component of the mechanisms by which α_2-agonists mediate the inhibition of insulin secretion (Cable *et al.*, 1995). However, noradrenaline induced inhibition appears to be mediated by an elevation of cGMP levels which probably result from α_2-adrenoceptor activation of islet guanylate cyclase through a guanine nucleotide regulatory protein (Vara & Rodrigue, 1991). G_i and G_o proteins might be involved and they mediate their action via activation of ATP sensitive potassium channel (Gillison *et al.*, 1997)

Pancreastatin

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

Amylin

Amylin is a 37 amino acid peptide hormone co-secreted with insulin from pancreatic β-cells. Amylin appears to control plasma glucose via several mechanisms that reduce the rate of glucose appearance in the plasma. Amylin limits nutrient inflow into the gut, limits nutrient flux from the gut to blood and by its ability to suppress glucagon secretion, it is predicted to modulate the flux of glucose from liver to blood. Amylin is absolutely or relatively deficient in type I-diabetes and in insulin requiring type II-diabetes (Young, 1997). Islet amyloid polypeptide (IAPP) or amylin inhibits insulin secretion via an autocrine effect within pancreatic islets (Tokoyama *et al.*, 1997). Amylin fibril formation in the pancreas may cause islet cell dysfunction and cell death in type II-diabetes mellitus.(Alfredo et al., 1994).

Adrenomedullin

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

Galanin

Galanin is a 29 amino acid neuropeptide localised in the intrinsic nervous system of the entire gastrointestinal tract and the pancreas of man and several animal species. (Scheurink *et al.*, 1992) . Among other functions galanin inhibits insulin release, (Ahren *et al.*, 1991), probably via activation of G-proteins (Renstrom *et al.*, 1996) by the mediation of activated galanin receptors. However, galanin receptors are not as effective as α_2-adrenergic receptors in activating G-proteins (Gillison *et al.*, 1997).

Macrophage migration inhibitory factor (MIF)

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

Other agents

Coenzyme Q_{10} improved insulin release (Conget *et al.*, 1996) and it may also have a blood glucose lowering effect (Anderson, *et al.*, 1997). Inositol hexa bisphosphate stimulates non Ca^{+} mediated and purine-Ca^{2+} mediated exocytosis of insulin by activation of protein kinase C (Efanov *et al.*, 1997). Small GTP-ases of the rab 3A family expressed in insulin secreting cells are also involved in the control of insulin release in rat and hamster (Regazzi *et al.*, 1996).

Neurotransmitters

Gamma amino butyric acid (GABA)

GABA (γ-amino butyric acid), is a prominent neurotransmitter, present in high concentrations in β-cells of islets of Langerhans. The GABA shunt enzymes, glutamate decarboxylase (GAD) and GABA-transaminase have also been localised in islet β-cells. Physiological studies on the effect of extracellular GABA on islet hormonal secretion have had variable results. GABA has an inhibitory effect on somatostatin (Annette *et al*, 1991)and glucagon secretion (Robert.*et al.*, 1995) and this may facilitate insulin secretion.

GABA shunt activity might be involved in insulin secretion, more over GABA and its metabolites may regulate proinsulin synthesis (Annette *et al.*, 1991). The concentration of GABA in the endocrine pancreas is reduced in STZ-induced diabetes (Gerber & Hare 1979), this points to the relevance of GABA in islet hormone release (Rex *et al.*, 1995). Acute oral administration of GABA analogue, baclofen caused significant increases in basal insulin, glucagon and growth hormone levels, perhaps through the mediation of the central nervous system (Nicola *et al.*, 1982).

Serotonin

Studies using metergoline a powerful and long acting anti serotonergic agent, suggest that there is serotonergic control of insulin secretion and that serotonin exerts different effects on insulin release according to different stimuli (Pontiroli *et al.*, 1975). Serotonin in high concentrations have been reported to inhibit glucose induced insulin secretion (Lindstrom *et al.*, 1983; Tabeuchi *et al.*, 1990). Serotonin receptors 5-HT_1 and $5HT_2$ receptors may regulate blood glucose levels by modifying the release of insulin (Chaouloff *et al.*, 1990). Peripheral 5-HT_{2A} receptors participates in glucose regulation probably via release of adrenomedullary catecholamines. 5-HT_{2C} and or 5-HT_{2B} receptor stimulation elicited hyperglycaemia (Sugimoto *et al.*, 1996).

Dopamine

High concentrations of dopamine in pancreatic islets can decrease glucose stimulated insulin secretion (Zern *et al.*, 1980; Tabeuchi *et al.*, 1990). L-DOPA the precursor of dopamine had similar effect to that of dopamine (Lindstrom *et al.*, 1983). Dopamine D3 receptors are implicated in the control of blood glucose levels (Alster & Hillegaart, 1996). Dopamine DAI receptors have also been reported to be present on pancreatic β-cells (Tabeuchi, *et al.*, 1990). These clearly indicate the role of dopamine in the regulation of pancreatic function.

Acetylcholine

Acetylcholine is a principle transmitter of the parasympathetic system. Acetylcholine, through vagal muscarinic and non vagal muscarinic pathways (Greenberg & Pokol, 1994) increases insulin secretion (Tassava *et al.*, 1992) via muscarinic receptors on pancreatic islet cells (Ostenson & Grill, 1987).

Neuropeptides

The insulin secretory response to enkephalins and morphine was rapid, corresponding to the first phase of glucose induced insulin release, suggesting that there might be opiate receptors in the islets and that opiod receptors could modulate insulin release (Green *et al.*, 1980). Beta endorphins has a positive effect on the enhancement of insulin secretion in normal subjects and patients with type II-diabetes mellitus (Giugliano *et al.*, 1987a), while met-enkephalin has a negative role in the regulation of insulin secretion in both normal and NIDDM subjects (Giugliano *et al.*, 1987b). Pituitary adenylate cyclase activating polypeptide stimulate insulin release in absence of increased intracellular Ca^{2+} (Tornoe *et al.*, 1997). Substance P, neurokinin A, pancreatic gastrin releasing peptide and vasoactive intestinal polypeptide may have a role in regulating pancreatic function (Bailey *et al.*, 1986). Secretin (Funakoshi *et al.*, 1985) cholecystokinin and human pancreatic polypeptide (Inui, 1985) peptide YY (Greenley *et al.*, 1988) may also be involved in pancreatic islet hormone secretion.

Central nervous system regulation of pancreatic insulin secretion

From the morphological and functional point of view, CNS does have a role in the regulation of pancreatic insulin secretion. Three types of nerve endings are reported within the pancreatic islets. They are sympathetic, parasympathetic and peptidergic nerves. The neurotransmitters found in such nerves are catecholamines and serotonin, acetylcholine and vasoactive intestinal polypeptide and cholecyctokinin respectively.

These nerve fibres enter the pancreas in association with the vascular supply. Adrenergic fibers innervate vessels, acini and islets; cholinergic nerves are found mainly in islets (Miller, 1981). Peptidergic nerves are present in both the exocrine and endocrine tissues of this gland and there is considerable interspecies variability as to which part receives a greater proportion of these fibers (Bishop, *et al.*, 1980). The nerve terminals simply end abruptly some 20-30 nm from the endocrine cells. This implies that neurotransmitters might affect several cells by diffusing through the extracellular space (Miller, 1981).

In vivo stimulation of the vagus nerve or an infusion of acetylcholine usually increases insulin secretion (Kaneto *et al.*, 1981). Vagotomy often has little effect on the basal hormone secretion, however, the amount of hormone released is greatly altered (Helman *et al.*, 1982).

It is well established that the autonomic fibres supplying the pancreas travel via the vagus and splanchinic nerves (Helman *et al.*, 1982). These nerves are clearly related to the ventral hypothalamus. The hypothalamus plays a vital role in the integration of neurohormonal function (Oommura & Yoshimatsu, 1984). The ventro-medial hypothalamic nuclei is considered as the sympathetic centre and the stimulation of this area decreases insulin secretion (Helman *et al.*, 1982). Leisions in the ventro-medial hypothalamus, resulted in behaviour alterations and morphological changes in pancreatic islets (Sclafani, 1981). Ventro-lateral hypothalamus is the parasympathetic centre, stimulation of which increases the circulating level of insulin (Helman 1982). Leisions in ventro-lateral hypothalamus results in decreased body weight; food intake, plasma insulin levels and decrease in islet size (Powley & Opsahl, 1976). Substantia nigra is one autonomic area in the central nervous system which plays an important role in controlling the structure and activity of the pancreatic islets. Leisions in substantia nigra resulted in reduced size and number of islet cell populations (Smith & Davis, 1983).

The recent demonstration, that central nervous system cell groups projecting into the pancreatic vagal motor neurones received inputs from adrenergic, noradrenergic and serotonergic neurones from the lower brain stem and a dopaminergic input from

paraventricular nucleus of hypothalamus (Lowey *et al.*, 1994), evidently shows the importance of central nervous system neurotransmitters in the pancreatic hormone secretion and their importance in glucose homeostasis.

Central nervous system borne hyperglycaemia is mediated via central noradrenegic pathways (Mc Caleb & Myers, 1982) by an activation of sympathoadrenal system. Plasma glucose also appears to be under separate serotonergic and dopaminergic control exerted via 5-HT_{1A} and DA_{D3} receptors respectively (Alster and Hillegaart, 1996). $GABA_A$ receptors in hypothalamus are also involved in the control of peripheral glucose metabolism, possibly by enhanced hepatic glucose production in fasted rat (Charles, 1995). Previous neuro antomical and physiological studies have indicated that nucleus ambiguous is one source of vagal motor neurons in the brain stem, that innervates the pancreas and which when stimulated, increases insulin release, indicating that plasma insulin levels are under tonic GABA inhibition (David *et al.*, 1982).

AGEING AND INSULIN SECRETION

It is well documented that ageing is associated with a progressive impairment of glucose tolerance (Davidson, 1979). Few laboratories have attributed the alterations in insulin secretion, with age to changes in diet rather than ageing *per se* (Hara *et al.*, 1992). Animals fed on a restricted diet showed a prolonged and higher secretory rate during first phase of insulin release when compared to animals fed *ad libitum* (Castro *et al.*, 1997).

Total glucose-stimulated insulin secretion has been described as being unchanged (Ruhe *et al.*, 1997), suppressed (Molina *et al.*, 1985)or increased (Curry *et al.*, 1984) as the animal ages. Resting pancreatic insulin stores appears not to be negatively affected by ageing (Howell *et al.*, 1965). However, in general, when insulin secretion is reported in terms of islet cell mass, ageing appears to result in decreased insulin secretory efficiency (Curry *et al.*, 1984).

Glucose induced time dependent potentiation of insulin release is impaired in the islets of mature and old Sprague-Dawley rats, confirming an early loss of sensitivity of

beta-cells to secretagogues during ageing (Bombara *et al.*, 1995). Intact pancreatic islets from old Wistar rats showed impaired glucose induced insulin release; glucose uptake and oxygen consumption than in the young or adult rats. Moreover, $^{45}Ca^{+}$ uptake and calmodulin content were decreased in pancreatic islets from older rats, which explained the impairment in glucose induced insulin release in ageing. No age related changes were observed in glucose induced $^{45}Ca^{+}$ efflux in pancreatic islets (Castro *et al.*, 1993). Alterations in glucose-stimulus / secretion coupling are not associated with changes in $K^{+}ATP$ channel mediated responsiveness in aged male Fischer rats (Ruhe *et al.*, 1993). It was suggested that the delay in the first phase of insulin secretion as a function of ageing might be due to a defect which lies distal to the elevation of $[Ca^{2+}]i$ (Komatsu *et al.*, 1991).

Recently it was demonstrated that, in Wistar rats ageing is associated with progressive decline in beta-cell activity. Despite increases in islet size, beta-cell number, the pancreatic insulin content, amount of insulin secreted and insulin mRNA levels declined significantly with age. Glucagon mRNA levels showed a modest decline with age where as somatostatin mRNA levels did not vary significantly (Perfetti *et al.*, 1995). Similar abnormalities were reported in ageing C57 BL/6J mice (Perfetti *et al.*, 1996). This progressive decline may represent the biological feature of the age dependent risk for the development of diabetes. Studies conducted in ageing human subjects show that alterations of pancreatic β-cell function independent of that seen with NIDDM occurred in relation to ageing. This may be a predisposing factor to the development of impaired glucose tolerance or NIDDM in elderly subjects (Shimizu *et al.*, 1996).

Insulin action was altered in rats between two and twenty months of age. The most pronounced impairment was in insulin binding to muscle membranes and maximal response of insulin-induced glucose disappearance rate occurs during early life stage (through maturation) and then a coupling defect seems to be superimposed with further ageing. (Haruo *et al* , 1988).

Glucose-stimulated insulin secretion was significantly inhibited by norepinephrine in all age groups of male Fischer 344 rats. There was no significant effect of ageing in the sensitivity or magnitude of inhibition at any of the concentrations of norepirephrine studied

(McDonald *et al.*, 1992). No differences between the binding of [^{3}H]-norepinephrine was noted in the receptors of neonatal and adult islets of rats. However, it was suggested that adrenergic system may play a greater role in the regulation of insulin release from neonatal than from adult islets (Gembal & Wojcikowski, 1993). Studies conducted in hamster pancreatic islets as a function of age showed that adult hamsters had higher number of α_2-adrenoceptors than the young animals, while no change in the affinity of receptors were noted (Lacombe *et al.*, 1993).

AGE RELATED ALTERATIONS OF NEUROTRANSMITTERS AND RECEPTORS IN THE BRAIN

Studies of structural, chemical and functional changes of the brain during the life-span shows that the mammalian brain undergoes an early period of growth, remains relatively stable during most of adulthood, and then gradually declines in senescence. The qualitative aspect of changes in the nervous system with age, may have a greater significance for function and survival.

Of all the events that occur in the ageing brain, subtle changes at the synaptic level may be of greatest importance. A slight imbalance of putative neurotransmitters or their associated enzymes and receptor sites at the synapse may result in a greater impairment of information processing than may be evident from the apparent loss of neurons. Thus, age dependent changes in neurotransmitters may lead not only to changes in the electrical activity of the brain, but also to changes in behaviour and function. Transmitter function may be altered during ageing in several ways: changes in the amount of precursor substance reaching the neuron, changes in the amount of enzymes present within a neuron or the accumulation of metabolites, decrease in number of synaptic processes or decrease in number or affinity or receptor sites for the neurotransmitter system (Samorajshi, 1981).

Neurotransmitters

Catecholamines

Enzymes:- Significant decrease with ageing, of catecholamine synthesising enzymes like tyrosine hydroxylase and dopamine decarboxylase have been reported in several brain regions of man (McGeer & McGeer 1976a) and rats (McGeer *et al.*, 1971). However, an increase in tyrosine hydroxylase and dopa-decarboxylase activity was reported in the hypothalamus, superior cervical ganglion and in the adrenal glands of rats and mice (Reis *et al.*, 1977). Enzymes for amine disposal, monoamine oxidase and catechol-o-methyl transferase showed age-related changes. Monoamine oxidase activity was increased in brain and heart in man and rats (Robinson, 1975) however, Oreland & Shasken,(1983) have reported a progressive decline in the activity. Catechol-o-methyl transferase activity decreased in brain and liver of rats (Stramentinoli, 1977).

Uptake of Catecholamines

Following release of amine transmitters, their substantial portion is taken up into the storage granules in the presynpatic neurons. This uptake process is also affected during ageing. Uptake of norepinephrine and dopamine in the synaptosomes of hypothalamus and striatum of mouse was decreased (Sun, 1976).

Concentrations and turnover

In man, dopamine content decreased in the caudate nucleus and putamen, hippocampus and mesencephalon (Adolfsson, *et al.*, 1979). Homovanillic acid, a dopamine metabolite in cortical areas could also be positively interrelated with age (Adolfsson, *et al.*, 1979). Norepinephrine content decreased slightly but significantly with age in the hind brain of man (Robinson, 1975) and in rats, NE and DA content in the hypothalamus and brain stem were found to be lower (Sun, 1976).

Serotonin

Serotonin content or turnover has been reported to show usually no change or slight increase (Simpkins *et al.*, 1977), although in occasional experiments a decrease in

serotonin content and tryptophan hydroxylase activity in some brain areas (e.g. raphe nucleus, ventral pontine nucleus) has been demonstrated (Meek *et al.*, 1977). Turnover rate of serotonin was found to be increased in the hypothalamus ((Simpkins, *et al.*, 1977). 5-Hydroxy indole acetic acid (5-HIAA), a metabolite of 5-HT was shown to increase in cerebrospinal fluid (Bowers & Gerbode, 1968), blood and brain (Robinson, 1975). There is consistent preclinical evidence that brain 5-HT and 5-HIAA concentrations are abnormal during natural ageing. 5-HT turnover is positively correlated with natural ageing, suggesting that ageing may alter 5-HT system in humans (Dursun *et al.*, 1997).

Gamma-aminobutyric acid (GABA)

GABA, an inhibitory neurotransmitter has also been studied in relation to ageing. The GABA synthesising enzyme, glutamic acid decarboxylase (GAD) (McGeer & McGeer, 1976a) has been reported to decline in several brain regions of man. Benzodiazepine binding sites and the $GABA_A$ binding sites of $GABA_A$ receptors complex from rat prefrontal cortical membranes were differentially affected by the ageing process (Ruano *et al.*, 1996).

Acetylcholine

Activity of enzymes responsible for biosynthesis (choline acetyl transferase, CAT) and hydrolysis (acetyl cholinesterase, AChE) of the acetylcholine were shown to decrease with ageing in various brain areas and other tissues in majority of the investigations. Thus, cortex in man (McGeer & Mcgeer 1976a) brain and heart in rats (Frolkis *et al.*, 1973) and hippocampus in mice (Vijayan, 1977) showed decrease in activities of both enzymes; CAT activity also decreased in the cortex, cerebellum (Mohan & Radha, 1978), Caudate (Meek *et al.*, 1977) and spinal cord (Timiras & Vernadakis, 1972) in rats.

Receptors

A variety of investigations have examined the effect of ageing on catecholamine receptor sensitivity in the central nervous system.

Dopamine Receptors

In the striatum and substantia nigra, there is a significant decrease in the ability of dopamine to stimulate the formation of cAMP with age. A decrease in dopamine receptors in the striatum of rats have been reported (Misra *et al.*, 1980).

Adrenergic receptors

The levels of β-adrenergic receptors appear to decline during the ageing process. In the cerebellum, striatum, brain stem, pineal gland and cerebral cortex, there is a significant loss of beta-adrenergic receptors with age (Misra *et al.*, 1980; Paulose *et al.*, 1982). Differential alterations in β-adrenergic receptor subtypes during ageing has been reported in the cerebellum with β_1-adrenergic receptors being increased and β_2-adrenoceptors decreased in older animals (Misra. *et al.*, 1980). Greenberg & Weiss, (1979) have studied changes in the adaptability of β-adrenergic receptors with age, and they have proposed that the reduced responsiveness observed may be related to a lower capability of brain tissue to synthesise β-adrenergic receptors. Alpha-adrenergic receptors in the cortex of Fischer rats were reported to have decreased as a function of age (Misra *et al.*, 1980).

Cholinergic receptors

A decrease in the cholinergic receptors in rat striatum , cortex, hippocampus and cerebellum have been reported (James & Kanungo, 1976)..

Serotonin receptors

5-HT_2 and 5-HT_{1C} receptors have been reported to be markedly increased during ontogeny in mice (Bryan *et al.*, 1991).

Adenylate Cyclase

Several brain areas including striatum, nucleus accumbens, tuberculum olfactorium and substantia nigra showed a marked decrease in the activity of adenylate cyclase (both basal and stimulated) by dopamine, apomorphrine or LSD during ageing; however, the retina of senescent rats showed an increased activity of dopamine-stimulated adenylate cyclase, which may be due to receptor denervation or supersensitivity in light deprivation

during ageing (Govonie *et al.*, 1977). cAMP levels in cerebral cortex stimulated by norepinephrine or electric pulses in anaesthetised rats were also three to four fold lower in older rats compared to the young controls (Berg & Zimerman, 1975).

ALTERATIONS IN MONOAMINES, THEIR METABOLITES AND RECEPTOR FUNCTION IN DIABETES MELLITUS:

BRAIN

Diabetes induced alterations in the levels of brain monoamines and their metabolites have been well documented. Diabetes alters the normal meal-related responses in hypothalamic catecholamine metabolism (Glanville & Anderson, 1986).

Norepinephrine and Epinephrine

Significant increase in the activities of the metabolising enzymes and levels of catecholamines during experimental diabetes have been reported (Gupta, *et al.*, 1992). The concentration of norepinephrine in several brain regions have been reported to have increased during the course of diabetes (Trulson & Himmel, 1985; Fushimi *et al.*, 1984; Chu *et al.*, 1986; Oreland & Shasken, 1983; Wesselman *et al.*, 1988; Garris, 1990; Lackovic *et al.*, 1990; Chen & Yang 1991; Tasaka *et al.*, 1992). However, norepinephrine levels were decreased in pons and medulla (Ramakrishna & Namasivayam, 1995), neocortex and caudal segment of the brain stem (Kulikov *et al.*, 1986) and hypothalamus (Shimizu, 1991). A decrease in the rate of metabolism of norepinephrine (MHPG/NE) was also observed in the brain of STZ treated animals (Bitar & DeSouza, 1990). Epinephrine levels showed significant increase in the striatum, hippocampus and hypothalamus of alloxan diabetic rats and these effects were reversed by insulin therapy (Ramakrishnan & Namasivayam, 1995).

Dopamine

Dopamine levels were significantly increased in the hippocampus, corpus striatum (Chu *et al.*, 1986) whole brain (Lackovic *et al.*, 1990; Chen & Yang, 1991), cerebral

cortex, hypothalamus and thalamic area of STZ- diabetic rats (Tasaka *et al.*, 1992; Shimizu, 1991). A decreased dopamine turn over rates has been reported in diabetic rats (Trulson *et al.*, 1983; Bellush *et al.*, 1991).

Serotonin

Serotonin levels have been reported to be lower in the neocortex, caudal segment of brain stem and hypothalamus of diabetic rats (Kulikov *et al.*, 1986; Chu *et al.*, 1986; Shimizu, 1991), accompanied with significant decreases in brain tryptophan concentrations (Mackenzie & Trulson, 1978) and in the rate of serotonin synthesis (Crandall *et al.*, 1981). The turn over rate of 5-HT to 5-HIAA in diabetic rats was also reported to be lower (Bellush, *et al.*, 1991). Corpus striatum showed no change in the levels of serotonin in the diabetic state (Chu *et al*, 1986). However, in diabetic mice, 5-HT levels were reported to have increased in the hypothalamus, hippocampus, pons-medulla and cortex progressively from short term to long term diabetics (Chen & Yang, 1991).

Adrenergic receptors

STZ-induced diabetes resulted in a increase in α_1-adrenergic receptors) while α_2-adrenergic receptors showed differential affinity in the hypothalamus and brain stem of rats (Pius, 1996). Significant alterations in brain beta-adrenergic receptor subtypes have been noted in the STZ-diabetic state. The density of β_1- but not β_2-adrenoceptors were reported to have increased in hypothalamus, thalamus and amygdala of the brain of the diabetic rats (Bitar & DeSouza, 1990). In genetically obese diabetic mice, all of the α_1 and α_2-adrenergic receptor populations were elevated in the regional brain samples, relative to controls while, β-adrenergic receptor populations were depressed compared with age matched controls (Garris, 1990).

Dopamine receptors

A differential regulation of dopamine receptor subtypes were reported. An increased D_2 receptor density has been discovered in some brain regions in diabetic state (Lozovsky *et al.*, 1981; Trulson & Himmel, 1983; Serri *et al.*, 1985) while, the dopamine

D_1 receptors were decreased in the striatum of alloxan diabetic rats (Salkovic & Lackovic, 1992).

Serotonin receptors

An increase in 5-HT_{1A} and $5HT_2$ receptors have been reported in the cerebral cortex of STZ diabetic rats (Sandrini *et al.*, 1997; Sumiyoshi *et al.*, 1997; Pius, 1996) while, serotonin receptors in hypothalamus switched to more of S_1-receptors in diabetic rats, (Pius, 1996).

PANCREAS AND PLASMA

Significant reduction in sympathetic nervous system activity in the interscapular brown adipose tissue, heart and pancreas of STZ-diabetic rats have been reported and this reduction in norepinephrine turnover was reversed by insulin therapy, but it was less marked in pancreas (Yoshida *et al.*, 1985). In Chinese hamsters, no significant increase in norepinephrine concentration was reported in hypothalamus, acinar pancreas, pancreatic islets and heart in diabetes (Feldman & Gerritsen, 1988).

Studies using perfused rat pancreas of NIDDM rat model, showed that the overflow of noradrenaline was 2-3 fold higher than from non diabetic rats. Levels of adrenaline were always low in the perfusates of non diabetic glands, but increased in diabetic glands. However, the pancreatic content of noradrenaline and adrenaline were similar in diabetic and non diabetic rats (Ostenson *et al.*, 1993).

In Vitro studies revealed that glucose induced β-cell secretion from islets isolated from STZ-diabetic rats were preserved, though lower than from normal rats (Ostenson & Grill, 1987). Moreover, the β-cells of STZ-diabetic islets tend to be more sensitive than β-cells of normal islets to α_2-adrenoceptor agonism (Ostenson *et al.*, 1989).

Serum catecholamine levels have been found to be elevated compared with healthy subjects (Christensen, 1979). Plasma and platelet monoamines were significantly increased in the human diabetics compared with age matched controls. Similar changes were noted in rat plasma and platelets (Jackson *et al.*, 1997). A low plasma DHPG/NE ratio in forearm venous blood was reported in patients with diabetic autonomic neuropathy (Christensen 1979).

SIGNAL TRANSDUCTION PATHWAYS

Alterations in G-proteins

In the hepatocytes and adipocytes of alloxan or streptozotocin treated diabetic rats, there appears to be a total loss of functional G_i. This has been assessed by the failure of either p(NH) ppG or GTP to inhibit adenylate cyclase activity in membranes derived from diabetic, but not from control animals. However, inhibitory coupling of receptors to adenylyl cyclase is not abolished in either intact hepatocytes or adipocytes from such diabetic animals (Sandhya & Mohan, 1997). In STZ-diabetic rats, hepatocytes had marked decrease in α_{i2}, α_{i3}, with moderate decrease in α_3 and no change in $\beta\gamma$ subunits (Bushfield *et al.*, 1990). The α_{i2} from diabetic hepatocytes was found phosphorylated, a key event which is suspected to be involved in impairing the function of α_{i2} in diabetics. Such a phosphorylation of α_{i2} is evidently absent in the hepatocytes from control animals. In alloxan induced diabetes, a functional decrease in all the α_i subunits have been noticed in hepatocytes, adipocytes and even in corpus striatum (Abbracchio *et al.*, 1989).

In non insulin dependent diabetics also decreased amounts of α_{i2} and α_{i3} have been reported in the platelet membranes (Livingstone *et al.*, 1991). A functional imbalance between G_s-protein and G_i/G_o-protein-mediated transduction mechanism with an increased efficacy for G_s activity, probably as a result of the loss of G_i/G_o inhibitory function has been found in the striatum and other tissues of diabetic animals (Abbracchio *et al.*, 1989). Diabetes is associated with development of a time related alteration of cerebral G_i/G_o proteins and this defect is probably due to modifications of G-protein structure or

physiological status affecting the coupling with membrane effector system and the sensitivity to PTX (Finco *et al.*, 1992). Basal adenylate cyclase activity was indeed increased in the retina of diabetic rats, consistent with the observed reduction of G_i/G_o inhibitory proteins. Such functional alterations of cAMP producing system were reversed by insulin therapy. Such a defect of G_i/G_o proteins could represent an early transductional damage in the development of diabetic neuropathy (Abbracchio *et al.*, 1991).

Adenylate cyclase

In the sciatic nerve of STZ-diabetic rats, the cAMP content was lower than those of controls whereas, cGMP levels were unaltered. The cAMP-phosphodiesterase activity was unaltered in the diabetic state. There was a positive correlation between cAMP content in the sciatic and motor nerve conduction velocity in both control and diabetic rats. This reduction in cAMP content in the peripheral nerve could be due to impairment of adenylate cyclase activity in the diabetic state, and might be involved in the pathogenesis of diabetic neuropathy (Shindo *et al.*, 1993). Strassheim *et al* (1990) have reported a reduced specific activity of adenylate cyclase in adipocyte membranes and enhanced stimulatory effect of isoprenaline. They suggested that diabetes brings about selective changes in the functioning of G_i in adipocyte membranes which removes the tonic GTP dependence of this G-protein. A decrease in the functional responses to cAMP increasing agents like β-adrenoceptor agonist, has been reported in diabetic hearts. This change is attributed to impaired phosphorylation of cardiac regulatory phosphoproteins including phospholamban (Gando, 1994). IDDM subjects have been reported to have a partial dysfunction of the beta-adrenoceptor-coupling to adenylate cyclase in mononuclear leukocytes (Sager *et al.*, 1991). No major alteration in the functionality of the adenylate cyclase in the pancreatic β-cells of diabetic rats have been reported. However, there is a defective glucose-induced cAMP generation, that could be due to a block in the activation of adenylate cyclase (Dachicourt, *et al.*, 1996).

Protein kinase C

An increased membrane bound protein kinase C activity has been proposed to be the reason for diabetic cardiomyopathy (Xiang & McNeil, 1992). The enhanced contractile response of aorta from diabetic rats to norepinephrine has been attributed to enhanced phosphoinositide metabolism via stimulation of α_1-adrenoceptors (Abebe & McCleod, 1991).

Phospholipase C (PLC)

The phospholipase C (PLC) activity have shown differential alterations in diabetic brain regions. The membrane fraction of the hypothalamus of STZ-diabetic rats did not show any change while, the enzyme activity was reduced by about 55% in the cytosolic fraction. In the brain stem of these rats, PLC activity was unaltered in the cytosolic fraction, while in the membrane fraction the activity of the enzyme could not be detected (Pius, 1996). PLC pathway is coupled to α_1-adrenergic, 5-HT_2, 5-HT_{1C} receptors and cholinergic receptors (Casey & Gilman, 1988; Berridge & Irivine, 1984).

Materials And Methods

MATERIALS AND METHODS

BIOCHEMICALS AND THEIR SOURCES

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

The following are the chemicals purchased from Sigma and used in this study

Streptozotocin, (±) norepinephrine, (−) − norepinephrine bitartarate salt, (±) epinephrine, normetanephrine, 5-hydroxytyramine, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-hydroxy indole acetic acid, homovanillic acid, sodium octyl sulfonate, ethylene glycol bis (β-aminoethyl ether)-EGTA, ethylene diamine tetra acetic acid-EDTA, HEPES - [n′ (2-hydroxy ethyl] piperazine-n′-[2-ethanesulfonic acid], ascorbic acid, catechol, pargyline, tris buffer, dithiothreitol, phentolamine, prazosin, yohimbine, propranolol, atenolol, RPMI 1640 auto mod. media, foetal calf serum (heat inactivated), collagenase type V, D-glucose, hydrocortisone, dexamethasone, forskolin, calcium chloride, bovine serum albumin fraction V and metyrapone.

DNAase I and Hisafe cocktail were purchased from Pharmacia (USA). Spiperone and clonidine were kind gifts from Janssen Research Foundation, Beerse, BELGIUM and Boehringer Ingelheim (W.GERMANY) respectively.

Radiochemicals

1-[7,8, ^{3}H] noradrenaline, specific activity 39 Ci/mmol (Amersham, England), cAMP [^{3}H] assay system. (Amersham, England) and Radioimmunoassay kit for Insulin - RIAK I (Bhabha Atomic Research Centre, Mumbai, India).

ANIMALS

Male Wistar rats of different age groups were purchased from Small animal breeding station, Veterinary College, Mannuthy and Central Institute Of Fisheries

Technology, Cochin. These rats were housed in groups of 2-3 in separate cages and fed lab chow and water *ad libitum*. They were maintained in twelve hour light and twelve hour dark cycles.

EXPERIMENTAL SET UP

Induction of diabetes mellitus in rats

Streptozotocin, dissolved in citrate buffer pH 4.5, was given as a single intrafemoral vein injection to the rats under light ether anaesthesia (Junod *et al.*, 1969). The dosage of streptozotocin varied from 50-100 mg./kg body weight, depending on the age of the rats. The young rats were found to be more resistant and had to be injected with a high dose to induce diabetes. The injections were given during the early hours of the day (7 a.m. to 9 a.m.) to avoid diurnal variations in responses. The control rats were given citrate buffer injection. The diabetic rats were divided into four groups. One diabetic group did not receive any treatment.

Insulin administration

One group of diabetic rats received subcutaneous injections of insulin daily during the entire period of the experiment. The dosage varied from 2-6 units depending on the concentration of blood glucose. A mixture of both plain and lente insulin (Boots, India) were given for better control (Sasaki and Bunag, 1983). Insulin injections were given between 3.00 p.m. and 5.00 p.m. The final insulin injection was given twelve hours prior to sacrifice.

Adrenalectomy

In order to investigate the role of adrenal glands on the diabetic state, a group of diabetic animals were unilaterally adrenalectomised (Waynforth, H.B.). The surgery was performed under light ether anaesthesia. Skin incision was made anteriorly below the peak of the "hump" of the back (i.e., about one quarter the distance between the last pair of ribs and the base of the tail). The muscle incision on the right side of the animal was made

immediately beneath the spinal muscles. This correlates with the anatomical positioning of the adrenals which were found medially and near the anterior poles of the kidneys. A pair of curved forceps was then inserted into the incision and the liver pushed away anteriorly by the convex surface of the forceps and the adrenal gland was brought out through the muscle incision by holding the periadrenal fat using the second forceps. The removal was effected by clamping the tissues and blood vessels at the base of the adrenals with both pairs of forceps. Usually bleeding was minimum and is of no consequence. The forceps were then drawn away and any fat or other tissues, minus the adrenal was returned into the abdominal cavity. The muscle incision was sutured and the wound sprinkled with antibiotic powder. The skin incision was then sutured and the antibiotic power was overlayered on the wound. Aseptic handling is required to reduce the risk of infections. The adrenalectomised rats were given 0.9% saline *ad libitum* along with food .

Metyrapone administration

The role of adrenal steroids in diabetic state was investigated by specifically blocking the adrenal steroid biosynthesis in a group of diabetic rats. Metyrapone (Metopirone) inhibits the enzyme steroid 11 β-hydroxylase, that converts 11-deoxy precursors into hydrocortisone, corticosterone and aldosterone. This drug specifically inhibits adrenal steroid biosynthesis.

Metyrapone was dissolved in normal saline (10 mg/ml stock). The drug was administered intraperitoneally at a dose of 30 mg/kg body weight after the induction of diabetes. Metyrapone treated rats were given 0.9% saline *ad libitum* along with food.

Sacrifice of the rats

The experimental rats were maintained for a period of 14 days. Body weight and blood glucose concentrations were periodically monitored. Rats were sacrificed by guillotine method between 9 a.m. and 10 a.m. The brain regions were rapidly dissected out on a chilled glass plate (Glowinski and Iversen, 1966). The brain regions-hypothalamus (Hypo), corpus striatum (CS), brain stem (BS), cerebellum (CB) and

cerebral cortex (CC) were immediately immersed into liquid nitrogen and stored at -70°C for various experiments.

BLOOD GLUCOSE ESTIMATIONS

The blood samples were collected as required by tail snipping method. The blood glucose were estimated using Glucose estimation kit (Merck). The spectrophotometric method using glucose oxidase-peroxidase reactions was followed.

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

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

The hydrogen peroxide formed in this reaction reacts with 4-aminoantipyrine and 4-hydroxybenzoic acid in the presence of peroxidase (POD) to form N-(-4-antipyryl)-p-benzo quinoneimine. The addition of mutarotase accelerates the reactions. The amount of dye formed is proportional to the glucose concentration. The absorbance was read at 505 nm. in (Milton Roy Genesys 5 Spectronic) Spectrophotometer.

Determination Of Brain Monoamine Concentrations

The monoamines were assayed according to Paulose *et al* (1988). The tissues from brain regions were homogenised in 0.4N Perchloric acid. The homogenate was centrifuged at 5000 g for 10 minutes at 4°C (Kubota refrigerated centrifuge) and the clear supernatant was filtered through 0.45 μm HPLC grade filters and used for HPLC analysis.

Norepinephrine (NE), epinephrine (EPI), dopamine (DA) 5-hydroxy tryptamine (5-HT), 5-hydroxyindole acetic acid (5-HIAA), 5-hydroxytryptophan (5HTP), normetanephrine (NMN) and homovanillic acid (HVA) were determined in high performance liquid chromatography (HPLC) with electrochemical detector (HPLC-EC) (Shimadzu , Japan) fitted with CLC-ODS reverse phase columns of 5 μm particle size,

4.6 mm internal diameter and 25 cm length. The mobile phase consisted 75 mM sodium dihydrogen orthophosphate, 1mM sodium octyl sulfonate, 50mM EDTA and 7% acetonitrile. The pH was adjusted to 3.25 with orthophosphoric acid, filtered through 0.45 μm filter (Millipore) and degassed. A Shimadzu (model 10 AS) pump was used to deliver the solvent at a rate of 1 ml/min. The catecholamines were identified by an amperometric detection using an electrochemical detector (Model 6A, Shimadzu, Japan) with a reduction potential of + 0.8 V, with the range set at 16 and a time constant of 1.5 seconds. Twenty microlitre aliquots of the acidified supernatant were injected into the system. The peaks were identified by relative retention times compared with external standards and quantitatively estimated using an integrator (Shimadzu, C-R6A - Chromatopac) interfaced with the detector. Data from different brain regions of the experimental and control rats were statistically analysed and tabulated.

Determination Of Protein Content

Protein content of the tissues used for radioreceptor assays, insulin secretion studies and cAMP determination were determined in (Milton Roy Genesys 5- Spectronic) Spectrophotometer using BSA as standard according to Lowry *et al* .,(1951).

When pancreatic islet cell suspension was used for protein determination, the cells were lysed in 0.1 N NaOH for 5 minutes before the assay. The denatured protein samples after cAMP determination, were digested overnight at 37°C in 0.1N NaOH and used for assay.

The principle of the assay is that protein reacts with Folin-ciocalteau reagent to give a coloured complex. The colour so formed is due to the reaction of the alkaline copper with the protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of colour depends on the amount of these aromatic amino acids present and will vary for different proteins.

The reagents used in the assay were 0.1% (w/v) copper sulphate pentahydrate, 0.2% (w/v) sodium potassium tartarate and 2% (w/v) sodium carbonate in 0.1 N sodium

hydroxide. Protein reagent was freshly prepared by mixing the above reagents in the ratio 1:1:100.

The assay tubes consisted of a blank and different concentrations of bovine serum albumin (1mg/ml) and unknown samples. The assay mixture consisted of appropriately diluted unknown samples to a volume of 0.5 ml with distilled water and 1 ml of protein reagent. The mixture was incubated at room temperature for 15 minutes. 0.25 ml of 2N Folin-Ciocalteau phenol reagent (1:1 diluted with distilled water) was added to the reagent and the incubation continued at room temperature for 40 minutes. The intensity of the purple blue colour formed was proportional to the amount of protein which was read in spectrophotometer at 660 nm.

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

BRAIN

Adrenergic Receptors

The binding studies were done according to U'prichard and Snyder (1977) with slight modifications.

Preparation Of The Rat Brain Particulate Fraction For Adrenergic Receptor Binding Studies

The tissue used for the assay is brain stem. The brain tissue was homogenised in 20 volumes of ice cold 50mM Tris/HCl buffer, pH 7.7 at 25°C, with teflon-glass homogeniser for thirty seconds. The homogenates were centrifuged twice at 45,000 g in a RP21 rotor in a Hitachi (SCP 85) ultracentrifuge for 16 minutes at 4°C, with rehomogenisation in fresh buffer between centrifugations. The final pellet was resuspended in 1 ml of Tris/HCl buffer pH 7.7 containing 1.0μM pargyline and incubated for 15 minutes at 25°C and then kept in ice.

Determination Of [^{3}H] Norepinephrine Binding In Brain Particulate Preparation

Ligand used was l-[7,8-^{3}H] norepinephrine, specific activity 39.0 Ci/mmol, stored under nitrogen at 5°C. Membrane binding assays were performed in 0.5 ml incubations containing 0.15 - 0.2 mg protein concentration of particulate preparation of brain stem, ascorbic acid-0.004%, catechol-1mM, disodium EDTA-0.1mM, dithiothreitol-10μM, 50mM Tris/ HCl buffer, pH 7.7; 2.5nM to 20nM of [^{3}H] norepirephrine with and without 100μM cold norepinephrine to determine non specific binding. After incubation at 37°C for 15 minutes, the contents of the tube were rapidly filtered through (Whatman) GF/B filters and washed thrice with 5ml of ice cold buffer containing 50mM Tris/HCl pH 7.7, 1.0mM catechol and 0.1% ascorbic acid using manifold filtering apparatus (Millipore). 5 ml of Hisafe cocktail (Pharmacia) was added to dried filters and kept overnight [^{3}H] norepinephrine bound to the membranes was determined by counting the samples in a liquid scintillation counter (Wallac 1409).

Displacement Analysis Using [^{3}H] Norepinephrine:

The cold antagonists used for competition binding assays with [^{3}H]-norepinephrine were prazosin for α_1-adrenergic, yohimbine for α_2–adrenergic, propranolol for β–adrenergic and atenolol for β_1–adrenergic receptors. The assay mixture (0.5 ml.) contained 0.15-2.0 mg. of the particulate membrane fraction, ascorbic acid-0.004%; catechol-1mM; disodium EDTA-0.1 mM; dithiothreitol-10μM; Tris/HCl buffer- 50 mM pH 7.7; 10nM [^{3}H] norepinephrine and 10^{-8} M to 10^{-4} M concentrations of various competing antagonists dissolved in ascorbic acid (0.0075%). The mixture was incubated for 15 minutes at 37°C. The reaction was stopped by filtering immediately through Whatman GF/B filters with three washes of 5 ml of ice cold buffer containing 50mM Tris/HCl buffer pH 7.7; 1mM catechol and 0.1% ascorbic acid, using manifold filtering apparatus (Millipore). [^{3}H]-norepinephrine bound to the membrane was determined as described earlier.

Analysis Of The Receptor Binding Data

The data was analysed according to Scatchard (1949). The data included both total and non specific binding at many concentrations of radioligand and the specific binding was calculated as the difference between the two. Two binding parameters maximal binding (B_{max}) and equilibrium dissociation constant (K_d) were derived by linear regression analysis by plotting the specific binding of the radioligand on X-axis and bound/free on Y-axis. The maximal binding is a measure of the total number of receptors present in the tissue and the equilibrium dissociation constant is the measure of the affinity of the receptors for the radioligand. The K_d is inversely related to receptor affinity or the strength of binding.

Displacement Curve Analysis

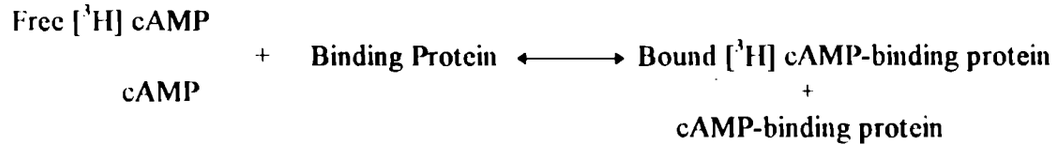
The receptor data was analysed using iterative non-linear regression using a computer program Prism, Graph Pad Software, Inc. USA. The concentration of competitor that competes for half the specific binding was defined as EC_{50}. It is same as the IC_{50}. In tables EC_{50} values are expressed as log EC_{50}. If the concentrations of unlabelled compound are equally spaced on a log scale the uncertainty of log EC_{50} will be symmetrical, but the uncertainty of EC_{50} will not be symmetrical (Unnerstall, 1990). The affinity of the receptor for the competing drug designated as the K_i and defined as the concentration of the competing ligand that will bind to half the binding sites at equilibrium in the absence of radioligand or other competitor (Cheng and Prusoff, 1973). The displacement data were analysed using one site versus two site binding models.

SECOND MESSENGER STUDIES

Assay Of cyclic Adenosine Monophosphate (cAMP)

cAMP assay kit (Amersham, England) was used. The assay is based on the competition between unlabelled cAMP and a fixed quantity of tritium labelled compound

for binding to a protein which has a high specificity and affinity for cAMP. The amount of labelled protein-cAMP complex formed is inversely related to the amount of unlabelled cAMP present in the assay sample. Measurement of the protein-bound radioactivity enables the amount of unlabelled cAMP in the sample to be calculated.



Separation of the protein bound cAMP from unbound nucleotide is achieved by adsorption of the free nucleotide on to a coated charcoal, followed by centrifugation. An aliquot of the supernatant is then removed for liquid scintillation counting. The concentration of unlabelled cAMP in the sample is then determined from a linear standard curve.

Tissue Preparation For cAMP Assay

The brain tissues were homogenised in acidic ethanol (1ml 1N HCl/100 ml ethanol) and allowed to stand for 5 minutes at room temperature. The homogenate was centrifuged at 13,000 g for 5 minutes at 4°C (Plastocraft refrigerated centrifuge). The supernatant was collected. The precipitate was washed with ethanol: water (2:1) and again centrifuged. The supernatants were combined and evaporated to dryness at 55°C under vacuum. The residue was dissolved in 0.5ml of Tris/EDTA buffer pH 7.5 containing 0.05M Tris and 4mM EDTA. The suspension was centrifuged to remove insoluble residues and the supernatant was used directly in the assay.

Assay Protocol

The tubes were placed on a water bath at 0°C. The assay mixture consisted of different concentrations of standard, [^{3}H] cAMP and binding protein in case of standards, buffer,[^{3}H]-cAMP and binding protein for zero blanks and unknown sample, [^{3}H]-cAMP

and binding protein for determination of unknown samples. The mixture was incubated at +2°C for two hours. Cold charcoal reagent was added to the tubes and the tubes were immediately centrifuged at 12,000g for 2 minutes at +2°C. Aliquots of the supernatant was immediately transferred to scintillation vials and mixed with cocktail T and counted in a liquid scintillation counter (Wallac 1409).

C_o/C_x is plotted on the Y-axis against pico moles of inactive cAMP on the X-axis of a linear graph paper, where C_o is the counts per minute bound in the absence of unlabelled cyclic AMP and C_x is the counts per minute bound in the presence of standard or unknown unlabelled cAMP. From the C_o/C_x value for the sample, the number of pico moles of inactive cAMP can be calculated.

PANCREATIC ISLETS

Isolation Of Islets And Cells

Islets of Langerhans were isolated from male Wistar rats of different age groups by standard collagenase digestion procedures (Howell and Taylor, 1968) using aseptic techniques.

The islets and cells were isolated in HEPES-buffered Earle's medium (EH) (Pipeleers *et al.*, 1985) with the following composition: 124mM NaCl, 5.4mM KCl, 1.8mM $CaCl_2$, 0.8mM $MgSO_4$, 1mM NaH_2PO_4, 14.3mM $NaHCO_3$, 10mM HEPES with 0.2% (w/v) BSA (Fraction V), equilibrated with 5% CO_2 and pH 7.30 at room temperature. Autoclaved triple distilled water was used for making up the medium and the medium was filtered through 0.22 μm filters (Millipore).

Splenic portion of the pancreas was aseptically removed into a sterile petri dish containing ice cold EH medium, and excess fat and blood vessels were removed. The pancreas were cut into small pieces and the pieces transferred to a sterile glass vial containing 2 ml of a solution of collagenase type V (1.5 mg/ml in EH), pH 7.4. The collagenase digestion was conducted for 15 minutes at 37°C in water bath shaker. The tissue digest was filtered through 500 μm nylon screen and the filtrate washed with three

successive centrifugations and resuspensions in cold EH medium.

The washed filtrate was transferred to a sterile petridish with a black base and examined under a dissection microscope. Islets visible as yellowish white spheres were hand-picked carefully by finely drawn pasture pipettes and aseptically transferred to 25 ml tissue culture flasks containing RPMI 1640, supplemented with 10% foetal calf serum, penicillin 100U/ml and streptomycin 100 µg/ml. The islets were then cultured overnight in a humidified atmosphere of 5% CO_2 in air at 37°C in an incubator.

Islets cultured overnight were harvested and washed by three sedimentations in calcium free EH medium containing 1mM EGTA and 2-10µg/ml DNAase. The suspension was maintained for 15 minutes at room temperature, continuously aspirated through a siliconized pasteur pipette. The cell dissociation was monitored using phase contrast microscope (Nikon). The islet-cell suspension was immediately diluted with 40 ml ice cold calcium free EH medium and filtered through a 63 µm nylon screen to remove occasional large cell clumps and undigested materials. The cells were then harvested by centrifugation, at 300 g for 6 minutes and rinsed in isotonic 10mM Tris buffer pH 7.4 before use in further experiments. Cell viability was tested by trypan blue exclusion method.

Determination Of [^{3}H] Norepinephrine Binding On Pancreatic Islet Cells

The procedure of Fyles, *et al*, (1987) was followed with modifications for determination of adrenergic receptors on pancreatic islet cells. 0.5ml of the incubation mixture consisted of 0.15mg protein of islet cell suspension, 10mM Tris/275mM sucrose buffer, pH 7.4 containing 1mM EGTA, 0.8mM ascorbic acid; 3mM catechol; 10mM magnesium chloride 2.5nM to 25nM [^{3}H] norepinephrine with and without 100µM cold norepinephrine, to determine non specific binding. after incubation at 37°C for 15 minutes, the contents of the tube were rapidly filtered through Whatman GF/B filters and washed thrice with 5 ml of ice cold 50 mM Tris/HCl buffer pH 7.4, using manifold filtering unit. [^{3}H] norepinephrine bound to the cell surface was determined in a liquid scintillation counter as described previously.

Displacement Analysis Using [^{3}H] Norepinephrine:

The cold antagonists used for competitive binding assays with [^{3}H]-norepinephrine were prazosin for α_1-adrenergic, yohimbine for α_2-adrenergic, propranolol for β-adrenergic and atenolol for β_1-adrenergic receptors.

The mixture (0.5ml) contained 0.15-2.0mg. protein of islet cell suspension, 10mM Tris/275mM sucrose buffer pH 7.4 containing 1mM EGTA, 0.8mM ascorbic acid, 3mM catechol, 10mM magnesium chloride, 5nM [^{3}H]-norepinephrine and 10^{-8} M to 10^{-4} M concentration of various competing antagonists dissolved in ascorbic acid. The mixture was incubated for 15 minutes at 37°C. The reaction was stopped by filtering immediately through Whatman GF/B filters with three washes of 5ml ice cold 50mM Tris / HCl buffer pH 7.4, using manifold filtering unit. [^{3}H] norepinephrine bound to the cell surface was determined using liquid scintillation counter as described earlier.

Insulin Secretion Studies

Islets were prepared by standard collagenase digestion method of Howell and Taylor (1968) using aseptic techniques and washed with HEPES-buffered Earle's medium (EH), composition already described. The islets were preincubated for 1 hour at 37°C in EH containing 4mM glucose. The islet suspension was centrifuged at 4°C at 500 g (Plastocraft refrigerated centrifuge). This is to remove the inherent insulin. The preincubated islets were then washed thrice with cold 10mM Tris/HCl buffer pH 7.4 and finally resuspended in the sample buffer. Static incubation was conducted. Each 0.5ml of incubation mixer contained 50µl of islet suspension, test substance plus 4mM glucose and 20mM glucose respectively in EH medium. The islets were incubated for 40 minutes at 37°C in an incubator. After incubation, the supernatant was removed by centrifugation at 10,000g for 10 minutes. The supernatant was stored at -20°C for insulin assay and the islet pellet was stored at -70°C for cAMP assay.

Radioimmuno Assay of Insulin

Principle of the assay

The radioimmuno assay method is based upon the competition of unlabelled insulin in the standard or samples and radio-iodinated (^{125}I) insulin for the limited binding sites on a specific antibody. At the end of incubation, the antibody bound and free insulin are separated by the second antibody, polyethylene glycol (PEG) aided separation method. Insulin concentration of samples is quantitated by measuring the radioactivity associated with bound fraction of sample and standards.

Assay Protocol

50 µl of standards (ranging from 0 to 200 µU/ml) 50 µl of Insulin free serum 50 µl insulin antiserum and volume made up to 250 µl with assay buffer were incubated overnight at +2°C. To this mixture 50µl of ^{125}I Insulin was added and again incubated at room temperature for 3 hours. This was followed by addition of 50µl of second antibody and 500µl of PEG. The tubes were vortexed and incubated for 20 minutes at the end of which they were centrifuged at 1500g for 20 minutes and the supernatant aspirated out and the radioactivity in the pellet was determined by liquid scintillation counting after pouring the cocktail into it. The unknown samples were assayed in the same way except that Insulin free serum was avoided. Instead, the mixture contained the same volume of buffer.

A standard curve was plotted with $\%B/B_0$ on the Y-axis and insulin concentration/ml on the X axis of a log-logit graph. $\%B/B_0$ was calculated as:

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

Insulin concentration sample can be directly read from the standard curve from the $\%B/B_0$ value.

Second Messengers

cAMP Assay

The islets stored after insulin secretion studies as well as islets from control and diabetic rats of different age groups were sonicated in 200µl of acid ethanol and centrifuged at 13,000 g for 10 minutes at +4°C. The supernatant was stored and the pellet was washed with 100µl of ethanol: water (2:1), recentrifuged and the supernatant was pooled with the first supernatant. This was evaporated to dryness at 55°C under vacuum. The residue was resuspended in 50 mM Tris/4mM EDTA buffer. The suspension was recentrifuged to sediment any undissolved residue and clear supernatant was used for the assay. Assay protocol was the same as the one described for brain tissues.

Determination Of Pancreatic Monoamines

Isolated pancreatic islets and whole pancreas were homogenised in 0.1.N. perchloric acid and the homogenate centrifuged at 1000 g for 10 minutes at +4°C. The supernatant was filtered through 0.22 µm filters and 20µl aliquots were used for the determination of monoamines using HPLC. The details of the HPLC analysis is the same as the one described for determination of brain monoamines.

Statistical Analysis Of The Data

Statistical valuations were done by ANOVA test using INSTAT (Ver.2.04a) Computer program.

Results

RESULTS

Body weight and blood glucose in different age groups of rats

The body weight and blood glucose levels of 6-10 week old were monitored (Table-1). There was a significant reduction ($p<0.01$) in body weight of diabetic rats compared to controls. No significant alterations were observed in other experimental groups. The blood glucose levels were significantly increased in ($p<0.001$) in diabetic rats compared to controls. No significant alteration in blood glucose levels were noted in other experimental groups.

There was a significant reduction ($p<0.05$) in body weight of diabetic rats compared to control in 40-60 week old rats (Table-2). The blood glucose was significantly elevated ($p<0.001$) compared to controls. Insulin treatment could not reverse the blood glucose levels in 40-60 week old rats unlike young rats. It remained significantly high ($p<0.001$) compared to controls.

In 72-104 week old rats there was a significant reduction in the body weight ($p<0.05$) of diabetic rats compared to controls while in other experimentals, no significant change was noted (Table-3). Blood glucose levels were significantly elevated in diabetic ($p<0.01$) compared to controls. Insulin therapy significantly reduced ($p<0.001$) the blood glucose levels compared to diabetics, but it remained significantly high ($p<0.05$) compared to controls. Adrenalectomy in diabetic rats also significantly brought down ($p<0.001$) the blood glucose levels compared to diabetic but remained significantly high ($p<0.05$) compared to control. Similarly metyrapone treatment to old diabetic rats, resulted in significant reduction ($p<0.001$) of blood glucose compared to diabetics. However, the blood glucose levels remained significantly high ($p<0.01$) compared to respective controls.

Brain monoamines and their metabolites as a function of age

Hypothalamus

There is a significant increase ($p<0.001$) in NE content in the hypothalamus of 40-60 week and 72-102 week old rats compared to 6-10 week old rats (Table-4). There was

a significant decrease ($p<0.01$) in the content of DA in 40-60 week old rats compared to 6-10week old rats. But this decrease was overcome in 72-102 week old rats, which showed a significant increase ($p<0.01$) compared to 40-60 week old rats. No significant change was noted in HVA content.

Serotonin (5-HT) content in 40-60 week decreased significantly ($p<0.01$) compared to 6-10 week old. However, no significant change was noted in the 5-HT content of 72-104 week old rats compared to 6-10 week old. 5-HT content in 72-104 week old decreased significantly ($p<0.01$) compared to 40-60 week old. No significant change was noted in the content of 5-hydroxytryptophan (5-HTP), a precursor of 5-HT. There was a significant increase ($p<0.001$) in 5-HIAA content in 40-60 week and 72-104 week old rats compared to 6-10 week old rats. However, 72-104 week old rats showed a significant reduction ($p<0.001$) in 5-HIAA content compared to 40-60 week old.

Brain Stem

There is a significant increase in the NE content ($p<0.01$) in 40-60 week old and 72-104 week old rats compared to 6-10 week old rats (Table-5). No significant change was noted in the DA content as a function of age. But HVA, showed age-related alterations. HVA content decreased significantly ($p<0.001$) in 40-60 week and 72-104 week old rats compared to 6-10 week olds. 5-HT content increased significantly ($p<0.01$) in 40-60 and 72-104 week old rats compared to 6-10 week old rats. No change in the 5-HTP content was noted in 40-60 week old rat compared to 6-10 week old rats. However, in 72-104 week old rats there was a significant reduction ($p<0.01$) compared to 6-10 week and 40-60 week old rats. 5-HIAA content increased significantly ($p<0.001$) in 40-60 week old rats compared to 6-10 week old rats. 72-104 week old rats did not show any significant change compared to 6-10 week old, however, 5-HIAA content decreased significantly ($p<0.001$) in 72-104 week old rats compared to 40-60 week old rats.

Corpus Striatum

There is a significant increase ($p<0.001$) in NE content in 40-60 week and 72-104 week old rats compared to 6-10 week old rats (Table-6). DA content showed a

significant reduction ($p<0.01$) in 40-60 week old and in 72-104 week old rats ($p<0.001$) compared to 6-10 week old. HVA content also decreased significantly ($p<0.01$) in 40-60 week and in 72-104 week old rats ($p<0.001$) compared to 6-10 week old. 5-HT content increased significantly ($p<0.001$) compared to 6-10 week old rats. 72-104 week old rats showed no significant change in 5-HT content compared to 6-10 week old rats. However, 72-104 week old rats showed significant decrease ($p<0.001$) in 5-HT content compared to 40-60 week old rat. 5-HTP content increased significantly ($p<0.001$) in 40-60 week old rats compared to 6-10 week old rats. 72-104 week old rats showed a significant increase ($p<0.01$) in 5-HTP compared to 6-10 week old rats, but 5-HTP content decreased significantly ($p<0.001$) compared to 40-60 week old rats. 5-HIAA content also increased significantly ($p<0.001$) in 40-60 week old and 72-104 week old rats compared to 6-10 week old. In 72-104 week old rats there was a significant decrease ($p<0.001$) in 5-HIAA compared to 40-60 week old rats.

Cerebral Cortex

No significant alteration in NE content was noted in 40-60 week old rats compared to 6-10 week old rats. However, in 72-104 week old rats, there was a significant increase ($p<0.01$) in NE content compared to 6-10 week and 40-60 week old rats (Table-7). DA content did not show any significant change. However, HVA showed an age-related decrease ($p<0.01$) in 40-60 week old and in 72-104 week old rats ($p<0.001$) compared to 6-10 week old rats. 72-104 week old rats showed a further decrease ($p<0.05$) in HVA content compared to 40-60 week old rats. 5-HT and 5-HTP did not show any significant alteration as a function of age. 5-HIAA content increased significaly ($p<0.01$) in 40-60 week old rats compared to 6-10 week old. 72-104 week old rats did not show any significant change compared to 6-10 week old however, there was a significant decrease ($p<0.01$) in 5-HIAA compared to 40-60 week old rats.

Cerebellum

There is a significant increase ($p<0.001$ and $p<0.01$) in NE content in 40-60 week old and 72-104 week old rats respectively compared to 6-10 week old (Table-8). DA

content did not show any significant change in 40-60 week old rats compared to 6-10 week old. However, 72-104 week old rats showed a significant increase ($p<0.05$) compared to 6-10 week and 40-60 week old rats. HVA content increased significantly ($p<0.01$) in 72-104 week old rats compared to 6-10 week old rats. In 40-60 week old rats, HVA content could not be detected. An age related increase in 5-HT content was noted. There was a significant increase ($p<0.001$) in 5-HT content in 72-104 week old rats compared to 40-60 week old rats. 5-HT content could not be detected in 6-10 week old rats. No significant alteration in the 5-HTP content was noted as a function of age. There was a significant decrease ($p<0.05$) in 5-HIAA in 40-60 week old and 72-104 week old rats compared to 6-10 week old rats.

Brain monoamines and their metabolites in diabetes as a function of age

Alterations in 6-10 week old rats

Hypothalamus

There is significant increase ($p<0.001$) in NE content in diabetic state compared to controls (Table-9). Insulin therapy, adrenalectomy and metyrapone treatment decreased the NE content significantly ($p<0.001$) compared to diabetic state. No significant change in DA content was noted. However, metyrapone treatment increased the DA content significantly ($p<0.05$) compared to control, adrenalectomised ($p<0.001$), diabetic ($p<0.001$) and insulin treated group ($p<0.01$). HVA content did not show any significant alteration in diabetic state compared to control. However, metyrapone treatment resulted in significant increase ($p<0.001$) in HVA content compared to control, diabetic, insulin, treated and adrenalectomised groups. 5-HT content decreased in diabetic state compared to controls, though not significantly. Insulin treatment elevated the 5-HT content compared to control. Adrenalectomy could not reverse the reduction in 5-HT. However, metyrapone treatment significantly increased ($p<0.001$) 5-HT content compared to control, diabetic, insulin treated and adrenalectomised groups. 5-HTP content decreased significantly ($p<0.001$) in all other groups compared to control. 5-HIAA content did not

show any significant alteration in all the groups, except adrenalectomised, which increased the 5-HIAA content significantly ($p<0.001$) compared to all other experimental groups.

Brain Stem

NE content increased significantly ($p<0.001$) compared to control. Insulin treatment, adrenalectomy and metyrapone treatment reversed the alteration in NE to control values (Table-10). There was a significant reduction ($p<0.001$) in NE content in all these groups compared to diabetics. There was no significant alteration in DA content in diabetic state, compared to controls. Metyrapone treatment resulted in significant increase ($p<0.001$) in DA content compared to other groups. HVA content also did not show any significant change in diabetic state. Insulin treatment and adrenalectomy decreased the HVA content significantly ($p<0.05$) compared to control. There was a reduction in the content of 5-HT in diabetic though not significantly compared to control. Insulin treatment reversed the 5-HT content to control values. Adrenalectomy showed no significant change in 5-HT levels in diabetic rats. However, metyrapone treatment significantly increased 5-HT values ($p<0.001$) compared to all other groups. 5-HTP content decreased in diabetic state, though not significantly compared to control. Insulin treatment did not reverse the 5-HTP content to control values. However, adrenalectomy significantly increased ($p<0.001$) the 5-HTP content compared to control, diabetic and insulin treated groups. 5-HTP content could not be detected in metyrapone treated groups. Insulin treatment decreased the turnover ratio though not significantly. Adrenalectomy resulted in significant increase ($p<0.001$) in the turnover ratio, compared to all other groups. 5-HIAA content also did not show any significant change in diabetic state compared to control. Insulin treatment significantly reduced 5-HIAA content ($p<0.01$) compared to diabetic and compared to control ($p<0.05$). Adrenalectomy resulted in a significant increase ($p<0.05$) in 5-HIAA compared to diabetic and control and compared to insulin treated groups ($p<0.001$). Metyrapone treatment significantly reduced ($p<0.001$) the 5-HIAA content compared to adrenalectomised, diabetic and control groups.

Corpus Striatum

A significant ($p<0.001$) increase in NE content was noted in diabetic state compared to control (Table-11). Insulin treatment, metyrapone treatment and adrenalectomy resulted in a significant decrease ($p<0.001$) in NE content compared to diabetic state. DA content showed a significant increase ($p<0.001$) compared to control. Insulin, metyrapone treatment and adrenalectomy showed a significant reduction ($p<0.001$) in DA content compared to diabetic state. HVA content decreased significantly in ($p<0.05$) in diabetic compared to control. Insulin therapy and adenalectomy could not reverse the HVA content to control values it remained significantly low ($p<0.05$) compared to control. Metyrapone treatment to diabetic rats, reversed HVA content to control values. HVA content in these groups increased significantly $p<0.05$ compared to all other groups. 5-HT content decreased in diabetic state, though not significantly compared to control. Insulin treatment and adrenalectomy could reverse the 5-HT content to near normal values. However, metyrapone treatment increased the 5-HT content significantly ($p<0.001$) compared to all other groups. 5-HTP content showed a significant increase ($p<0.05$) in diabetics compared to control. Insulin therapy and adrenalectomy significantly reduced ($p<0.05$) the 5-HTP content in diabetic rats to near normal values. Metyrapone, did reduce the 5-HTP content in the diabetic state though not significantly. 5-HIAA content increased in diabetic state compared to control, though not significantly. Insulin treatment significantly reduced ($p<0.001$) the 5-HIAA content compared to diabetics. Adrenalectomy resulted in a significant increase ($p<0.001$; $p<0.002$ and $p<0.01$) in 5-HIAA content compared to control, insulin treated and diabetic groups respectively. Metyrapone, like insulin, reversed the 5-HIAA content, significantly ($p<0.001$) compared to diabetic and adrenalectomised groups.

Cerebral cortex

No significant change in NE content was noted in the diabetic state (Table-12). Insulin treatment, resulted in significant reduction in NE content compared to diabetic and control groups respectively. Adrenalectomy significantly increased ($p<0.001$) the NE content compared to control, diabetic and insulin treated groups. Metyrapone treatment,

on the other hand, resulted in a significant decrease in NE content ($p<0.001$) compared to adrenalectomised group, it also significantly increased ($p<0.05$) the NE content compared to insulin treated groups. DA content increased in the diabetic state though not significantly, compared to control. Insulin treatment and adrenalectomy reduced the DA content, though not significantly compared to control and diabetic groups. Metyrapone treatment significantly increased ($p<0.001$) the DA content compared to control, diabetic insulin treated and adrenalectomised groups. HVA content decreased in diabetic state though not significantly. Insulin treatment further decreased the HVA content. Adrenalectomy did not produced any change compared to diabetic state in HVA content. Metyrapone treatment increased, the HVA content significantly ($p<0.05$, $p<0.05$ and $p<0.001$) compared to diabetic, adrenalectomised and insulin treated groups respectively. 5-HT content decreased in diabetic state, though not significantly compared to control. Insulin treatment and adrenalectomy had no significant effect on 5-HT content. Metyrapone treatment significantly increased ($p<0.05$) the 5-HT content, compared to control, diabetic, insulin treated and adrenalectomised groups. No significant change in 5-HTP content was noted in the diabetic state. 5-HTP content could not be detected in insulin and metyrapone treated groups. Adrenalectomy significantly reduced the 5-HTP content ($p<0.05$) compared to control and diabetic groups. 5-HIAA content did not show any significant change compared to control in diabetic as well as adrenalectomised groups. However, insulin and metyrapone treatment significantly reduced ($p<0.01$) 5-HIAA content in diabetic and adrenalectomised groups.

Cerebellum

There was a significant ($p<0.001$) increase in NE content compared to control, in diabetics (Table-13). Insulin, metyrapone and adrenalectomy significantly reduced ($p<0.001$) the NE content compared to diabetic groups. DA content did not show any significant change in diabetic group. Insulin treatment increased the DA content significantly compared to diabetic ($p<0.01$) and compared to control ($p<0.05$). Adrenalectomy further decreased the DA content significantly ($p<0.01$) compared to

insulin treated. Metyrapone treatment, significantly increased ($p<0.001$) the DA content compared to control, diabetic, insulin treated and adrenalectomised groups. On the whole, no significant alteration in the HVA content was noted in all the experiment groups.

Monoamines and their metabolites in 40-60 week old rats

Hypothalamus

The NE content decreased significantly ($p<0.01$) in diabetic compared the control (Table-14). Insulin treatment increased the NE content, but it remained significantly low ($p<0.05$) compared to controls. No significant change was noted in DA content in diabetic state compared to control. However, insulin treatment significantly increased ($p<0.001$) the DA content compared to control and diabetic. HVA content decreased significantly ($p<0.01$) in the diabetic compared to control. Insulin therapy, increased the HVA content, but it remained significantly high ($p<0.05$) compared to control. 5-HT content increased in diabetic state, though not significantly compared to control and insulin treated groups. 5-HTP did not show any significant change. There is a significant reduction ($p<0.05$) in 5-HIAA content compared to control and insulin therapy reversed the 5-HIAA level to near normal values.

Brain Stem

There is a significant increase , ($p<0.001$) in NE content in diabetic compared to control (Table-15). NE content could not be detected in insulin treated groups. No significant change was noted in DA and HVA content. There is a significant reduction ($p<0.01$) in 5-HT content in diabetics compared to controls and insulin therapy reversed the 5-HT content to near normal values. 5-HTP content increased significantly ($p<0.001$) compared to control and insulin treated groups. No significant change in 5-HIAA content was noted.

Corpus Striatum

A significant reduction ($p<0.001$) in NE content was observed in diabetic rats compared to control (Table-16). Insulin therapy further decreased the NE content ($p<0.001$) compared to control. DA content increased significantly ($p<0.01$) compared to control. Insulin therapy decreased the DA content significantly ($p<0.05$) compared to diabetic. HVA content did not show any significant change. There is a significant reduction ($p<0.001$) in 5-HT content in diabetic state compared to control and insulin treated group. 5-HTP content did not show any significant change in diabetic state. However insulin therapy resulted in a significant increase ($p<0.05$) in 5-HTP content compared to control and diabetic group. No significant change was noted in the 5-HIAA content in diabetic state. Insulin treatment increased the 5-HIAA content significantly ($p<0.01$ and $p<0.05$) compared to diabetic and control groups.

Cerebral Cortex

No significant alteration in NE content was observed in diabetic state (Table-17). DA content increased significantly ($p<0.001$) in diabetic compared to control. Insulin therapy decreased the DA content significantly ($p<0.001$) compared to diabetic group but it remained significantly high ($p<0.001$) compared to control. HVA content decreased significantly ($p<0.05$) in diabetic compared to control. Insulin treatment significantly increased ($p<0.001$) and ($p<0.05$) the HVA content compared to diabetic and control groups respectively. 5-HT content decreased in diabetes, though not significantly. 5-HTP content decreased significantly ($p<0.001$) in diabetics compared to control and insulin treated groups. A significant decrease ($p<0.05$) in 5-HIAA content was observed in the diabetic state compared to control. Insulin therapy, increased the 5-HIAA content significantly ($p<0.001$) compared to diabetics.

Cerebellum

There was a significant increase ($p<0.01$) in NE content in diabetes compared to control (Table-18). Insulin therapy decreased the NE content significantly ($p<0.05$) compared to diabetics. There was a significant increase ($p<0.01$) in DA content in

diabetics and insulin treated group compared to control. A significant increase ($p<0.01$) in 5-HT content was noted in the diabetic state compared to control. Insulin therapy partially reversed the 5-HT levels. 5-HTP did not show any significant change. A significant increase ($p<0.05$) in 5-HIAA content was noted in both diabetic and insulin treated groups.

Monoamines And The Metabolites In 72-104 Week Old Rats

Hypothalamus

In this age group of rats also, there was a significant ($p<0.01$) reduction in NE content compared to control (Table-19). Insulin treatment had no effect in reversing the NE content, it remained significantly low ($p<0.01$) compared to control. Adrenalectomy also resulted in significant reduction ($p<0.001$, $p<0.01$ and $p<0.05$) in NE content compared to control, insulin treated and diabetic groups respectively. Metyrapone treatment further decreased ($p<0.001$) the NE content compared to control, diabetic and insulin treated groups, also there was a significant reduction ($p<0.01$) in NE content compared to adrenalctomised groups. DA content in diabetes decreased significantly ($p<0.01$) compared to control. Insulin treatment and adrenalectomy resulted in an increase in DA content, but the values remained significantly lower ($p<0.05$) than the control. No significant alteration in HVA content was noted in the diabetic rats. In adrenalectomised, and metyrapone treated rats, HVA content could not be detected. No significant change was noted in the 5-HT and 5-HTP content in all the experimental groups. 5-HIAA content decreased significantly ($p<0.001$) compared to control and insulin treated groups. Adrenalectomy and metyrapone treatment, though increased the 5-HIAA content, the values remained significantly low ($p<0.001$) compared to control and insulin treated groups.

Brain Stem

There is a significant decrease ($p<0.001$) in NE content in diabetic state compared to control and insulin treated groups (Table-20). Adrenalectomy and metyrapone

treatment did not reverse the NE content. It remained significantly low ($p<0.001$) compared to control and insulin treated groups. No significant alteration in the DA content was noted in all the experimental groups except adrenalectomised, where there was a significant reduction ($p<0.05$) in DA content compared to control and insulin treated groups. HVA content did not show any significant change in diabetic state compared to control and metyrapone treated groups. Insulin treatment and adrenalectomy significantly decreased ($p<0.001$) the HVA content compared to control and diabetic groups. There was a significant reduction in 5-HT content, in diabetes compared to the control. Insulin, adrenalectomy and metyrapone treated groups, increased the 5-HT content significantly ($p<0.001$) compared to diabetic but compared to controls, these groups had significantly low ($p<0.001$) 5-HT content. No significant change was noted in 5-HTP content. No significant alteration was noted in the 5-HIAA content in diabetes compared to control. However, insulin treatment, significantly increased ($p<0.01$) the 5-HIAA content compared to all other groups.

Corpus Striatum

There is a significant increase ($p<0.01$) in NE content compared to control (Table-21). Insulin treatment further increased the NE content significantly ($p<0.001$) compared to all other groups. In adrenalectomised and metyrapone treated groups, there was a significant reduction ($p<0.001$) in NE content compared to all other groups. No significant change in DA and HVA content was noted in all the experimental groups. There was no significant change in 5-HT content in diabetic state compared to control. Insulin treatment significantly increased the 5-HT content ($p<0.01$ and $p<0.001$) compared to control and diabetic groups respectively. No significant change was noted in the 5-HTP content in the diabetic state. No significant change was noted in the 5-HIAA content in diabetic group compared to control and insulin treated groups. While in the adrenalectomised and metyrapone treated groups there was a decrease in the 5-HIAA content ($p<0.01$) compared to control, diabetic and insulin treated groups.

Cerebral Cortex

No significant change was noted in the NE, DA, 5-HT, 5-HTP, 5-HIAA and HVA content in diabetic state compared to control (Table-22).

Cerebellum

No significant change was noted in the NE content in diabetic compared to control (Table-23). However, adrenalectomy and metyrapone treatment significantly decreased ($p<0.001$) the NE content compared to diabetic. No significant change was noted in DA content in diabetic state compared to control. However, HVA content decreased significantly ($p<0.001$) compared to control. HVA content could not be detected in all other groups. 5-HT content decreased significantly ($p<0.001$) in diabetic rats compared to control. 5-HT content could not be detected in adrenalectomised and metyrapone treated groups. 5-HTP content increased significantly ($p<0.001$) compared to control. In all other experimental groups, 5-HTP content could not be detected. 5-HIAA content increased significantly ($p<0.01$) in diabetes compared to control. Insulin therapy could not reverse the change in 5-HIAA content which remained significantly high ($p<0.01$) compared to control. Adrenalectomy decreased the 5-HIAA content significantly ($p<0.01$) compared to diabetic. Metyrapone treatment significantly reduced ($p<0.001$) the 5-HIAA content compared to diabetic, insulin treated and adrenalectomised groups.

[^{3}H] Noradrenergic receptor binding in brain stem

There is a differential regulation in the noradrenergic receptor binding parameters as a function of age in diabetic state (Table-24; Fig. 1). On comparing control young and old, there is a decrease in the B_{max} of total noradrenergic receptors in old rats. A decrease in the K_d ($p<0.05$) was noted in old rats. In the diabetic state, both young ($p<0.01$) and old rats ($p<0.01$) showed significant increase in the B_{max} compared to respective controls. The K_d value in young diabetics decreased significantly ($p<0.05$) compared to control, while in old diabetics there was no significant change. Insulin therapy could reverse the B_{max} value to control values. In insulin treated young diabetic rats, the K_d further

decreased ($p<0.01$) compared to control and ($p<0.01$) compared to diabetic young while in the old, the K_d increased ($p<0.01$) compared to control and ($p<0.01$) compared to diabetic old.

cAMP content in brain stem

In old animals, there is a significant increase ($p<0.01$) in cAMP content compared to young controls (Table-25). However, in the diabetic state, there was no change in cAMP content in old rats. Young diabetic rats showed a significant increase ($p<0.01$) in cAMP content compared to control young. Old diabetic rats showed significant decrease ($p<0.001$) in cAMP content compared to young diabetic rats. Insulin therapy, partially reversed the increase in cAMP content in young rats, but it remained significantly high ($p<0.05$) compared to controls. In old rats, insulin therapy increased the cAMP content. In comparison with young insulin treated diabetic rats, there was a significant decrease ($p<0.05$) in cAMP content in insulin treated diabetic rats.

Displacement Analysis Using [^{3}H] Norepinephrine In Brain Stem

[^{3}H] NE Vs. Prazosin: (Table-26; Figures 2 & 3)

Prazosin is an α_1-adrenergic receptor antagonist. On comparing the binding properties of control young and old rats, we see a shift in affinity with a loss of the low affinity site in the old, and a decrease in the strength of binding at the high affinity site. In diabetic young rats, the affinity was shifted towards the high affinity range with decreased strength of binding. No significant alteration was noted in old diabetic rats. Insulin treated old diabetic rats did not show any displacement. Insulin treated young diabetic rats displayed higher strength of binding at the low affinity site.

[^{3}H] NE Vs. Yohimbine: (Table-27; Figures 4 & 5)

Yohimbine is an α_2-adrenergic receptor antagonist. In control young rats, the curve best fitted for two sites with an increased strength of binding at the low affinity site.

In old rats, however, there was a loss in the low affinity site with decrease in the strength of binding at the high affinity site. In diabetic young rats, there was a shift more towards high affinity with a decrease in binding strength at the low affinity site. Insulin treated diabetic young did not show reversal to control. Diabetic old rats did not show significant change compared to control. Insulin treated old diabetic rats hardly gave any displacement.

[^{3}H] NE Vs. Propranolol: (Table-28; Figures 6 & 7)

Propranolol is a non-specific β-adrenergic blocker. In young control rats, the curve best fitted for two sites, with increased strength of binding at the low affinity site. However, in the control old rats, the curve best fitted for a single site with loss of the low affinity site. Diabetic young and old rats showed similar changes as in old control rats. There was a shift in affinity with increased strength of binding at the high affinity site. The low affinity site was lost. Insulin treatment could not reverse these effects. In old diabetic rats, there was a shift in the affinity state towards the low affinity site, with increased strength of binding compared to control old. Insulin treatment partially reversed this alteration.

[^{3}H] NE Vs. Atenolol: (Table- 29; Figures 8 & 9)

Atenolol is a β_1-adrenergic receptor antagonist. In young rats, there was hardly any displacement of [^{3}H] NE by atenolol. In old rats, the curve best fitted for a single high affinity site. However, in young diabetic rats, there was expression of this class of receptors and the curve fitted for one site. Old diabetic rats also showed single high affinity site, but the strength of binding decreased for this site. Insulin treatment did not reverse these alterations in both young and old rats.

Monoamines and their metabolites in whole pancreas

An age related decrease in NE content was noted old rats ($p<0.05$) compared to young (Table-30). In the diabetic state NE content did not show any significant change in

the young while in the old rats, there was a significant increase in NE content ($p<0.05$). Insulin therapy, more or less reversed the NE content, to control values in both age groups. There was a significant decrease ($p<0.05$) in NE content in metyrapone treated old diabetic rats compared to old diabetics, insulin treated diabetic old and metyrapone treated diabetic young. Epinephrine did not show any significant change in diabetic state. In old, metyrapone treated group, the EPI values were significantly decreased ($p<0.05$) compared to insulin treated olds. Normetanephrine (NMN) a metabolite of NE showed significant age related decrease ($p<0.001$) in old compared to young. The same trend was observed in young diabetic where NMN decreased significantly ($p<0.001$). In old diabetics no significant change was noted. Insulin and metyrapone treatment had little effect on NMN content.

Dopamine (DA) content in whole pancreas decreased as a function of age. In old rats, DA content decreased significantly ($p<0.01$) compared to young. Diabetic young rats also showed a significant decrease ($p<0.05$) compared to control young. DA content was unchanged in old diabetic rats. Insulin treatment further decreased the DA content in young rats ($p<0.001$) compared to controls. Insulin treatment in old rats increased the DA content ($p<0.01$) compared to young insulin treated. Metyrapone treatment reversed the DA content to near normal values in young rats. Metyrapone treated old rats showed no significant change in DA content. Homovanillic acid (HVA), a metabolite of dopamine was analysed. There was no change in HVA content as a function of age.

Serotonin (5-HT) did not show any significant change in all the experimental groups. There was a significant increase in 5-HIAA content, a metabolite of 5-HT, in old ($p<0.05$) compared to young. However, no significant change was noted in the diabetic state as a function of age. Insulin and metyrapone treatment also had no significant effect on 5-HIAA, except metyrapone treatment in old, where there was a significant reduction ($p<0.05$) in 5-HIAA compared to control.

Catecholamines in isolated pancreatic islets

NE levels in old control islets were significantly ($p<0.05$) higher than in young control islets, while EPI values in the old were significantly ($p<0.01$) lower compared to

the young (Table-31). NE content increased significantly in both young ($p<0.001$) and old ($p<0.01$) diabetic rats compared to respective controls. EPI content decreased significantly ($p<0.05$) in young islets compared to control. However, in old diabetic rats there was a significant ($p<0.001$) increase in the EPI content compared to controls. Insulin treatment partially reduced the increased NE content but did not reverse the increase in NE levels in, young diabetic islets. It remained significantly ($p<0.05$) high compared to control. NE could not be detected in insulin treated old diabetic islets. The EPI content in the young was completely reversed by insulin treatment, while in the old diabetic islets, there was a significant ($p<0.01$) increase in EPI content compared with controls with no trace of NE.

[^{3}H] Noradrenergic receptor binding in isolated pancreatic islets cell suspension

Our results (Table-32; Fig. 10) indicate a significant increase ($p<0.01$) in the maximal binding of [^{3}H] NE to receptors in old islets compared to young islets with no significant age related difference in the dissociation constant (K_d) or affinity. In the STZ-diabetic state, there was a significant increase in the maximal binding [^{3}H] NE to receptors in young islets ($p<0.01$) and old islets ($p<0.001$) compared to respective controls. The dissociation constant also increased significantly ($p<0.001$) in both young and old diabetes i.e., affinity of the receptors to its agonist was lowered. Insulin treatment completely reversed the alteration in K_d in both groups. However, in the case of B_{max} young rats showed reversal, while old rats showed only partial reversal.

cAMP content in isolated pancreatic islets

In the diabetic state significant age related changes were noted (Table-33). In old diabetic islets no significant change was noted. While in young diabetics cAMP levels were significantly higher ($p<0.001$) compared to both control young and diabetic old rats. Insulin treatment reversed the cAMP levels to near normal values in young rats. In old rats insulin treatment increased cAMP significantly ($p<0.05$).

Displacement Analysis Using [^{3}H]Norepinephrine In Isolated Pancreatic Islets

[3H] NE Vs. Prazosin: (Table- 34; Figures 11 & 12)

Prazosin is an α_1-adrenergic receptor antagonist. No age-related changes were noted in the affinity status of these receptors in young and old rats. In diabetic young rats, a stronger binding of the ligand to higher affinity site was observed. However, in old diabetics, the shift was towards the low affinity site with increased strength of binding. Insulin treatment resulted in loss of the low affinity sites from diabetic rats. The strength of binding at the high affinity site was decreased in insulin treated rats compared to diabetic rats, while the strength of binding decreased in the young insulin treated rats at the high affinity site compared to the diabetic young.

[^{3}H] NE Vs. Yohimbine: (Table- 35; Figures 13 & 14)

Yohimbine is an α_2-adrenergic receptor antagonist. In young control rats, the curve fitted best for two sites with greater strength of binding at the low affinity site. However, in the old control rats, the curve fitted best for one site model, with the loss of the low affinity site. In young diabetic rats, eventhough the curve fitted for two site model the ligand bound strongly at the low affinity site. In diabetic old rats, the curve fitted for single site, with a shift towards low affinity and the ligand bound to this site strongly than in controls. Insulin treatment brought about reciprocal changes in young and old. Insulin treatment to young diabetic rats resulted in loss of the low affinity site and the strength of binding of the ligand to the site also decreased. Insulin treatment to old rats, resulted in the appearance of an additional site at the low affinity range with increased strength of binding of the ligand to this site.

[^{3}H] NE Vs. Propranolol: (Table- 36; Figures 15 & 16)

Propranolol is a non-specific β-adrenergic blocker. In young and old rats, the curve fitted for single site, however, the ligand bound strongly at this site of old animals compared to the young. In diabetic state, both young and old rats revealed two sites a high affinity site and a low affinity site. The ligand bound strongly at the low affinity site.

In the old diabetic rats, the affinity shifted towards higher affinity with decreased strength of binding at the high affinity site, while in the young, the curve shifted to lower affinity site with increased strength of binding of the ligand. Insulin treatment to old and young diabetic rats partially reversed the alterations.

[^{3}H] NE Vs. Atenolol: (Table- 37; Figures 17 & 18)

Atenolol is a β_1-adrenergic receptor antagonist. In young and old rats, the curve fitted for two site model. However, the ligand bound strongly in the low affinity site of older rats compared to young. In diabetic state, in both young and old rats, the low affinity site was lost. However, the ligand bound with higher strength at the high affinity site, compared to the respective controls. Insulin treatment in young rats reversed the alterations while in the old rats, the alterations were not reversed, instead the affinity shifted to the low affinity range with increased strength of binding of the ligand for the site.

Glucose induced insulin secretion and cAMP production as a function of age *in vitro*

. There is a significant decrease ($p<0.05$) in the amount of insulin secreted by the islets of the rats compared to young at both 4mM and 20mM glucose concentration respectively (Table-38). However, both young and old islets were able to increase the secretion of insulin in response to 20mM glucose in a similar fashion though not significantly. Pancreatic islets of old rats showed significant increase ($p<0.001$) in cAMP content when incubated with 4mM glucose compared to young rats. At 20mM glucose, both young and old islets showed significant increase ($p<0.001$ and $p<0.01$ respectively) in cAMP content, however, it should be noted that the extent of increase was more marked ($p<0.001$) in young islets compared to old.

Effect of hydrocortisone and dexamethasone on glucose induced insulin secretion as a function of age *in vitro*

Hydrocortisone and dexamethasone are synthetic corticosteriods. Dexamethasone is a potent glucocorticoid. Addition of these at 10^{-6}M concentration resulted in increase in glucose induced insulin secretion while at 10^{-4}M, it appeared that these hormones exerted an inhibitory effect on insulin secretion in both young and old rats (Figure-19).

Effect of dopamine (DA) on glucose induced insulin secretion as a function of age *in vitro*

Dopamine exerted a differential influence on old and young islets (Figure 20) at 10^{-5}M concentration, dopamine had no influence at 4mM glucose but at 20mM glucose, it had an inhibitory effect on insulin secretion from old rats. In young rats dopamine appeared to have a stimulatory effect at both 4mM and 20mM glucose respectively. Spiperone is also a dopamine D_2-receptor antagonist, to see if dopamine action is mediated via these receptors, we blocked the D_2-receptors with spiperone and observed the insulin secretion. In old islets, there was no significant change in insulin secretion at 4mM glucose, while at 20mM glucose, D_2-receptors seems to have a role in insulin secretion in old rats, blocking D_2-receptors resulted in a rise in insulin secretion. In young rats, D_2-receptors appeared to have an inhibitory effect only at 20mM glucose concentration. At 4mM glucose, forskolin stimulated insulin secretion in spite of the presence of dopamine significantly while, at 20mM glucose, it did not have much influence. In young islets, forskolin had stimulatory effect only under high glucose concentration.

TABLE-1

BODY WEIGHT AND BLOOD GLUCOSE IN 6-10 WEEK OLD RATS

Animal Status	Body weight (grams)		Blood Glucose (mg / dl blood)	
	Initial Day	Final Day	Initial Day	Final Day
Control	68.00 ± 7.33	84.00 ± 9.4	88.67 ± 3.74	83.91 ± 0.25
Diabetic	73.00 ± 4.03	55.00 ± 5.00 **	88.50 ± 4.50	217.29 ± 2.96 ***
Diabetic + Insulin	65.00 ± 5.00	78.52 ± 5.23	80.56 ± 3.26	85.45 ± 4.56
Diabetic + Adrenalectomy	75.00± 5.00	100.00 ± 9.94	94.55 ± 8.02	99.12 ± 3.99
Diabetic + Metyrapone	115.00 ± 5.05	140 ± 10.43	74.37 ± 0.74	75.28 ± 2.76

Mean ± S.E.M. of 4-6 separate determinations.

** $p<0.01$ compared to initial day. ; *** $p<0.001$ compared to initial day.

TABLE-2

BODY WEIGHT AND BLOOD GLUCOSE IN 40-60 WEEK OLD RATS

Animal Status	Body weight (grams)		Blood Glucose (mg / dl blood)	
	Initial Day	Final Day	Initial Day	Final Day
Control	280.00 ± 10.00	280.00 ± 5.77	87.64 ± 4.12	75.35 ± 8.97
Diabetic	300.00 ± 5.20	246.60 ± 7.63 *	119.82 ± 11.96	377.21 ± 5.11 ***
Diabetic + Insulin	320.00 ± 1.14	276.66 ± 12.05	115.45 ± 2.35	255.41 ± 5.41 ***

Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to initial day.; *** $p<0.001$ compared to initial day.

TABLE-3

BODY WEIGHT AND BLOOD GLUCOSE IN 72-104 WEEK OLD RATS

Animal Status	Body weight (grams)		Blood Glucose (mg / dl blood)	
	Initial Day	Final Day	Initial Day	Final Day
Control	340.00 ± 11.55	356.66 ± 14.53	138.00 ± 11.62	128.66 ± 2.60
Diabetic	336.66 ± 8.55	280.00 ± 5.16 *	132.91 ± 5.98	591.32 ± 34.47 ***
Diabetic + Insulin	353.33 ± 4.03	353.33 ± 4.03	130.19 ± 8.23	205.27 ± 38.25 * †††
Diabetic + Adrenalectomy	330.00 ± 5.77	313.33 ± 8.82	141.37 ± 9.91	222.45 ± 12.55 * †††
Diabetic + Metyrapone	333.33 ± 3.30	316.30 ± 10.27	135.08 ± 11.55	300.04 ± 6.39 ** †††

Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to initial day; ** $p<0.01$ compared to initial day; *** $p<0.001$ compared to initial day.
††† $p<0.001$ compared to diabetic.

TABLE-4

MONOAMINES AND METABOLITES AND IN HYPOTHALAMUS AS A FUNCTION OF AGE

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
6-10 week old	2.11 ± 0.11	1.29 ± 0.29	0.29 ± 0.06	0.38 ± 0.14	0.27 ± 0.05	2.71 ± 0.17
40-60 week old	4.87 ± 0.04 ***	0.35 ± 0.04 **	0.34 ± 0.03	0.84 ± 0.03 **	0.45 ± 0.05	9.34 ± 0.31 ***
72-104 week old	5.22 ± 0.38 ***	1.36 ± 0.07 †††	0.18 ± 0.04	0.35 ± 0.01 †††	0.45 ± 0.09	4.63 ± 0.03 *** †††

Values are expressed as nmoles/gram wet weight tissue.
Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to 6-10 week old ;** $p<0.01$ compared to 6-10 week old;
*** $p<0.001$ compared to 6-10 week old,††† $p<0.001$ compared to 40-60 week old;
††† $p<0.001$ compared to 40-60 week old

TABLE-5

MONOAMINES AND METABOLITES IN BRAIN STEM AS A FUNCTION OF AGE

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
6-10 week old	1.57 ± 0.43	0.37 ± 0.04	1.72 ± 0.79	0.52 ± 0.10	0.26 ± 0.05	2.42 ± 0.17
40-60 week old	3.85 ± 0.45 **	0.27 ± 0.06	0.10 ± 0.04 ***	1.83 ± 0.40 **	0.26 ± 0.05	6.46 ± 0.99 ***
72-104 week old	3.60 ± 0.23 **	0.33 ± 0.05	0.15 ± 0.01 ***	2.05 ± 0.03 **	0.07 ± 0.01 **†	2.86 ± 0.40 †††

Values are expressed as nmoles/gram wet weight tissue.
Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to 6-10 week old ;** $p<0.01$ compared to 6-10 week old ;
*** $p<0.001$ compared to 6-10 week old† $p<0.05$ compared to 40-60 week old ;
††† $p<0.001$ compared to 40-60 week old

TABLE-6

MONOAMINES AND METABOLITES IN CORPUS STRIATUM AS A FUNCTION OF AGE

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
6-10 week old	0.50 ± 0.14	24.13 ± 2.24	3.56 ± 0.47	0.52 ± 0.09	0.36 ± 0.07	1.46 ± 0.23
40-60 week old	1.23 ± 0.10 ***	17.42 ± 0.82 **	2.36 ± 0.06 **	1.22 ± 0.03 ***	3.08 ± 0.14 ***	5.11 ± 0.06 ***
72-104 week old	1.32 ± 0.04 ***	14.45 ± 0.62 ***	1.65 ± 0.04 ***	0.65 ± 0.07 †††	1.21 ± 0.21 ** †††	2.99 ± 0.10 *** †††

Values are expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to 6-10 week old ;** $p<0.01$ compared to 6-10 week old;
*** $p<0.001$ compared to 6-10 week old,
††† $p<0.001$ compared to 40-60 week old

TABLE-7

MONOAMINES AND METABOLITES IN CEREBRAL CORTEX AS A FUNCTION OF AGE

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
6-10 week old	1.41 ± 0.14	0.59 ± 0.16	0.78 ± 0.07	0.80 ± 0.46	0.29 ± 0.05	2.50 ± 0.39
40-60 week old	1.41 ± 0.36	0.43 ± 0.02	0.45 ± 0.09 **	1.13 ± 0.12	0.43 ± 0.12	4.34 ± 0.55 **
72-104 week old	2.36 ± 0.12 *†	0.50 ± 0.10	0.19 ± 0.03 ***†	0.68 ± 0.23	0.14 ± 0.05	1.88 ± 0.32 ††

Values are expressed as nmoles/gram wet weight tissue.
Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to 6-10 week old ;** $p<0.01$ compared to 6-10 week old ;
*** $p<0.001$ compared to 6-10 week old, † $p<0.05$ compared to 40-60 week old;
†† $p<0.01$ compared to 40-60 week old·

TABLE-8

MONOAMINE AND METABOLITES IN CEREBELLUM AS A FUNCTION OF AGE

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
6-10 week old	0.90 ± 0.13	0.32 ± 0.11	0.15 ± 0.03	N.D.	0.14 ± 0.07	1.00 ± 0.20
40-60 week old	3.63 ± 0.29 ***	0.48 ± 0.06	N.D.	0.13 ± 0.05	0.11 ± 0.01	0.45 ± 0.04 *
72-104 week old	2.07 ± 0.29 ** †††	0.91 ± 0.20 * †	0.34 ± 0.01 **	0.30 ± 0.01 †††	0.06 ± 0.01	0.58 ± 0.06 *

Values are expressed as nmoles/gram wet weight tissue.
Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to 6-10 week old **$p<0.01$ compared to 6-10 week old;
*** $p<0.001$ compared to 6-10 week old, † $p<0.05$ compared to 40-60 week old;
††† $p<0.001$ compared to 40-60 week old

TABLE-9

MONOAMINES AND METABOLITES IN HYPOTHALAMUS OF 6-10 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	2.11 ± 0.11	1.29 ± 0.29	0.29 ± 0.06	0.38 ± 0.14	0.27 ± 0.05	2.71 ± 0.17
Diabetic	25.75 ± 1.06 ***	0.45 ± 0.08	0.27 ± 0.01	0.12 ± 0.05	0.05 ± 0.02 ***	2.90 ± 0.19
Diabetic + Insulin	2.14 ± 0.42 ###	0.92 ± 0.32	0.27 ± 0.13	1.10 ± 0.36	0.07 ± 0.01***	2.21 ± 0.40
Diabetic + Adrenalectomy	1.12 ± 0.68 ###	0.58 ± 0.34	0.13 ± 0.03	0.14 ± 0.03	0.09 ± 0.04 ***	5.46 ± 0.39 ††† *** ###
Diabetic + Metyrapone	2.44 ± 0.67 ###	2.19 ± 0.29 * ### ††	1.08 ± 0.09 *** ### ††† ###	6.30 ± 1.27*** ###††† ###	0.08± 0.01 ***	2.65 ± 0.21 ###

Values are expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations. * $p<0.05$ compared to control; *** $p<0.001$ compared to control; ‡‡‡ $p<0.001$ compared to diabetic; †† $p<0.01$ compared to insulin treated; ††† $p<0.001$ compared to insulin treated; ††† $p<0.001$ compared to adrenalectomised.

TABLE-10

MONOAMINES AND METABOLITES IN BRAIN STEM OF 6-10 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	1.57 ± 0.43	0.37 ± 0.04	1.72 ± 0.79	0.52 ± 0.10	0.26 ± 0.05	2.42 ± 0.17
Diabetic	27.73 ± 1.40 ***	0.41 ± 0.05	0.84 ± 0.05	0.21 ± 0.03	0.09 ± 0.05	2.79 ± 0.24
Diabetic + Insulin	0.41 ± 0.07 ‡‡‡	0.19 ± 0.08	0.04 ± 0.01 *	0.68 ± 0.08	0.074 ± 0.02	1.02 ± 0.43 * ‡‡
Diabetic + Adrenalectomy	1.95 ± 1.22 ‡‡‡	0.35 ± 0.10	0.12 ± 0.02 *	0.20 ± 0.07	3.99 ± 0.72 *** ‡‡‡ †††	3.74 ± 0.36 * ‡‡‡ †††
Diabetic + Metyrapone	1.46 ± 0.29 ‡‡‡	1.02 ± 0.03 *** ‡‡‡ †††	1.53 ± 0.48	5.25 ± 1.49 *** ‡‡‡ †††	N.D.	1.71 ± 0.22 * ‡‡‡

Values are expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to control; *** $p<0.001$ compared to control ; ‡‡ $p<0.01$ compared to diabetic
‡‡‡ $p<0.001$ compared to diabetic; ††† $p<0.001$ compared to insulin treated
††† $p<0.001$ compared to adrenalectomised.

TABLE-11

MONOAMINES AND METABOLITES IN CORPUS STRIATUM OF 6-10 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	0.50 ± 0.14	24.13 ± 2.24	3.56 ± 0.47	0.52 ± 0.09	0.36 ± 0.07	1.46 ± 0.23
Diabetic	36.68 ± 13.27 ***	44.23 ± 1.75 ***	1.54 ± 0.034 *	0.13 ± 0.05	3.75 ± 1.42 *	2.72 ± 0.35
Diabetic + Insulin	0.12 ± 0.01 ‡‡	11.74 ± 2.20 ‡‡	1.36 ± 0.23 *	1.00 ± 0.01	0.84 ± 0.30 '	1.07 ± 0.16 ‡‡
Diabetic + Adrenalectomy	1.43 ± 0.30 ‡‡	16.68 ± 3.84 ‡‡	1.21 ± 0.04 *	0.49 ± 0.08	0.72 ± 0.16 '	3.16± 0.24 ††† *** ‡
Diabetic + Metyrapone	0.50± 0.08 ‡‡	22.45 ± 5.38 ‡	3.54 ± 1.21 ' ††	5.15 ± 1.77 *** ‡‡ ††† ‡	1.75 ± 0.74	1.04 ± 0.37 ‡‡ ‡

Values are expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations.

* $p<0.05$ compared to control; ** $p<0.01$ compared to control ;*** $p<0.001$ compared to control; ‡ $p<0.05$ compared to diabetic ;‡‡ $p<0.01$ compared to diabetic ; ‡‡‡ $p<0.001$ compared to diabetic ; † $p<0.05$ compared to insulin treated; ††† $p<0.001$ compared to insulin treated; † $p<0.05$ compared to; adrenalectomised; †† $p<0.01$ compared to adrenalectomised; ; ††† $p<0.001$ compared to adrenalectomised

TABLE-12

MONOAMINES AND METABOLITES IN CEREBRAL CORTEX OF 6-10 WEEK OLDRATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	1.41 ± 0.14	0.59 ± 0.16	0.78 ± 0.07	0.80 ± 0.46	0.29 ± 0.05	2.50 ± 0.39
Diabetic	1.15 ± 0.10	2.88 ± 0.94	0.40 ± 0.04	0.15 ± 0.03	0.31 ± 0.07	2.47 ± 0.43
Diabetic + Insulin	0.30 ± 0.07 *** ‡	0.16 ± 0.06	0.09 ± 0.03	0.54 ± 0.12	N.D.	0.64 ± 0.14 ** ‡
Diabetic + Adrenalectomy	2.69 ± 0.28 *** ‡ †††	0.16 ± 0.04	0.50 ± 0.08	0.09 ± 0.05	0.09 ± 0.01 * †	2.35 ± 0.46 ††
Diabetic + Metyrapone	0.89 ± 0.04 ‡	9.01 ± 1.61 *** ‡ ††† ‡	1.37 ± 0.06 † †	4.94 ± 2.41 * † †	N.D.	0.86 ± 0.05 ** ‡ †

Values are expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations. * $p<0.05$ compared to control ; ** $p<0.01$ compared to control ;*** $p<0.001$ compared to control ; ‡ $p<0.05$ compared to diabetic; ‡‡ $p<0.01$ compared to diabetic; ‡‡‡ $p<0.001$ compared to diabetic ; † $p<0.05$ compared to insulin treated ; †† $p<0.01$ compared to insulin treated; ††† $p<0.001$ compared to insulin treated; † $p<0.05$ compared to; adrenalectomised;††† $p<0.001$ compared to adrenalectomised.

TABLE-13

MONOAMINES AND METABOLITES IN CEREBELLUM OF 6-10 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	0.90 ± 0.13	0.32 ± 0.11	0.15 ± 0.03	N.D.	0.14 ± 0.07	1.00 ± 0.20
Diabetic	16.63 ± 2.64 ***	0.14 ± 0.06	0.25 ± 0.17	0.05 ± 0.01	0.12 ± 0.04	0.67 ± 0.09
Diabetic + Insulin	0.22 ± 0.13 ‡‡	0.88 ± 0.14 *‡	0.10 ± 0.04	0.25 ± 0.07	0.04 ± 0.01 **‡	0.36 ± 0.06 **
Diabetic + Adrenalectomy	1.45 ± 0.13 ‡‡	0.05 ± 0.02 ††	0.10 ± 0.01	0.23 ± 0.13	N.D.	0.69 ± 0.09
Diabetic + Metyrapone	0.73 ± 0.06 ‡‡	3.23 ± 0.23 ***‡‡ †††m	0.62 ± 0.27	0.68 ± 0.05 ‡‡ ††	0.13 ± 0.11 ††	0.31 ± 0.11 *

Values are expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to control ; ** $p<0.01$ compared to control ;*** $p<0.001$ compared to control ;‡‡ $p<0.01$ compared to diabetic ; ‡‡‡ $p<0.001$ compared to diabetic; †† $p<0.01$ compared to insulin treated; ††† $p<0.001$ compared to insulin treated; †† $p<0.01$ compared to adrenalectomised ; ††† $p<0.001$ compared to adrenalectomised.

TABLE-14

MONOAMINES AND METABOLITES IN THE HYPOTHALAMUS OF 40-60 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	4.87 ± 0.04	0.35 ± 0.04	0.34 ± 0.03	0.84 ± 0.03	0.45 ± 0.05	9.34 ± 0.31
Diabetic	2.06 ± 0.83 **	0.24 ± 0.04	0.15 ± 0.03 **	1.17 ± 0.14	0.57 ± 0.19	7.01 ± 0.89 *
Diabetic + Insulin	3.32 ± 0.04 *	1.10 ± 0.05 ††† ***	0.24 ± 0.03 *	0.79 ± 0.20	0.63 ± 0.44	8.04 ± 0.09

Values are expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations
* $p<0.05$ compared to control ; ** $p<0.01$ compared to control ;
*** $p<0.001$ compared to control
††† $p<0.001$ compared to diabetic

TABLE-15

MONOAMINES ANDMETABOLITES IN BRAIN STEM OF 40-60 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	3.85 ± 0.45	0.27 ± 0.06	0.10 ± 0.04	1.83 ± 0.40	0.26 ± 0.05	6.46 ± 0.99
Diabetic	15.09 ± 0.60 ***	2.77 ± 2.46	0.12 ± 0.04	0.58 ± 0.09 **	11.66 ± 3.00 ***	3.54 ± 1.34
Diabetic + Insulin	N.D.	1.18 ± 0.79	0.09 ± 0.01	2.55 ± 1.43	0.43 ± 0.03 †††	5.23 ± 0.87

Values are expressed as nmoles/gram wet weight tissue.
Mean ± S.E.M. of 4-6 separate determinations.
**$p<0.01$ compared to control; *** $p<0.01$ compared to control; ††† $p<0.001$ compared to diabetic

TABLE-16

MONOAMINES AND METABOLITES IN CORPUS STRIATUM OF 40-60 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	1.23 ± 0.01	17.42 ± 0.82	2.36 ± 0.06	1.22 ± 0.03	3.08 ± 0.14	5.11 ± 0.06
Diabetic	0.55 ± 0.03 ***	43.55 ± 5.98 **	3.22 ± 0.53	0.93 ± 0.01 ***	4.21 ± 0.88	5.45 ± 0.24
Diabetic + Insulin	0.33 ± 0.16 **	25.68 ± 6.84 †	3.24 ± 0.97	1.22 ± 0.07 †††	10.31 ± 2.86 *†	7.83 ± 0.95 *††

Values are expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to control ;** $p<0.01$ compared to control ; *** $p<0.001$ compared to control
† $p<0.05$ compared to diabetic ; †† $p<0.01$ compared to diabetic ; ††† $p<0.001$ compared to diabetic

TABLE-17

MONOAMINES AND METABOLITES IN THE CEREBRAL CORTEX OF 40-60 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	1.41 ± 0.36	0.43 ± 0.02	0.45 ± 0.09	1.13 ± 0.12	0.43 ± 0.12	4.34 ± 0.55
Diabetic	2.10 ± 0.11	3.50 ± 0.10 ***	0.19 ± 0.01 *	0.87 ± 0.20	0.12 ± 0.04 **	2.93 ± 0.14 *
Diabetic + Insulin	1.64 ± 0.43	2.05 ± 0.09 *** †††	0.67 ± 0.07 * †††	1.42 ± 0.04	0.46 ± 0.02 †	5.37 ± 0.29 †††

Values are expressed as nmoles/gram wet weight tissue.
Mean ± S.E.M. of 4-6 separate determinations.

* $p<0.05$ compared to control ; ** $p<0.01$ compared to control ;
*** $p<0.001$ compared to control † $p<0.05$ compared to diabetic ;
††† $p<0.001$ compared to diabetic

TABLE-18

MONOAMINES AND METABOLITES IN THE CEREBELLUM OF 40-60 WEEK OLD RATS

Animal Status	NE	DA	5-HT	5-HTP	5-HIAA
Control	3.63 ± 0.29	0.48 ± 0.06	0.13 ± 0.05	0.11 ± 0.01	0.45 ± 0.04
Diabetic	40.97 ± 14.37**	1.44 ± 0.27**	0.38 ± 0.07 **	0.15 ± 0.02	1.50 ±0.35 *
Diabetic + Insulin	1.08 ± 0.38 †	1.24 ± 0.09 **	0.24 ± .02	0.33 ± 0.20	1.58 ± 0.34*

Values are expressed as nmoles/gram wet weight tissue.
Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to control;
** $p<0.01$ compared to control; † $p<0.05$ compared to diabetic.

TABLE-19

MONOAMINES AND METABOLITES AND IN HYPOTHALAMUS OF 72-104 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	5.22 ± 0.38	1.36 ± 0.07	0.18 ± 0.04	0.35 ± 0.01	0.45 ± 0.09	4.63 ± 0.03
Diabetic	3.58 ± 0.14 **	0.34 ± 0.04 *	0.16 ± 0.03	0.44 ± 0.03	0.54 ± 0.22	1.60 ± 0.30 ***
Diabetic + Insulin	3.80 ± 0.28 ***	0.70 ± 0.19 *	0.09 ± 0.01	0.53 ± 0.03	0.38 ± 0.03	4.43 ± 0.32 ‡‡
Diabetic + Adrenalectomy	2.15 ± 0.66 ***‡ ††	0.75 ± 0.28 *	N.D.	0.48 ± 0.12	0.96 ± 0.62	2.23 ± 0.51 *** †††
Diabetic + Metyrapone	0.75 ± 0.04 *** ‡‡ ††† †	N.D.	N.D.	0.64 ± 0.4	0.45 ± 0.05	3.01 ± 0.16 *** ‡ ††

Values are expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations.

* $p<0.05$ compared to control ; ** $p<0.01$ compared to control ;*** $p<0.001$ compared to control .

‡ $p<0.05$ compared to diabetic; ‡‡‡ $p<0.001$ compared to diabetic ; †† $p<0.01$ compared to insulin treated; ††† $p<0.001$ compared to insulin treated ; †† $p<0.01$ compared to adrenalectomised

TABLE-20

MONOAMINES AND METABOLITES IN BRAIN STEM OF 72-104 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	3.60 ± 0.23	0.33 ± 0.05	0.15 ± 0.01	2.05 ± 0.03	0.07 ± 0.01	2.86 ± 0.40
Diabetic	1.63 ± 0.12 ***	0.27 ± 0.05	0.13 ± 0.01	0.30 ± 0.01 ***	0.19 ± 0.05	2.30 ± 0.14
Diabetic + Insulin	3.42 ± 0.38 ‡‡‡	0.30 ± 0.03	0.09 ± 0.01 **‡ †	0.98 ± 0.18 ***‡‡‡	0.22 ± 0.11	4.55 ± 0.34 **‡‡
Diabetic + Adrenalectomy	1.55 ± 0.25 *** †††	0.12 ± 0.03 *†	0.04 ± 0.01 ***‡‡‡ ††	0.97 ± 0.12 ***‡‡‡	0.36 ± 0.27	2.16 ± 0.28 ††
Diabetic + Metyrapone	1.54 ± 0.03 *** †††	0.25 ± 0.07	0.11 ± 0.01 *‡‡‡	0.65 ± 0.08 ***‡	0.26 ± 0.23	2.36 ± 0.56 ††

Values are expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations.
* $p<0.05$ compared to control ; ** $p<0.01$ compared to control ;*** $p<0.001$ compared to control;
‡ $p<0.05$ compared to diabetic ; ‡‡ $p<0.01$ compared to diabetic ;‡‡‡ $p<0.001$ compared to diabetic;
† $p<0.05$ compared to insulin treated; †† $p<0.01$ compared to insulin treated;
††† $p<0.001$ compared to insulin treated; ††† $p<0.001$compared to adrenalectomised.

TABLE-21

MONOAMINES AND METABOLITES IN CORPUS STRIATUM OF 72-104 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	1.32 ± 0.04	14.45 ±0.62	1.65 ± 0.04	0.65 ± 0.07	1.21 ± 0.21	2.99 ± 0.10
Diabetic	1.85 ± 0.14 **	10.09 ± 2.73	1.49 ± 0.15	0.29 ± 0.01	1.31 ± 0.13	3.49 ± 0.15
Diabetic + Insulin	2.52 ± 0.25 *** ‡‡	12.39 ± 0.40	1.47 ± 0.17	1.18 ± 0.18 ** ‡‡	2.08 ± 0.28	3.13 ± 0.08
Diabetic + Adrenalectomy	0.37 ± 0.07 *** ‡‡ †††	18.04 ± 5.18	2.00 ± 0.15	0.41 ± 0.19 ††	3.02 ± 1.69	1.76 ± 0.03 * †
Diabetic + Metyrapone	0.30 ± 0.01 *** ‡‡ †††	7.89 ± 1.93	2.44 ± 0.31 †	0.60 ± 0.13 ††	1.18 ± 0.08	1.13 ± 0.75 ** ‡‡ †††

Values are expressed as nmoles/gram wet weight tissue Mean ± S.E.M. of 4-6 separate determinations.
* p<0.05 compared to control; ** p<0.01 compared to control ;*** p<0.001 compared to control . ‡ p<0.05 compared to diabetic; ‡‡ p<0.01 compared with diabetic; ‡‡‡ p<0.001 compared to diabetic ; † p<0.05 compared to insulin treated ;†† p<0.01 compared to insulin treated; ††† p<0.001 compared to insulin treated.

TABLE-22

MONOAMINES AND METABOLITES IN CEREBRAL CORTEX OF 72-104 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	2.36 ± 0.12	0.50 ± 0.10	0.19 ± 0.03	0.68 ± 0.23	0.14 ± 0.05	1.88 ± 0.32
Diabetic	4.39 ± 1.78	0.30 ± 0.18	0.24 ± 0.10	2.29 ± 1.69	0.22 ± 0.07	1.99 ± 0.21
Diabetic + Insulin	2.20 ± 0.32	1.41 ± 0.01	0.24 ± 0.04	0.10 ± 0.02	0.12 ± 0.02	1.97 ± 0.45
Diabetic + Adrenalectomy	0.76 ± 0.05 ‡	0.55 ± 0.01	0.16 ± 0.10	0.46 ± 0.02	0.17 ± 0.04	1.10 ± 0.48
Diabetic + Metyrapone	0.76 ± 0.26 ‡‡	0.82 ± 0.72	0.49 ± 0.38	0.26 ± 0.04	1.40 ± 1.12	1.60 ± 0.01

Values are expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations
‡ $p<0.05$ compared to diabetic; ‡‡ $p<0.01$ compared to diabetic

TABLE-23

MONOAMINES AND METABOLITES IN CEREBELLUM OF 72-104 WEEK OLD RATS

Animal Status	NE	DA	HVA	5-HT	5-HTP	5-HIAA
Control	2.07 ± 0.29	0.91 ± 0.20	0.34 ± 0.01	0.30 ± 0.01	0.06 ± 0.01	0.58 ± 0.06
Diabetic	3.01 ± 0.73	0.53 ± 0.18	0.15 ± 0.01 ***	0.06 ± 0.01 ***	0.18 ± 0.03 ***	1.17 ± 0.05 **
Diabetic + Insulin	2.20 ± 0.59	0.56 ± 0.07	N.D.	0.11 ± 0.01	N.D.	1.13 ± 0.02 **
Diabetic + Adrenalectomy	0.50 ± 0.03 **‡‡†	0.35 ± 0.01	N.D.	N.D.	N.D.	0.37 ± 0.27‡‡
Diabetic + Metyrapone	0.26 ± 0.10 *‡‡†	N.D.	N.D.	N.D.	N.D.	0.10 ± 0.01 ‡‡

Values are expressed as nmoles/gram wet weight tissue.

Mean ± S.E.M. of 4-6 separate determinations.

* $p<0.05$ compared to control ; ** $p<0.01$ compared to control ;*** $p<0.001$ compared to control; ‡‡‡ $p<0.001$ compared to diabetic;† $p<0.05$ compared to insulin treated.

TABLE-24

[^{3}H] NORADRENERGIC RECEPTOR BINDING PARAMETERS IN BRAIN STEM

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	Young	Old	Young	Old	Young	Old
B_{max} (fmoles/mg protein)	174.25 ± 4.25	105.33 ± 6.36	358.00 ± 57.37$^{\#}$	320.00 ± 11.55*	152.66 ± 27.08	150.00 ± 50.33
K_d (nM)	11.58 ± 0.41	9.05 ± 0.45†	9.25 ± 0.17†	8.97 ± 0.41	5.11 ± 0.97$^{\P\#}$	12.45 ± 0.20**¶¶

Mean ± S.E.M. of 3-4 separate determinations B_{max} - Maximal Binding, K_d - Dissociation constant

* $p< 0.05$ Compared to control old
** $p< 0.01$ Compared to control old
† $p< 0.05$ Compared to control young
$^{\#}$ $p< 0.01$ Compared to control young
¶¶ $p< 0.01$ Compared to diabetic old
¶ $p< 0.01$ Compared to diabetic young

TABLE-25

cAMP CONTENT IN BRAIN STEM

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	Young	Old	Young	Old	Young	Old
cAMP (pmoles/mg protein)	0.11 ± 0.04	0.27 ± 0.05**	4.83 ± 1.12**	0.24 ± 0.08¶¶¶	1.76 ± 0.78*	0.45 ± 0.13†

Mean ± S.E.M. of 3-4 separate determinations

* $p< 0.05$ Compared to control young
** $p< 0.001$ Compared to control young
† $P<0.05$ Compared to diabetic+insulin young
¶¶¶ $p< 0.001$ Compared to diabetic young

TABLE-26

BINDING PARAMETERS OF [^{3}H] NE Vs PRAZOSIN IN BRAIN STEM

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD
BEST FIT MODEL	2	1	2	1	2	-
$LogEC_{50}$ 1	-8.367	-9.209	-9.282	-9.158	-8.081	-
$LogEC_{50}$ 2	-4.179	-	-4.150	-	-3.532	-
Ki 1 (M)	2.3 e-009	2.93 e-010	2.51 e-010	3.29 e-010	2.81e-009	-
Ki 2 (M)	3.55 e-005	-	3.4 e-005	-	9.92 e-005	-

TABLE-27

BINDING PARAMETERS OF [^{3}H] NE Vs YOHIMBINE IN BRAIN STEM

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD
BEST FIT MODEL	2	1	I	1	1	-
$LogEC_{50}$ 1	-8.316	-8.562	-8.993	-8.22	-9.036	-
$LogEC_{50}$ 2	-5.348	-	-	-	-	-
Ki 1 (M)	2.59 e-009	1.3 e-009	4.88 e-010	2.85 e-009	3.11 e-010	-
Ki 2 (M)	2.41 e-006	-	-	-	-	-

TABLE-28

BINDING PARAMETERS OF [^{3}H] NE Vs PROPRANOLOL IN BRAIN STEM

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD
Best Fit Model	2	1	1	I	1	1
$LogEC_{50}$ 1	-9.126	-9.137	-8.531	-7.275	-7.916	-9.238
$LogEC_{50}$ 2	-4.374	-	-	-	-	-
Ki 1 (M)	4.02 e-010	3.47 e-010	1.42 e-009	2.51 e-008	4.1 e-009	3.21 e-010
Ki 2 (M)	2.27 e-005	-	-	-	-	-

TABLE-29

BINDING PARAMETERS OF [^{3}H] NE Vs ATENOLOL IN BRAIN STEM

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD
BEST FIT MODEL	-	1	1	1	1	2
$LogEC_{50}$ 1	-	-7.281	-8.637	-7.836	-8.439	-8.429
$LogEC_{50}$ 2	-	-	-	-	-	-5.057
Ki 1 (M)	-	2.49 e-008	1.11 e-009	6.90 e-009	I.23 e-009	2.07 e-009
Ki 2 (M)	-	-	-	-	-	4.86 e-006

TABLE-30

MONOAMINES AND METABOLITES IN WHOLE PANCREAS

	CONTROL		DIABETIC		DIABETIC + INSULIN		DIABETIC + METYRAPONE	
	Young	Old	Young	Old	Young	Old	Young	Old
NE	1.24 ±0.04	0.62 ± 0.06 *	1.38 ± 0.17	1.20 ± 0.03 ‡	1.16 ± 0.07	0.98 ± 0.01	0.96 ± 0.23	0.44 ± 0.05 ‡ ††
EPI	0.22 ±0.02	0.77 ± 0.18	0.43 ± 0.22	0.73 ± 0.16	0.51 ± 0.30	0.98 ± 0.02	0.59 ± 0.11	0.34 ± 0.03 †
DA	0.33 ± 0.03	0.17 ± 0.02 **	0.20 ± 0.05 **	0.17 ± 0.02	0.08 ± 0.02 ***♣♣	0.25 ± 0.01 **	0.26 ± 0.05 **	0.10 ± 0.01 **††
HVA	0.08 ± 0.01	0.08 ±0.01	0.24 ± 0.01	0.11 ± 0.04	0.28 ± 0.20	0.13 ± 0.02	0.06 ± 0.01	N.D.
5-HT	0.08 ± 0.01	0.28 ± 0.06	0.22 ± 0.02	0.28 ± 0.12	N.D.	0.03 ± 0.02	0.02 ± 0.01	0.06 ± 0.01
5-HIAA	0.14 ± 0.01	2.75 ± 0.73 *	0.93 ± 0.62	1.10 ± 0.82	1.07 ± 0.07	0.76 ± 0.12	0.26 ± 0.03	0.54 ± 0.28 ‡
NMN	0.52 ± 0.02	0.07 ± 0.03 ***	0.22 ± 0.02 ***	0.10 ± 0.03	0.20 ± 0.05 ***	0.068 ±0.01 ••	N.D.	0.07 ± 0.01

Values expressed as nmoles/gram wet weight tissue. Mean ± S.E.M. of 4-6 separate determinations.

* $p<0.05$ compared to control young.; ** $p<0.01$ compared to control young ; *** $p<0.001$ compared to control young; ‡ $p<0.05$ compared to control old; † $p<0.05$ compared to old insulin treated ; †† $p<0.01$ compared to old insulin treated ; * $p<0.05$ compared to young metyrapone treated; **$p<0.01$ compared to young metyrapone treated † $p<0.05$ compared to old diabetic ; • $p<0.05$ compared to young insulin treated; •• $p<0.01$ compared to young insulin treated; ♣♣ $p<0.01$ compared to old metyrapone treated .

TABLE-31

CATECHOLAMINES IN ISOLATED PANCREATIC ISLETS

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	Young	Old	Young	Old	Young	Old
NE (nmoles/mg islet protein)	0.83 ± 0.07	2.37 ± 0.52*	3.89 ± 0.14***	4.27 ± 0.34##	2.28 ± 0.02*	N.D.
EPI (nmoles/mg islet protein)	2.42 ± 0.02	0.07 ± 0.03***	0.86 ± 0.01*	0.46 ± 0.05###	2.50 ± 0.25†	1.28 ± 0.55#

Mean ± S.E.M. of 3-4 separate determinations

* $p < 0.05$ Compared to control young

*** $p < 0.001$ Compared to control young

\# $p < 0.01$ Compared to control old

\### $p < 0.001$ Compared to control old

† $p < 0.05$ Compared to diabetic young

TABLE-32

[^{3}H] NORADRENERGIC RECEPTOR BINDING PARAMETERS IN ISOLATED PANCREATIC ISLET CELL SUSPENSION

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	Young	Old	Young	Old	Young	Old
B_{max} (fmoles/mg protein)	330.00 ± 15.23	626.25 ± 14.40$^{\#\#}$	730.00 ± 25.50$^{\#\#}$	1410.00 ± 90.00***	233.00 ± 66.50	850.00 ± 50.00*
K_d (nM)	9.95 ± 1.42	12.44 ± 0.34	35.42 ± 13.83$^{\#\#}$	31.95 ± 4.20***	10.17 ± 2.67	12.67 ± 0.17

Mean ± S.E.M. of 3-4 separate determinations B_{max}-Maximal Binding, K_d-Dissociation constant

$^{*}p< 0.05$ Compared to diabetic old

$^{***}p< 0.001$ Compared to control old

$^{\#\#}p< 0.01$Compared to control young

$^{\#\#\#}p< 0.001$Compared to control young

TABLE-33

cAMP CONTENT IN PANCREATIC ISLETS

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	Young	Old	Young	Old	Young	Old
cAMP (pmoles/mg islet protein)	1.03 ± 0.05	2.18 ± 1.11	8.18 ± 0.73***	0.92 ± 0.12†††	2.45 ± 0.92	4.30 ± 1.60†

Mean ± S.E.M. of 3-4 separate determinations

*** $p< 0.001$ Compared to control young

† $p< 0.05$ Compared to control old

††† $p< 0.001$ Compared to diabetic young

TABLE-34

BINDING PARAMETERS OF [^{3}H] NE Vs PRAZOSIN IN PANCREATIC ISLETS

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD
BEST FIT MODEL	2	2	2	2	1	1
$LogEC_{50}$ 1	-8.4	-8..759	-9.39	-7.926	-8.719	-9.457
$LogEC_{50}$ 2	-5.23	-5.064	-5.65	-5.11	-	-
Ki 1 (M)	2.65 e-009	1.24 e-009	3.57 e-010	1.02 e-008	1.28 e-009	2.50 e-010
Ki 2 (M)	3.89 e-006	6.15 e-006	1.96 e-006	6.72 e-006	-	-

TABLE-35

BINDING PARAMETERS OF [^{3}H] NE Vs YOHIMBINE IN PANCREATIC ISLETS

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD
BEST FIT MODEL	2	1	2	1	1	2
$LogEC_{50}$ 1	-8.778	-8.324	-8.768	-7.857	-8.99	-9.032
$LogEC_{50}$ 2	-5.174	-	-3.642	-	-	-5.31
Ki 1 (M)	1.11 e-009	3.38 e-009	1.49 e-009	1.20 e-008	6.72 e-010	6.66 e-010
Ki 2 (M)	4.45 e-006	-	0.000199	-	-	3.53 e-006

TABLE-36

BINDING PARAMETERS OF [^{3}H] NE Vs PROPRANOLOL IN PANCREATIC ISLETS

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD
BEST FIT MODEL	1	1	2		1	1
$LogEC_{50}$ 1	-9.41	-8.25	-8.486	-9.367	-8.836	-9.549
$LogEC_{50}$ 2	-	-	-3.201	-4.043	-	-
Ki 1 (M)	2.57 e-010	4.02 e-009	2.86 e-009	3.72 e-010	9.77 e-010	2.03 e-010
Ki 2 (M)	-	-	0.000551	7.83 e-005	-	

TABLE-37

BINDING PARAMETERS OF [^{3}H] NE Vs ATENOLOL IN PANCREATIC ISLETS

	CONTROL		DIABETIC		DIABETIC+INSULIN	
	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD
BEST FIT MODEL	2	2	1	1	2	1
$LogEC_{50}$ 1	-9.22	-9.394	-9.028	-9.09	-9.188	-7.326
$LogEC_{50}$ 2	-5.49	-4.69	-	-	-4.345	-
Ki 1 (M)	3.98 e-010	2.87 e-010	8.21 e-010	7.01 e-010	4.61 e-010	3.38 e-008
Ki 2 (M)	2.13 e-006	1.46 e-005	-	-	3.03 e-005	-

TABLE-38

GLUCOSE INDUCED INSULIN SECRETION AND cAMP PRODUCTION
In Vitro

	YOUNG		OLD	
	4mM Glucose	20mM Glucose	4mM Glucose	20mM Glucose
Insulin (μ Units/mg islet protein)	190.38 ± 22.15	245.57 ± 30.14	36.38 ± 12.04*	70.88 ±19.50$^{\#}$
cAMP(p moles/ mg islet protein)	0.078 ± 0.01	0.86 ± 0.01***	0.42 ± 0.03***	0.52 ± 0.02$^{\#\#\dagger\dagger}$

Mean ± S.E.M. of 3-4 separate determinations
*p< 0.05 Compared to 4 mM glucose young ***p< 0.001 Compared4 mM glucose young
$^{\#\#}$ P<0.05 Compared to 20 mM glucose young †† p< 0.01 Compared to 4 mM glucose old

Figure-1

[^{3}H] NE SCATCHARD IN THE BRAIN STEM IN DIABETES AS A FUNCTION OF AGE

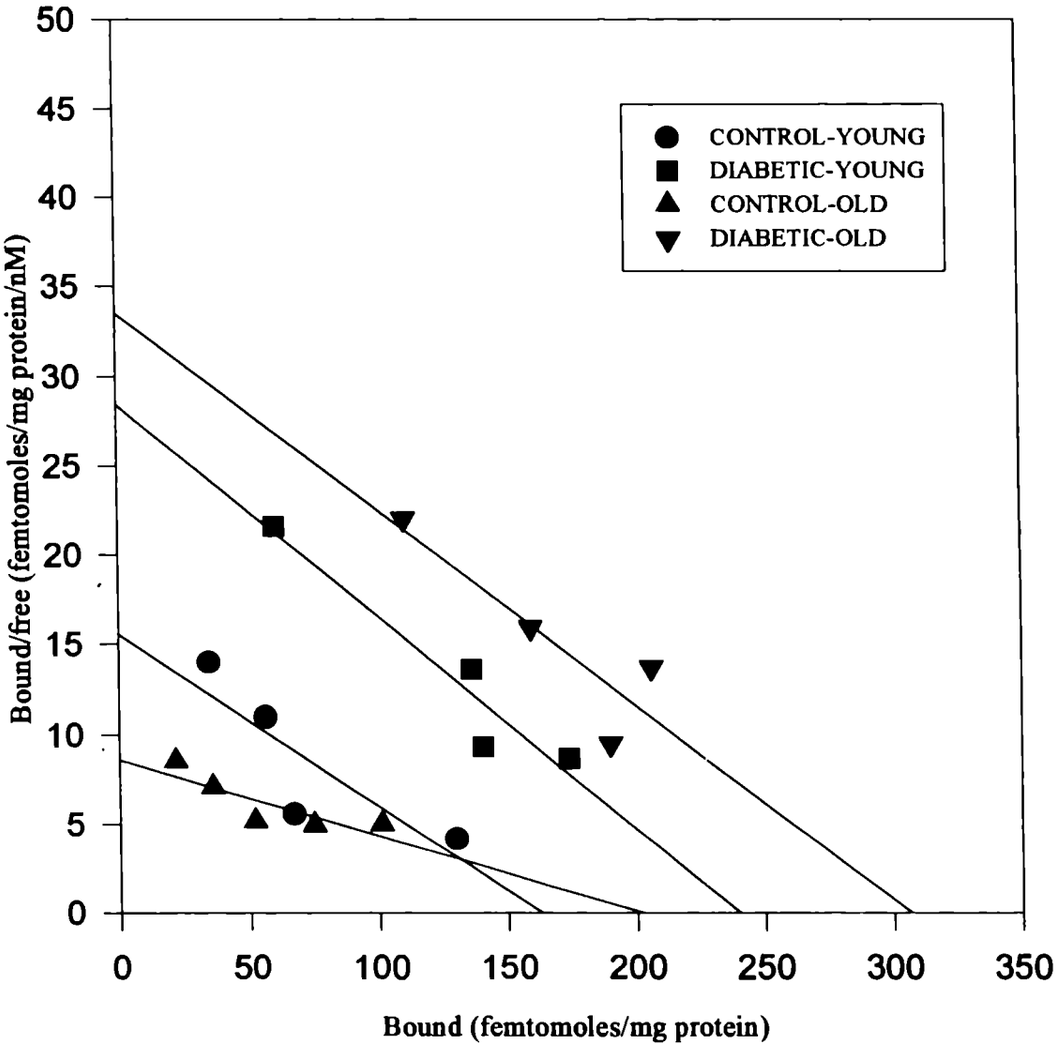


Figure-2

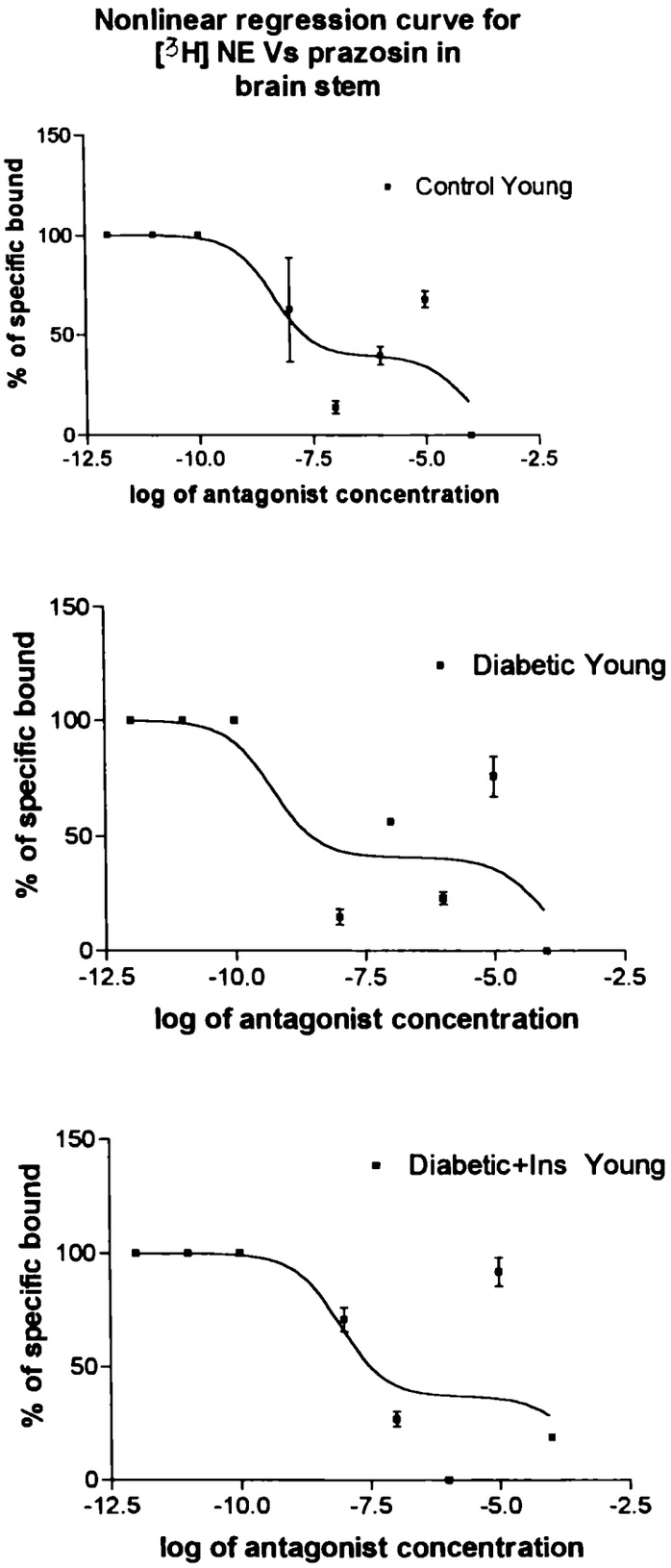
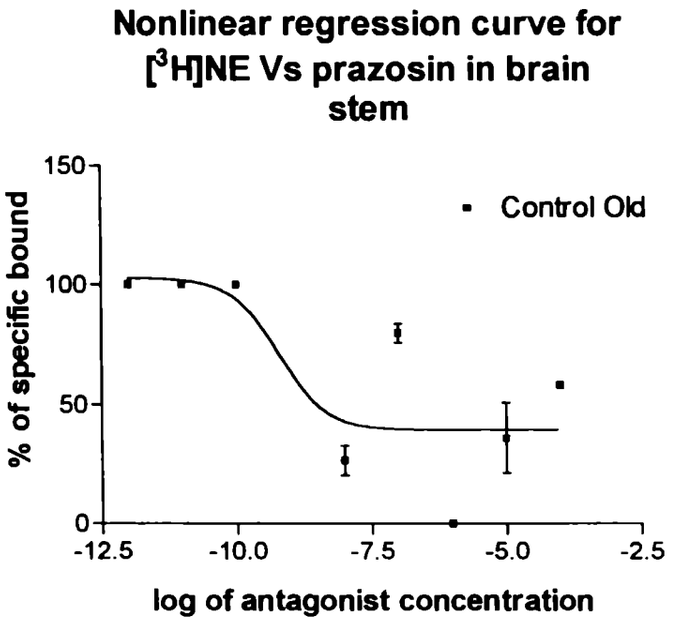

Nonlinear regression curve for
[3H] NE Vs prazosin in
brain stem
Control Young
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Diabetic Young
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Diabetic+Ins Young
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

Figure-3


Nonlinear regression curve for [3H]NE Vs prazosin in brain stem
150
100
50
0
% of specific bound
Control Old
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

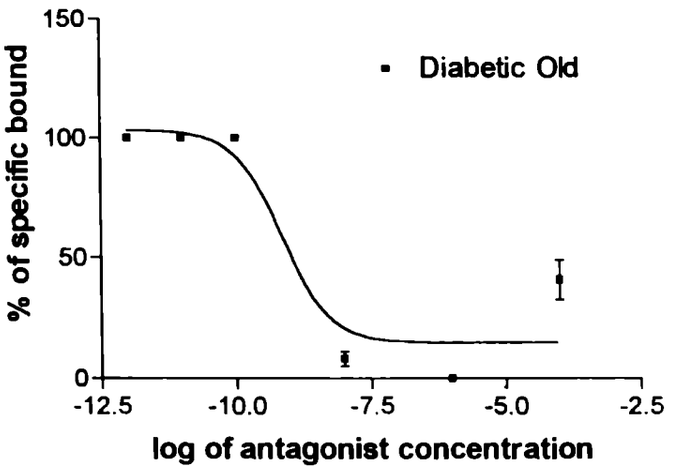

150
100
50
0
% of specific bound
Diabetic Old
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

Figure-4

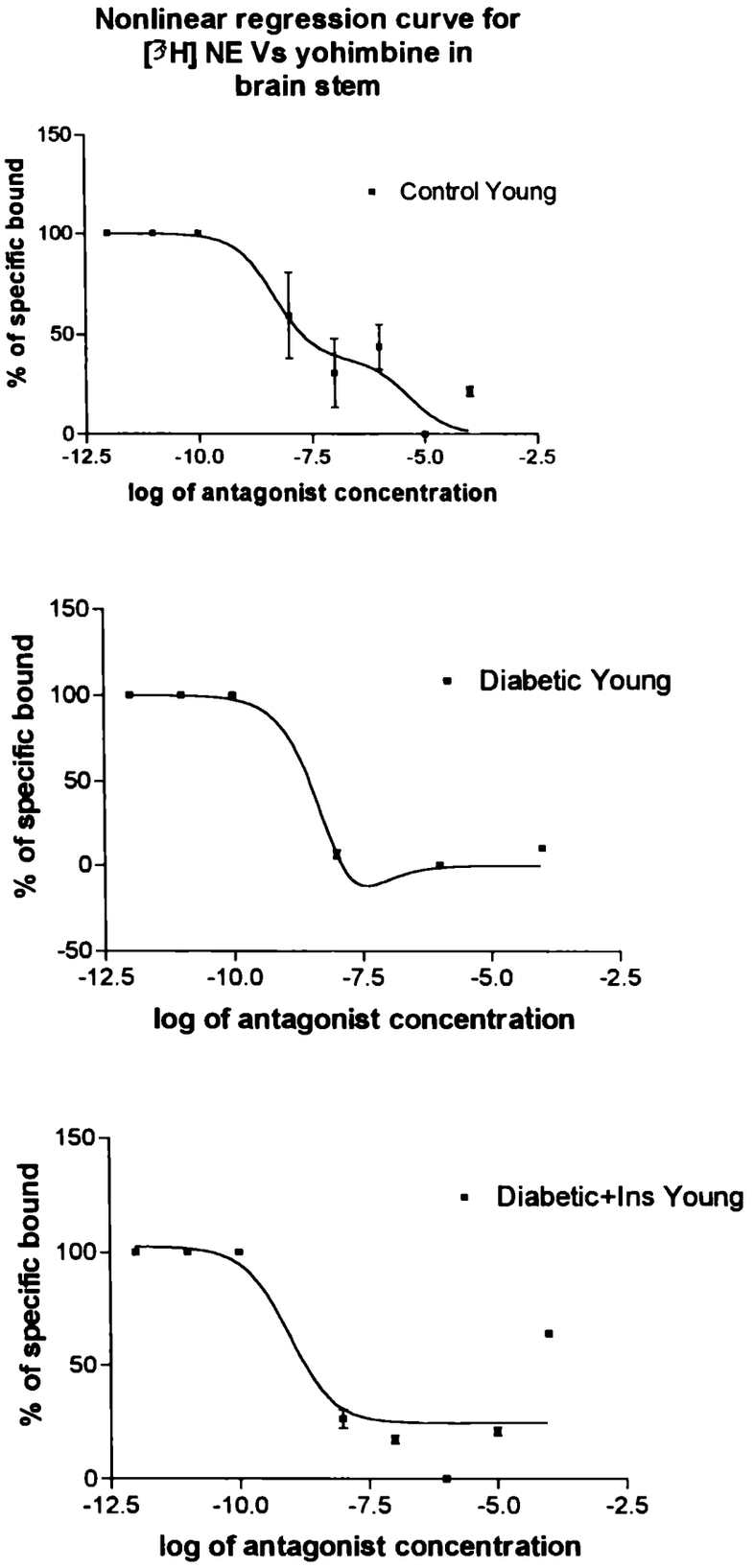
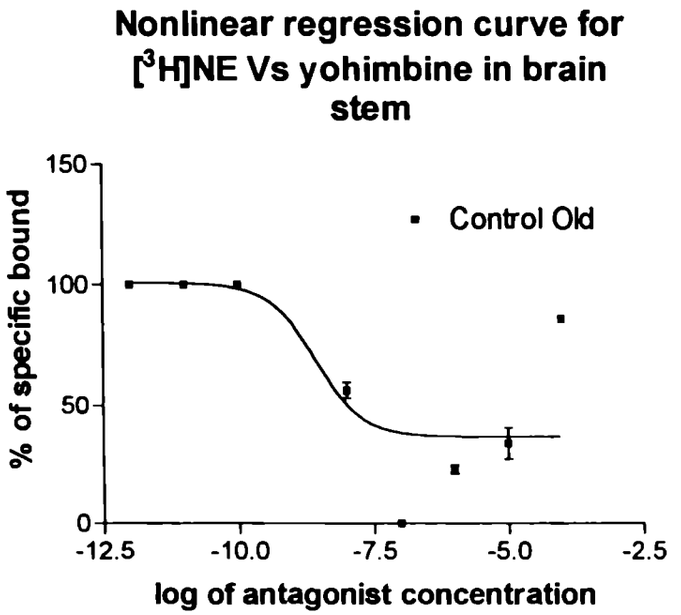

Nonlinear regression curve for
[3H] NE Vs yohimbine in
brain stem
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Control Young
% of specific bound
150
100
50
0
-50
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Diabetic Young
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Diabetic+Ins Young

Figure-5


Nonlinear regression curve for [3H]NE Vs yohimbine in brain stem
150
100
50
0
% of specific bound
Control Old
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

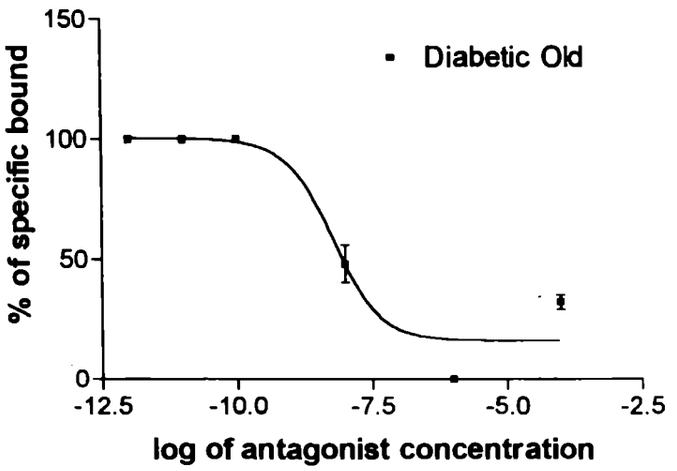

150
100
50
0
% of specific bound
Diabetic Old
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

Figure-6

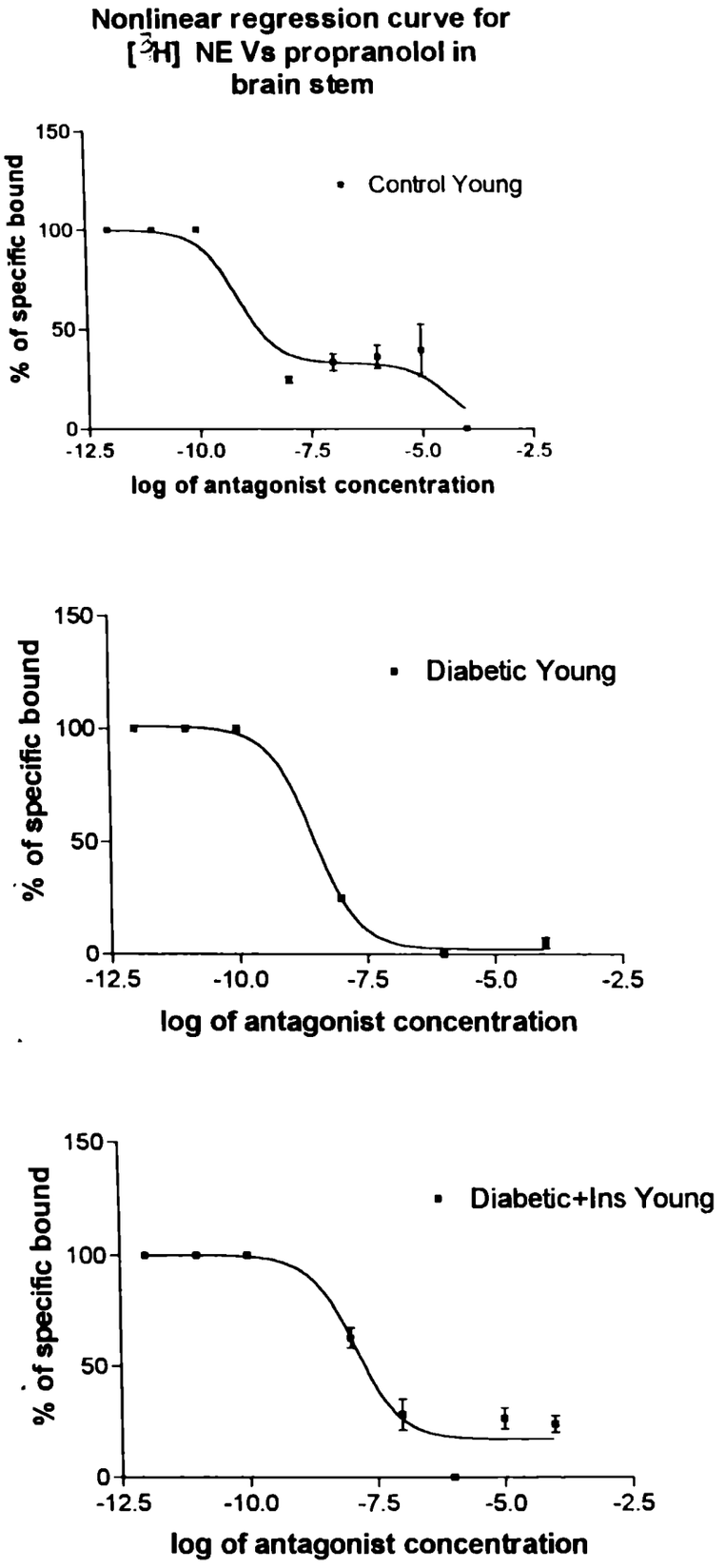

Nonlinear regression curve for
[3H] NE Vs propranolol in
brain stem
150
100
50
0
% of specific bound
Control Young
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Diabetic Young
Diabetic+Ins Young

Figure-7

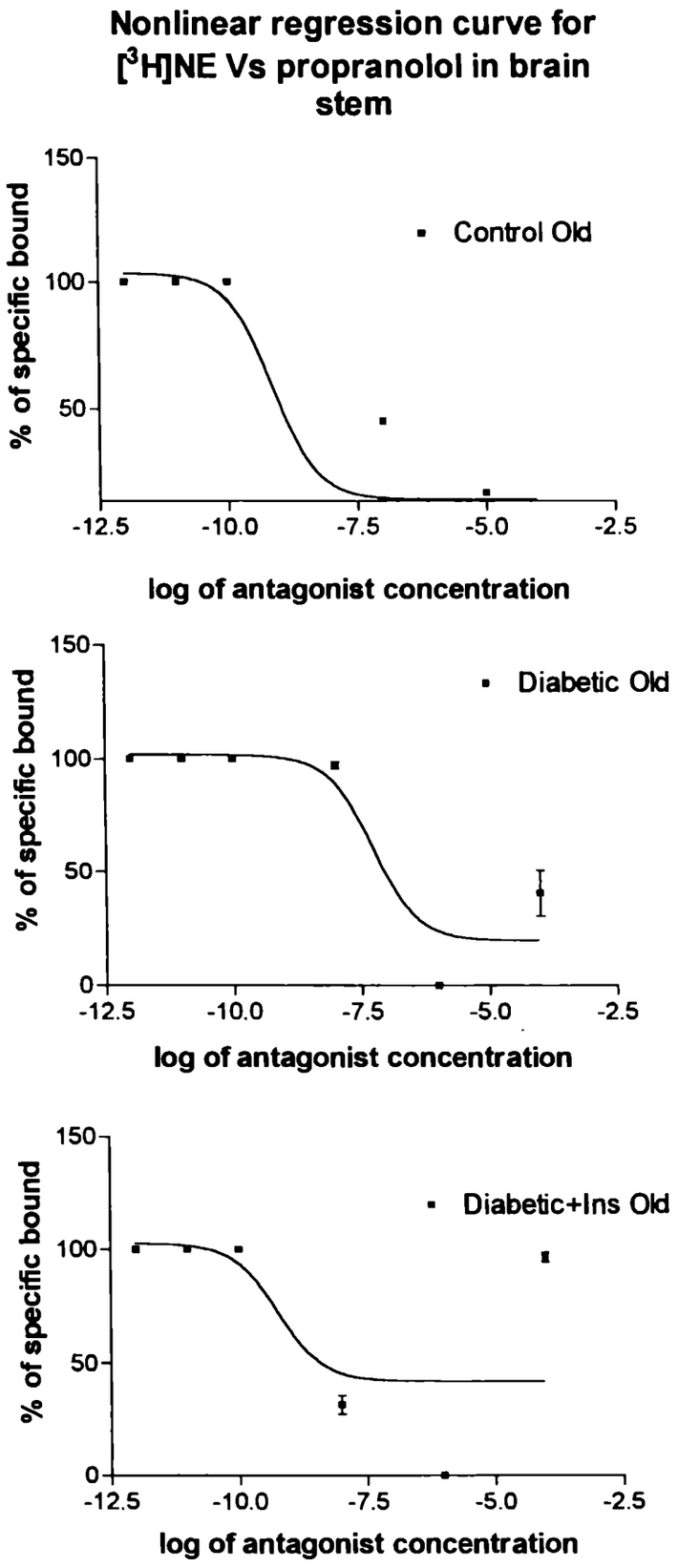
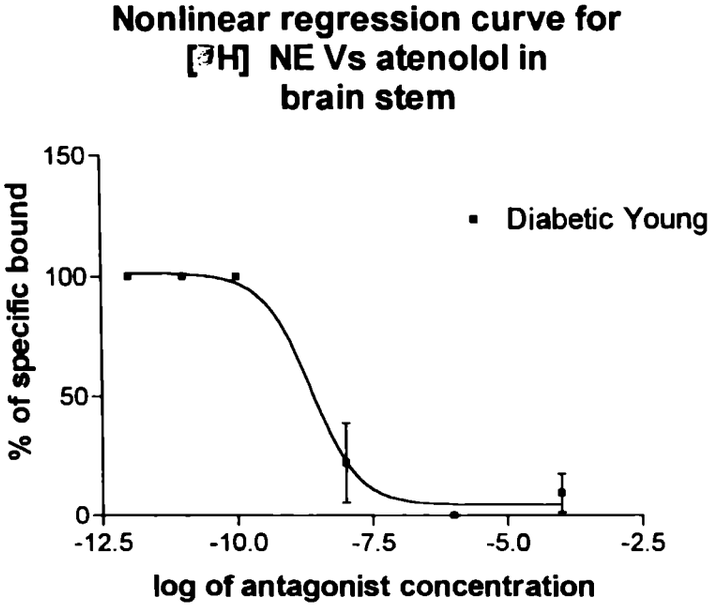

Nonlinear regression curve for [3H]NE Vs propranolol in brain stem
% of specific bound
150
100
50
0
Control Old
Diabetic Old
Diabetic+Ins Old
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

Figure-8


Nonlinear regression curve for
[³H] NE Vs atenolol in
brain stem
150
100
50
0
% of specific bound
Diabetic Young
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

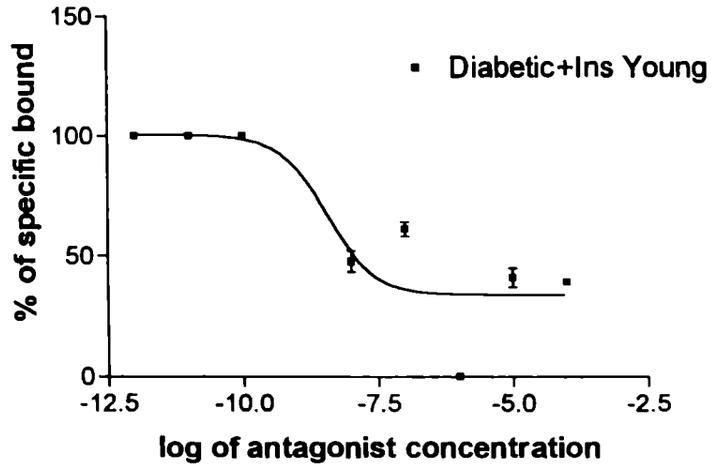

150
100
50
0
% of specific bound
Diabetic+Ins Young
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

Figure-9

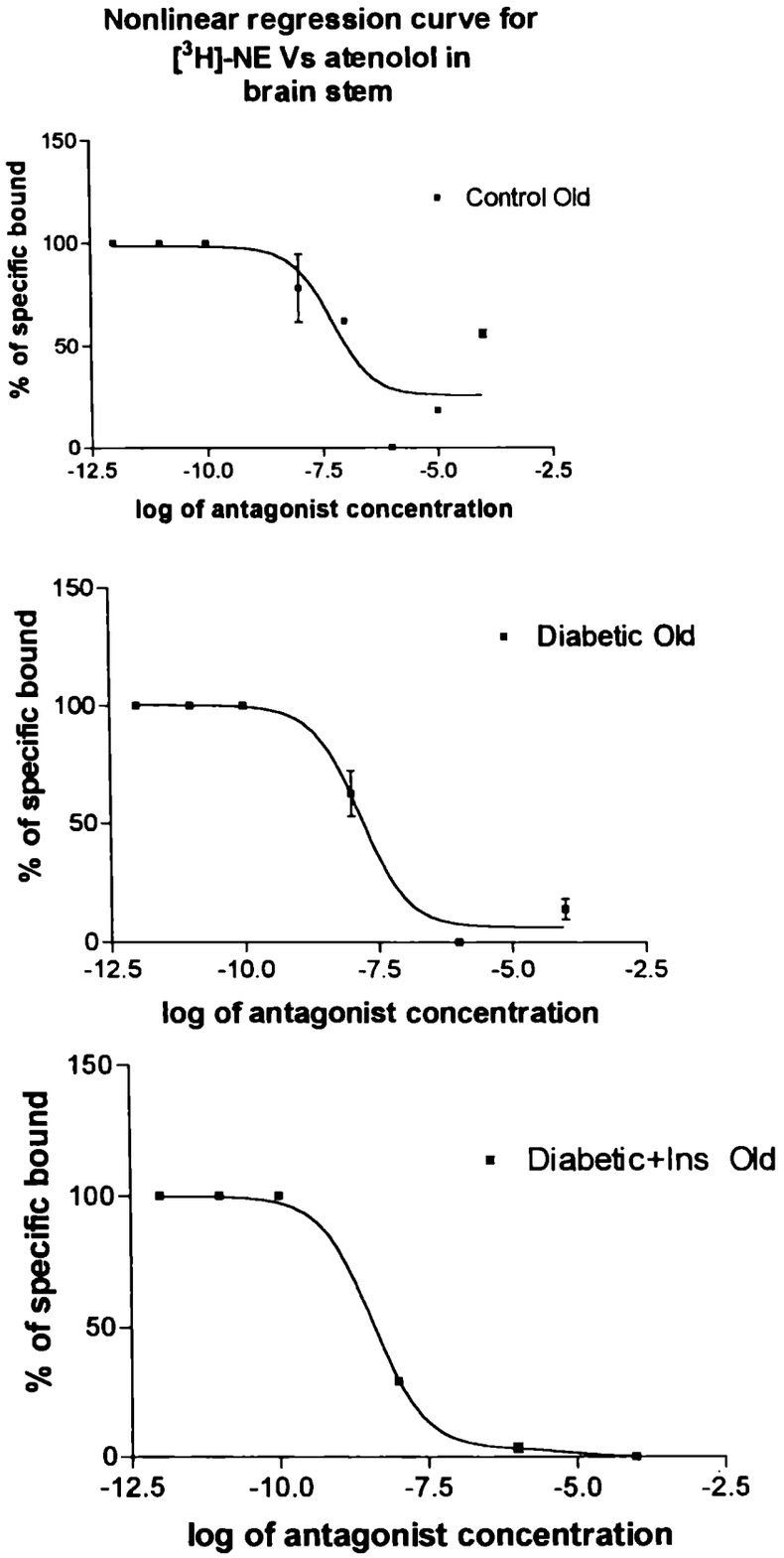

Nonlinear regression curve for
[3H]-NE Vs atenolol in
brain stem
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Control Old
Diabetic Old
Diabetic+Ins Old

Figure-10

]^{3}H] NE SCATCHARD IN PANCREATIC ISLETS IN DIABETES AS A FUNCTION OF AGE

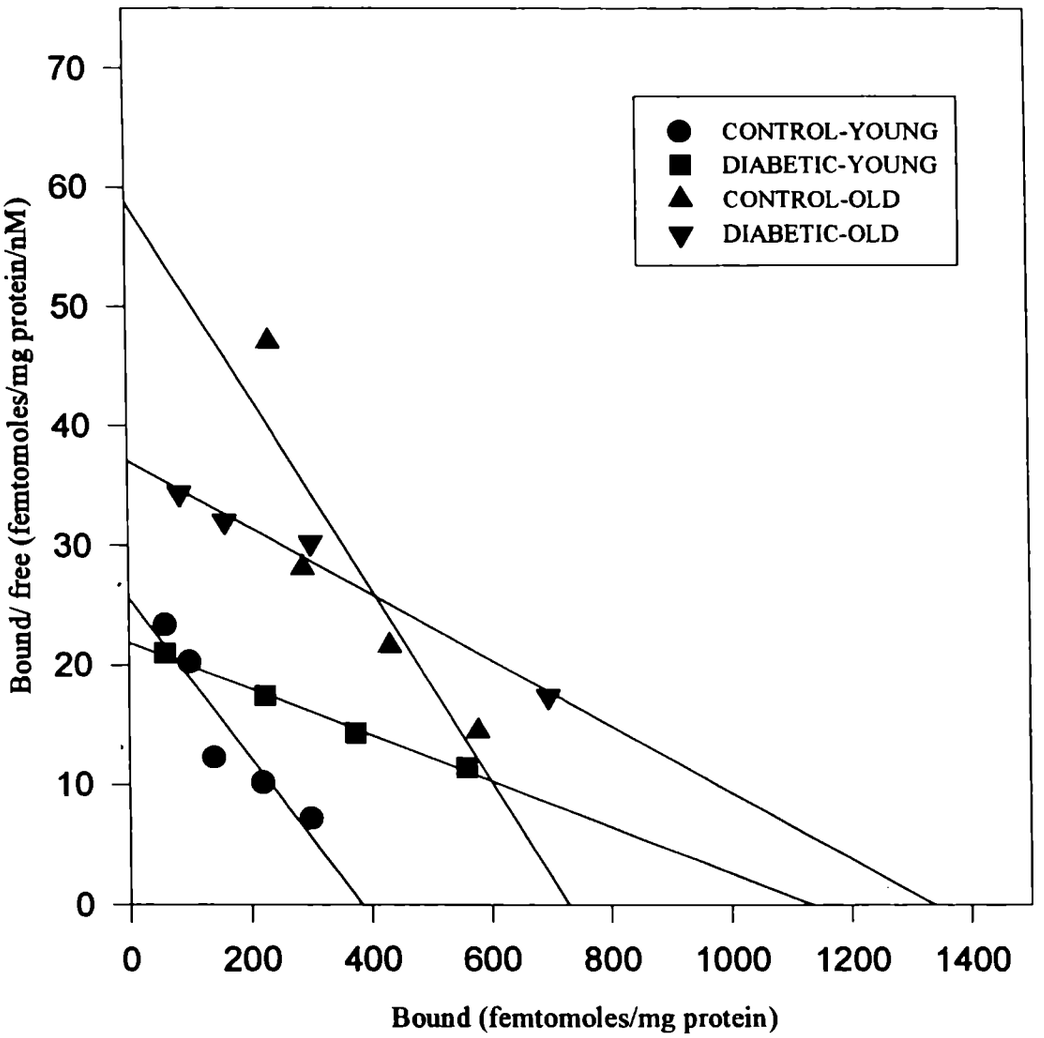


Figure-11

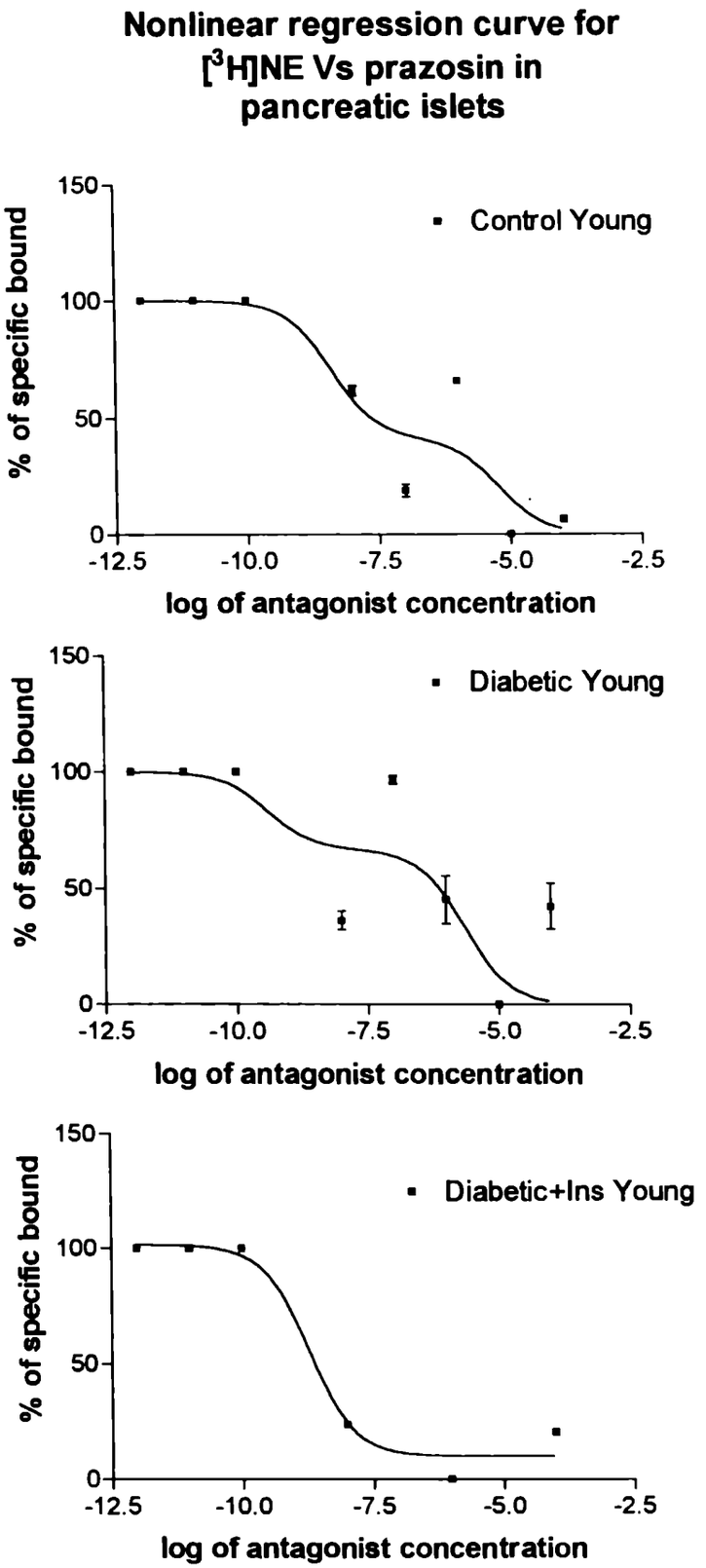

Nonlinear regression curve for
[3H]NE Vs prazosin in
pancreatic islets
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Control Young
Diabetic Young
Diabetic+Ins Young

Figure-12

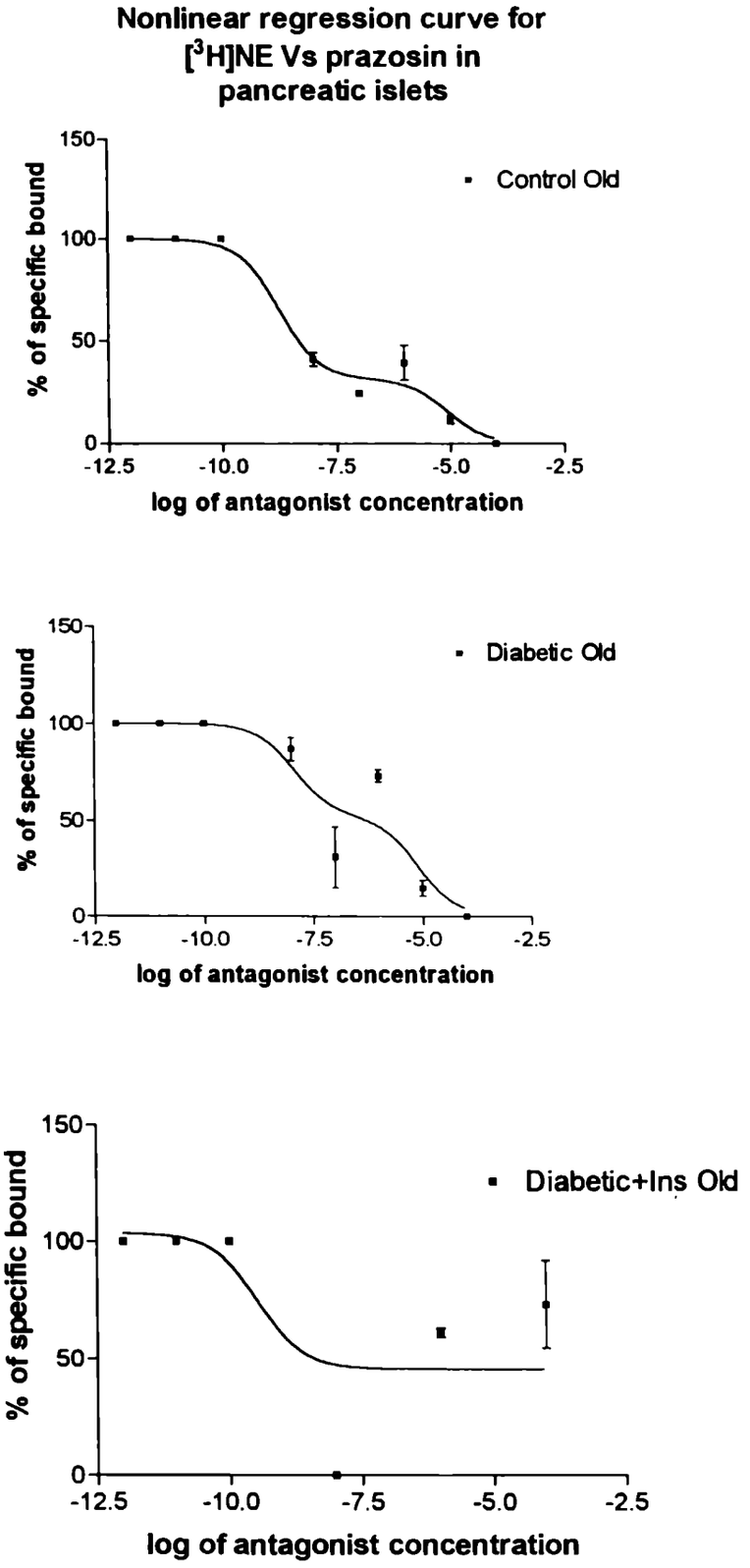

Nonlinear regression curve for
[3H]NE Vs prazosin in
pancreatic islets
Control Old
Diabetic Old
Diabetic+Ins Old
% of specific bound
log of antagonist concentration
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5

Figure-13

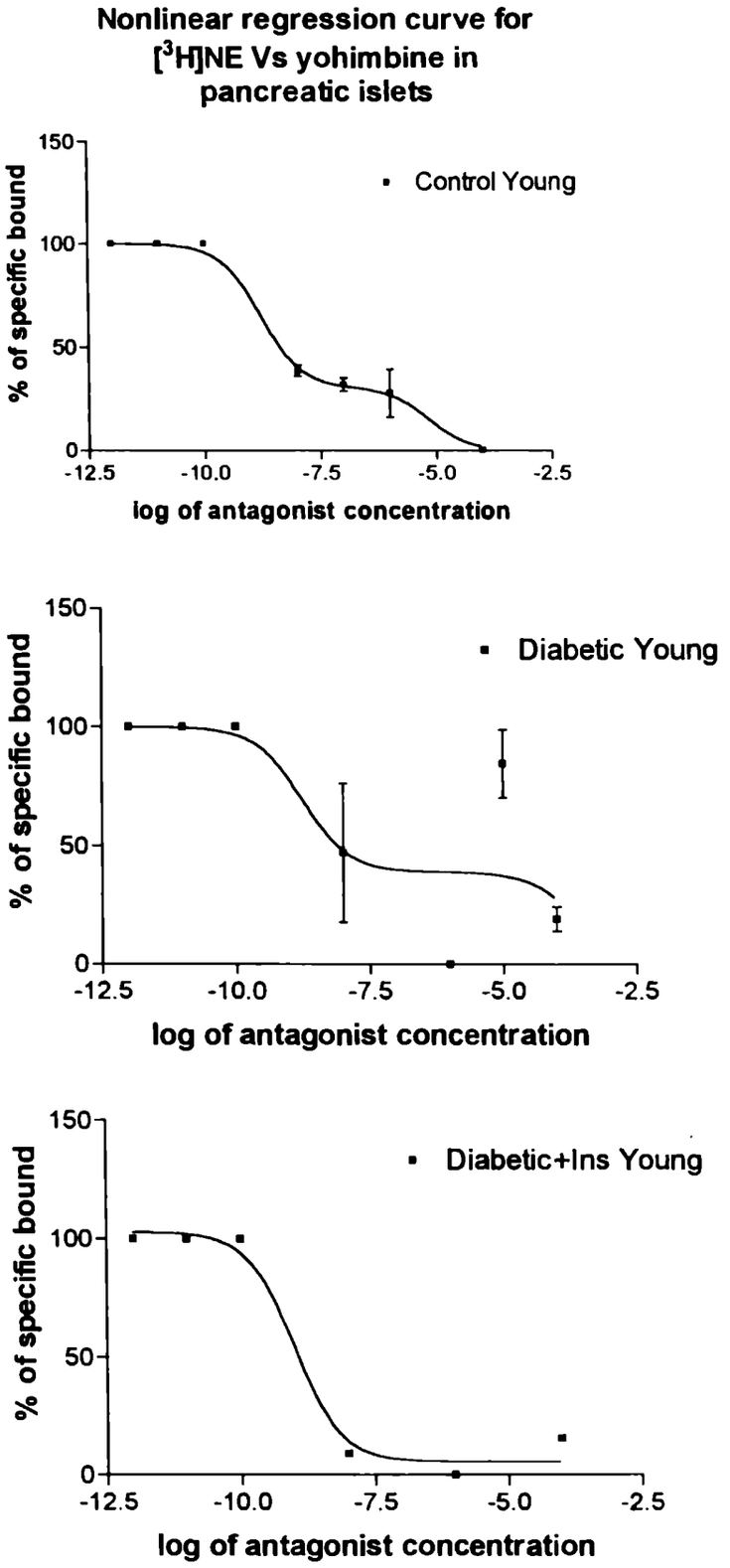

Nonlinear regression curve for
[3H]NE Vs yohimbine in
pancreatic islets
Control Young
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Diabetic Young
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Diabetic+Ins Young
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

Figure-14

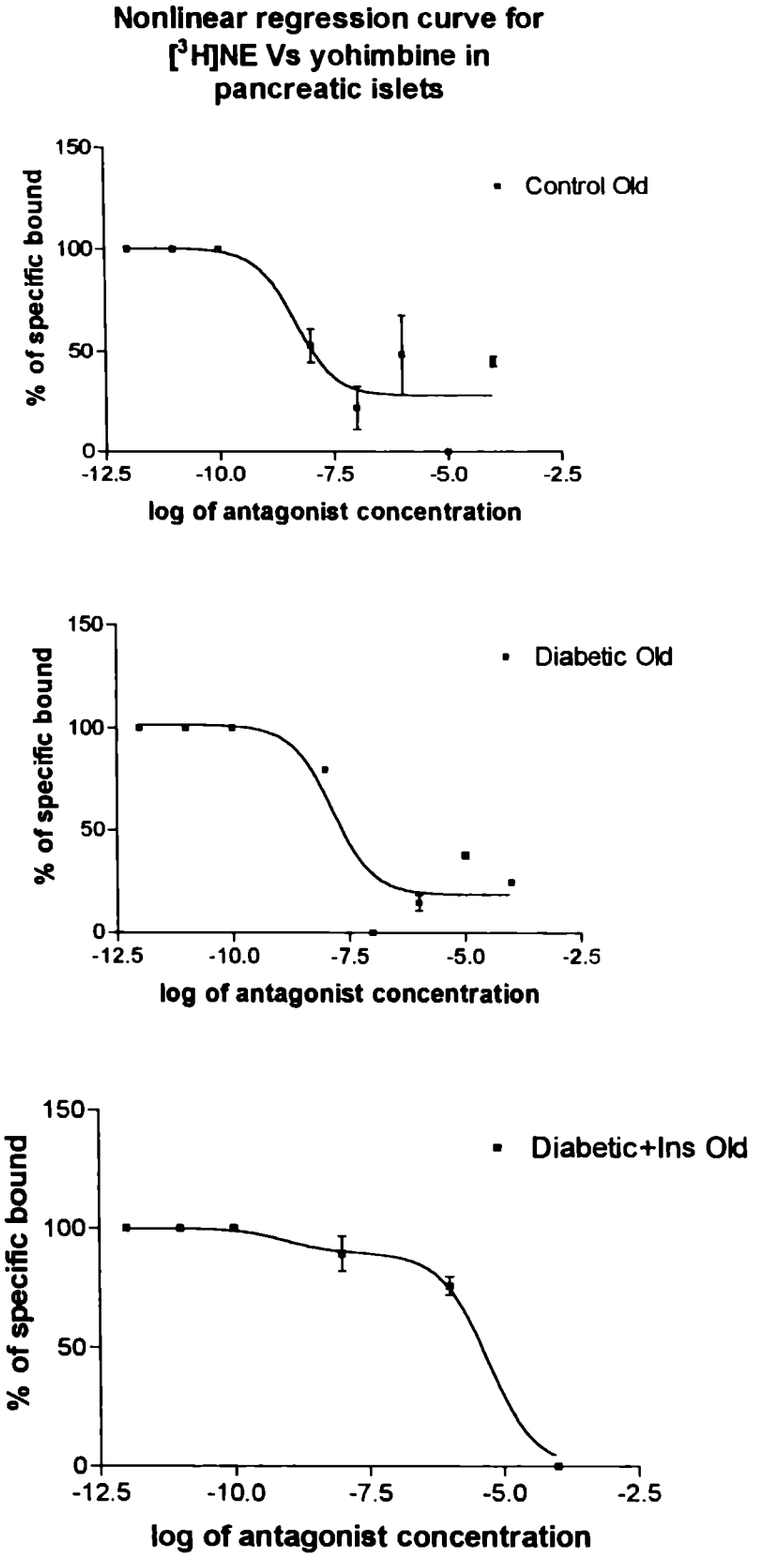

Nonlinear regression curve for
[3H]NE Vs yohimbine in
pancreatic islets
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Control Old
Diabetic Old
Diabetic+Ins Old

Figure-15

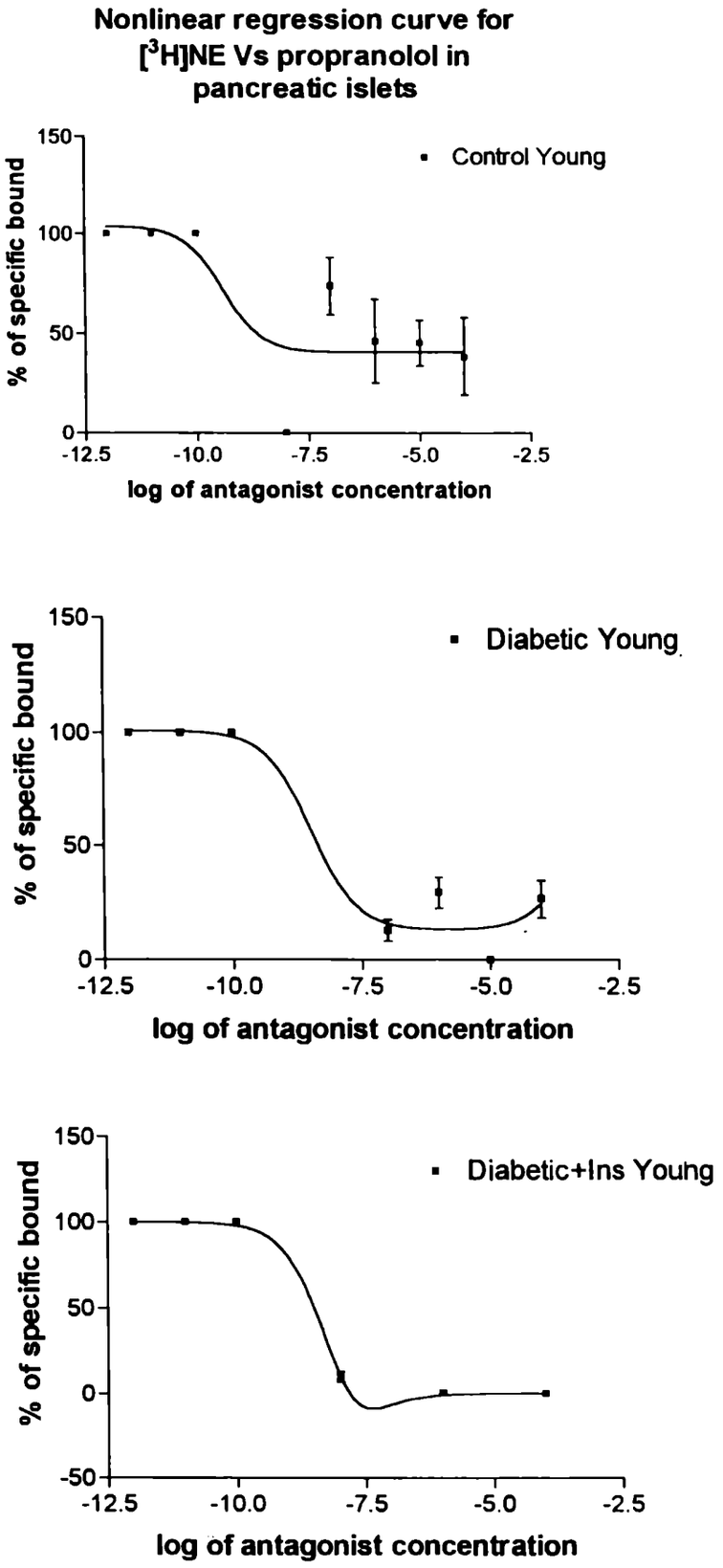

Nonlinear regression curve for
[3H]NE Vs propranolol in
pancreatic islets
Control Young
Diabetic Young
Diabetic+Ins Young
% of specific bound
150
100
50
0
-50
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

Figure-16

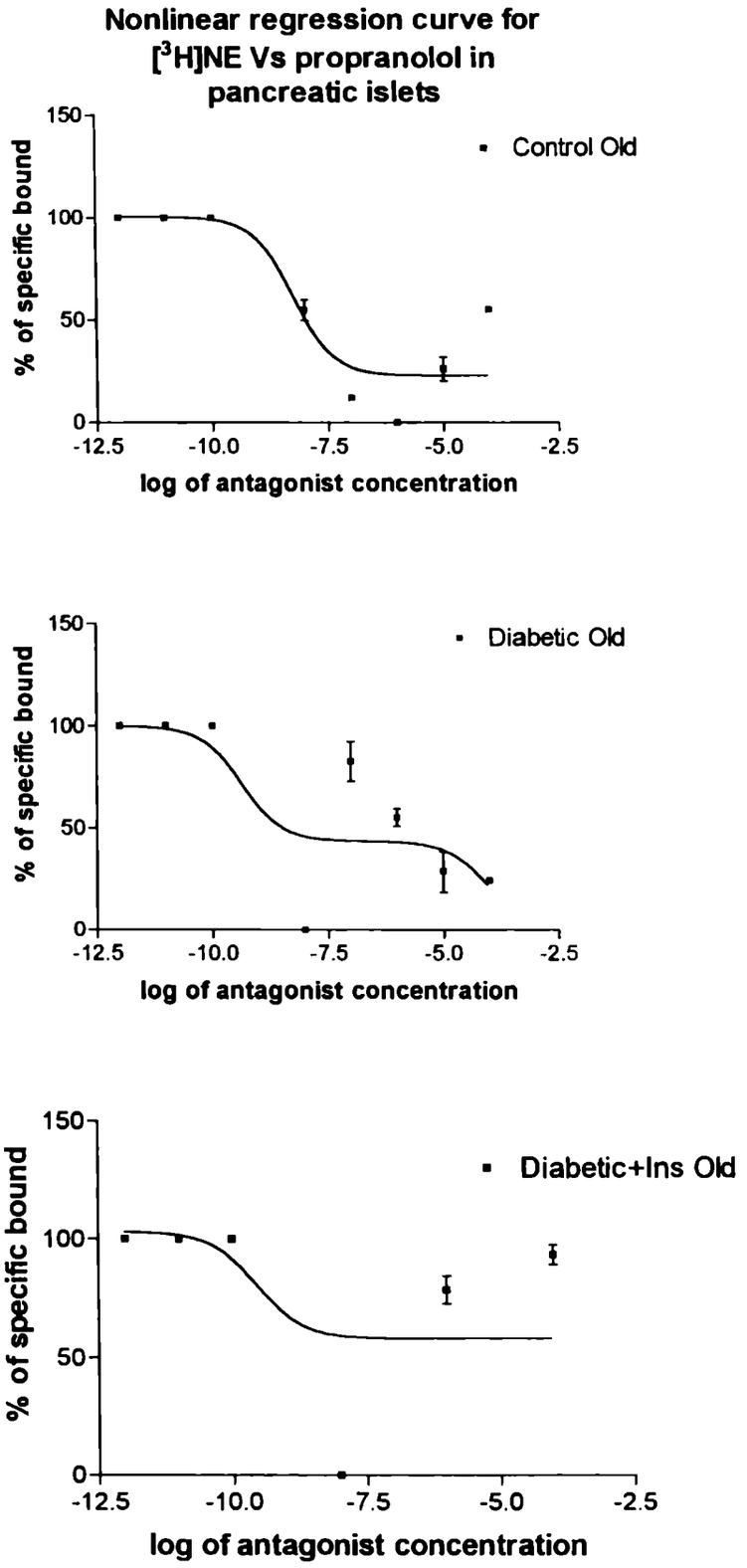

Nonlinear regression curve for
[3H]NE Vs propranolol in
pancreatic islets
Control Old
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Diabetic Old
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Diabetic+Ins Old
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

Figure-17

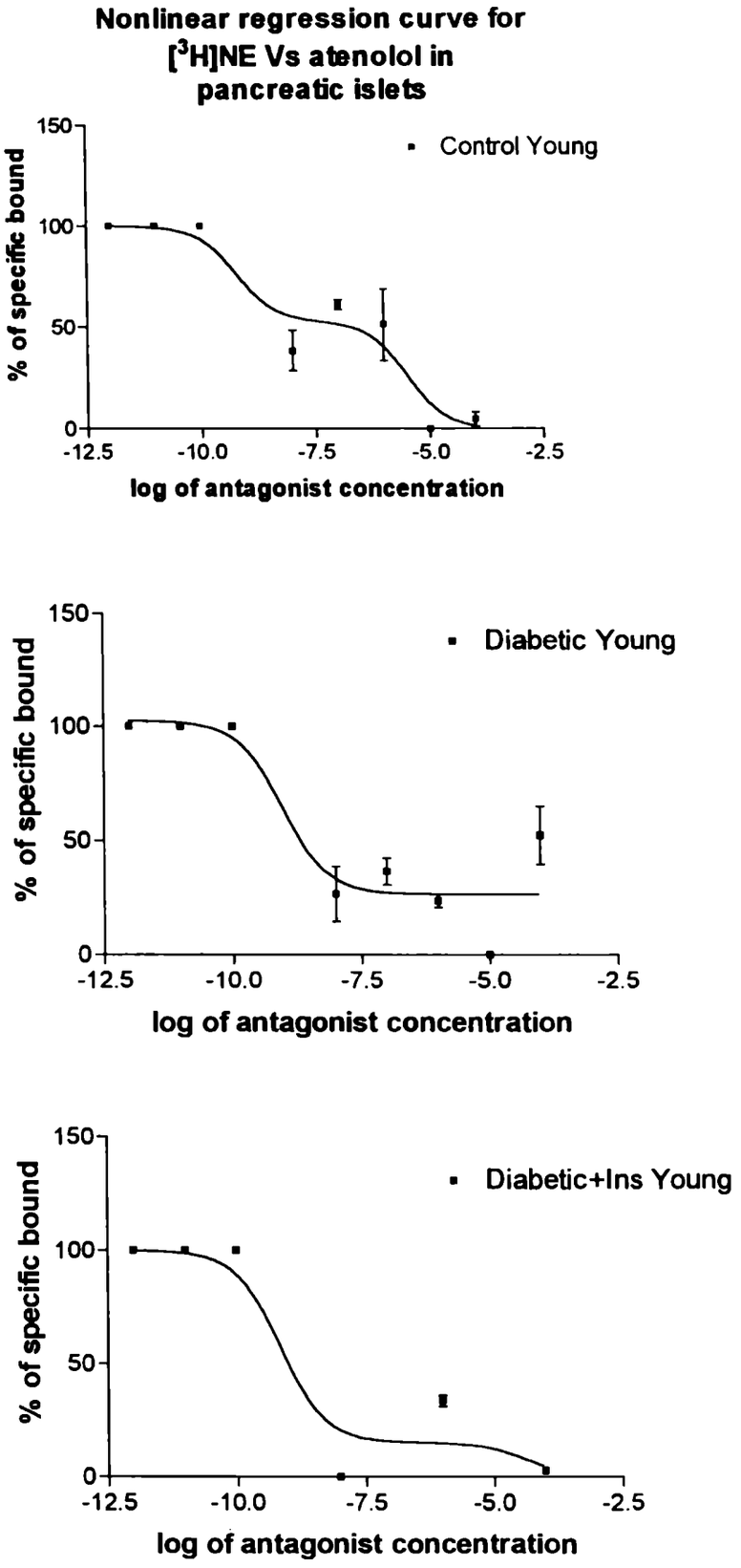

Nonlinear regression curve for
[3H]NE Vs atenolol in
pancreatic islets
Control Young
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Diabetic Young
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration
Diabetic+Ins Young
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

Figure-18

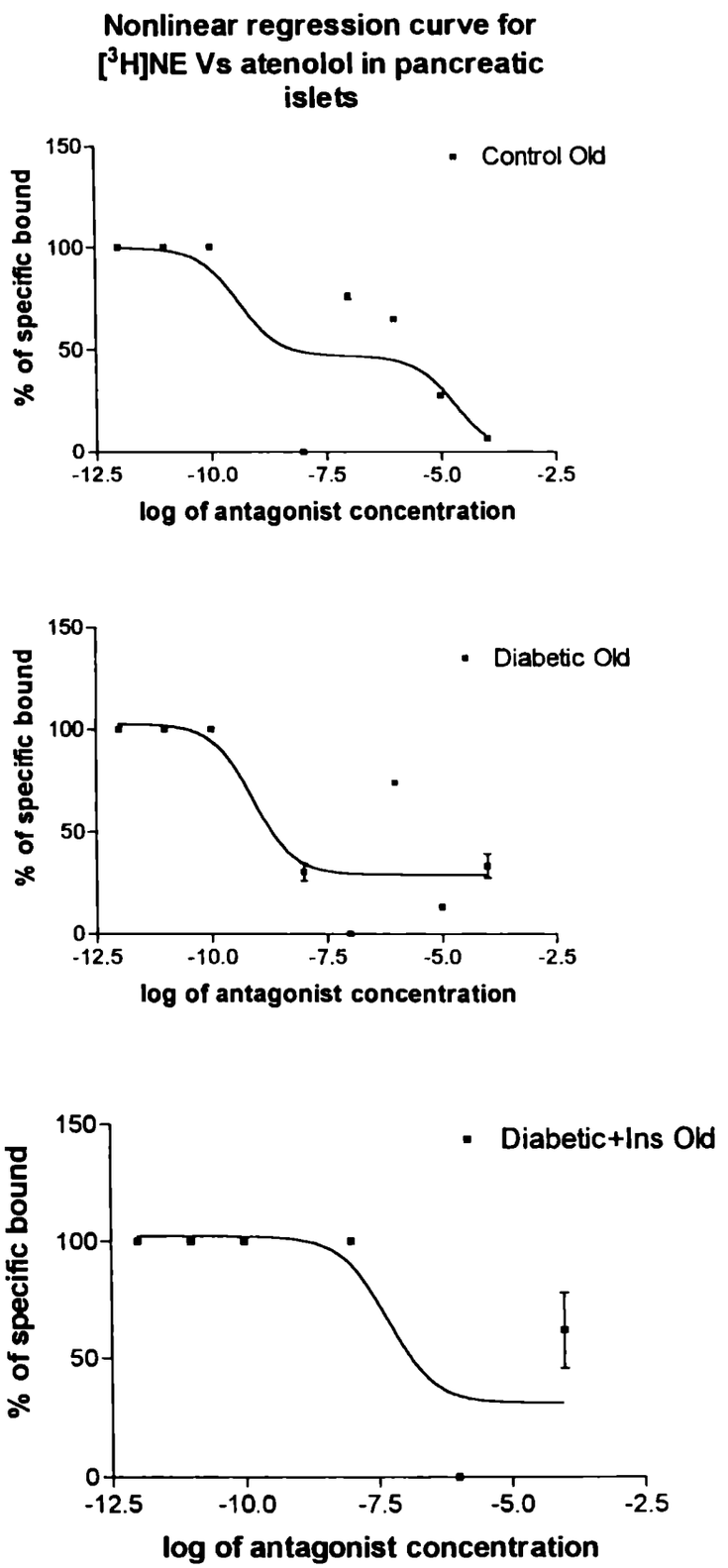

Nonlinear regression curve for [3H]NE Vs atenolol in pancreatic islets
Control Old
Diabetic Old
Diabetic+Ins Old
% of specific bound
150
100
50
0
-12.5
-10.0
-7.5
-5.0
-2.5
log of antagonist concentration

Figure-19

EFFECT OF CORTICOSTEROIDS ON GLUCOSE INDUCED INSULIN SECRETION AS A FUNCTION OF AGE *IN VITRO*

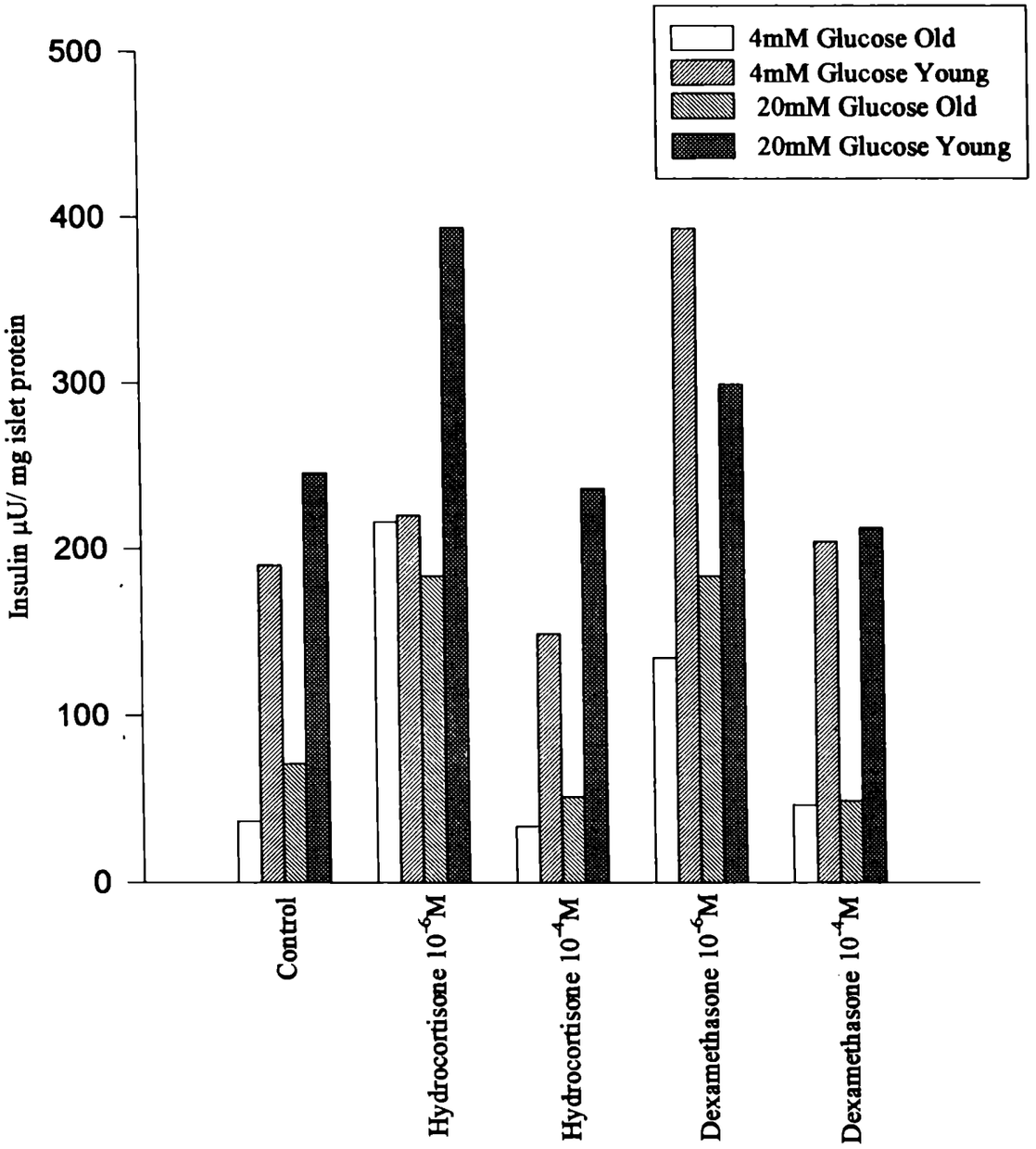
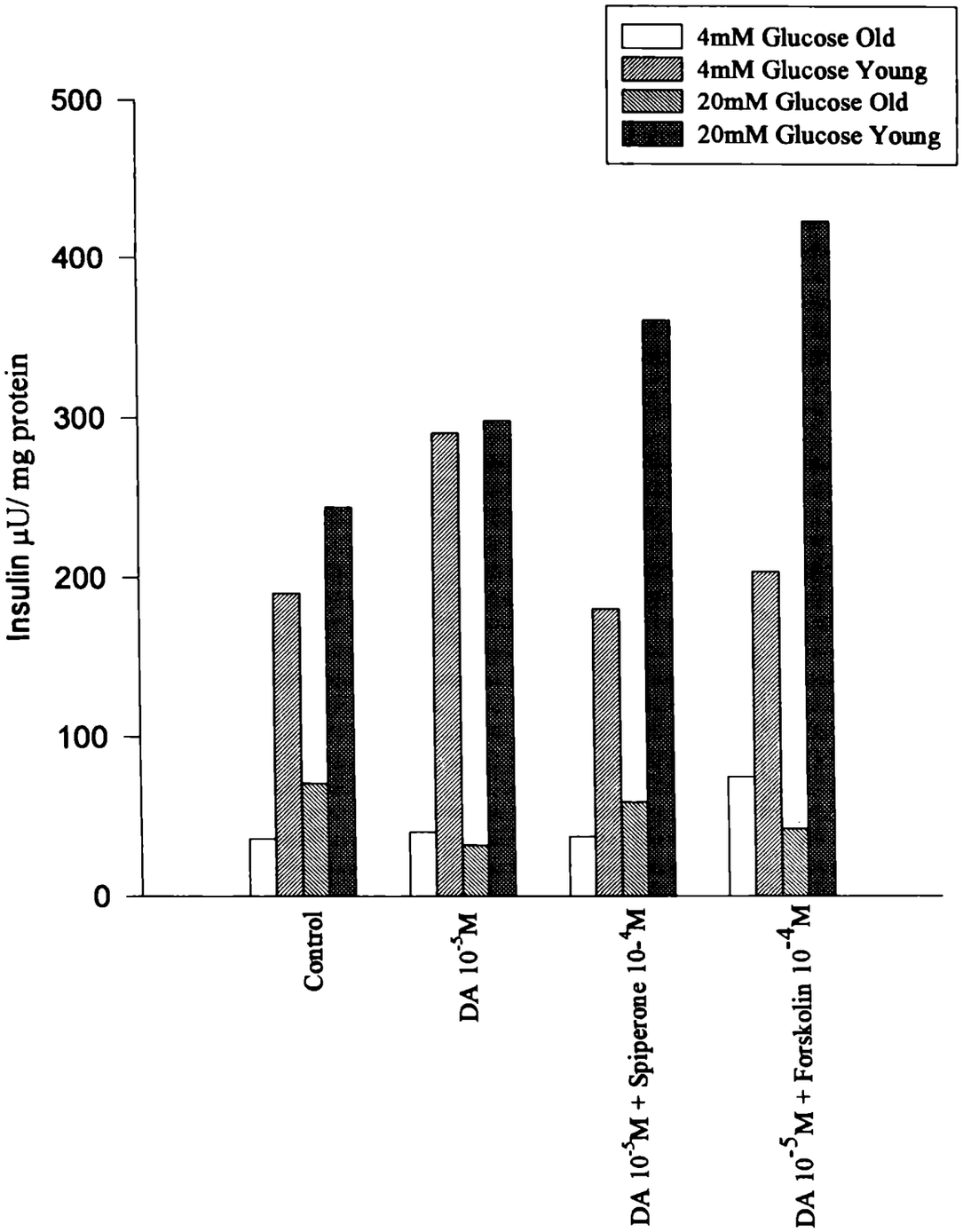


Figure-20

EFFECT OF DOPAMINE ON GLUCOSE INDUCED INSULIN SECRETION AS A FUNCTION OF AGE *IN VITRO*



Discussion

DISCUSSION

Blood Glucose and Bodyweight

Diabetes mellitus is essentially a disease of hyperglycaemia. Administration of streptozotocin results in hyperglycaemia. This is due to the marked destruction of insulin secreting pancreatic islet β-cells by streptozotocin (Junod *et al* 1969). Our results are in agreement with the various reports of marked hyperglycaemia and reduction of body weight in streptozotocin administered rats compared to vehicle injected controls. Hyperglycaemia occurs as a result of increased glycogenolysis, decreased glycogenesis, increased gluconeogenesis, impaired glucose transport across membranes and almost complete suppression of the conversion of glucose into fatty acids via acetyl-CoA. Reduction in body weight in diabetes is due to wastage of body tissues resulting from the altered metabolic function. The limited β-cell regeneration occurring in STZ-treated young rats (Weir *et al,* 1981) and decrease in insulin secretory efficiency (Curry *et al,* 1984) as animal ages, could account for the marked differences in the level of hyperglycaemia noted, as a function of age in diabetic rats. Central noradrenergic pathway by activation of sympatho-adrenal system can elecit hyperglycaemia (McCaleb & Myers; 1982). Concentration of norepinephrine in several brain regions have been reported to have increased during the course of diabetes (Tasaka *et al* , 1992). It is well documented that (Christensen, 1979, Jackson *et al.,* 1997) there is an elevation of circulating catecholamines during diabetes both in human and rats. Also, adrenodemelludation (Christine *et al.,* 1993) and adrenalectomy have been shown to have attenuated the STZ-induced hyperglycaemia (Yang & Lin, 1995). Our results clearly indicate the role of adrenal hormones and adrenergic receptors in glucose homeostasis and cellular function in diabetes. Blocking of corticosteroid biosynthesis, and adrenalectomy in diabetic rats resulted in significant decrease in the levels of corticosteroids and catecholamines, which may arrest the increased glycogenolysis and gluconeogenesis observed in diabetes, thus bringing about a control in hyperglycaemia.

Brain Monoamines and their metabolites as a function of age

The glucose homeostasis is dependent on central noradrenergic (McCaleb & Myers, 1982) serotonergic and dopaminergic control (Alster & Hillegaart, 1996). We, therefore investigated the changes occurring in the monoamines and their metabolites in various regions of the brain in three age groups of rats in order to understand its functional correlation in the development of diabetes mellitus in the elderly. Our results show that there is a significant age-related increase in NE content in the hypothalamus, brain stem, corpus striatum, cerebral cortex and cerebellum. A progressive increase in adrenal medullary hormones with age have been reported (Veith *et al.*, 1986). Garris (1990) have reported an age-related increase in NE in various brain regions of genetically obese diabetic mice C57 BL/KsJ Mice. Reis *et al.*, (1977) have reported an increased tyrosine hydroxylase, a key enzyme in the catecholamine biosynthesis, activity in the hypothalamus and adrenal glands of rats as a function of age. This is in agreement with our finding of increased NE content in the ageing brain . Also a progressive decline of the catecholamine degrading enzymes monoamine oxidase (Oreland & Shasken, 1983) and catechol-o-methyl transferase activity in brain of rats (Stramentioli, 1977) with age has been reported. We propose that increased noradrenergic activity in the ageing brain could be a factor leading to impaired glucose tolerance and insulin secretory efficiency seen in the aged.

Our results are concordant with the previously reported findings of decreased DA content in striatum of rats (Finch, 1971). A decrease in dopamine receptors in the striatum has also been reported (Misra *et al*, 1980). Also it has been reported that in striatum and substantia nigra, there is a significant decrease in the ability of dopamine to stimulate the formation of cAMP with age. Substantia nigra is one autonomous area of the central nervous system which plays an important role in controlling the structure and activity of the pancreatic islets. Studies have shown that pancreatic islets from rats with lesions of the substantia nigra are reduced in both size and number compared to age matched controls, accompanied with specific change in islet hormone content and islet function (Smith & Davis, 1983). Moreover, a decreased dopamine synthesis rate (Trulson & Himmel, 1983) and decreased dopamine D_1 receptors in the corpus striatum of diabetic

rats (Salkvoic & Lackovic, 1992) have been reported. Therefore, its logical to presume that the impairment of central dopaminergic system has a significant role in the development of adult onset diabetes mellitus.

Serotonin content did not show any change or showed slight increase in various brain regions studied. This is in agreement with already published reports (Simpkins *et al*, 1977) on the effect of ageing on serotonin content. Serotonergic system play a role in glucose homeostasis via 5-HT_{1A} receptors (Alster & Hillegaart, 1996) and an increase in 5-HT_{1A} and $5HT_2$ receptors have been reported in STZ-diabetic rats (Sandrini *et al.*, 1997). Increased levels of 5-HT in aged rats might mediate a regulatory role in insulin function via mediation of 5-HT_{1A} and $5HT_2$ receptors.

Brain monoamines and their metabolites in diabetes as a function of age

Though considerable amount of literature is available on diabetes induced alterations in the brain monoamines, very few comparative reports are there on the alterations of brain monoamines in diabetic state in young and old rats. Regional brain norepinephrine concentrations and adrenergic receptors have been reported to show age and diabetes associated alterations in genetically obese diabetic (C57 BL/KsJ) mice, (Garris, 1990) where regional brain NE levels in diabetic state were chronically elevated as compared to those of age-matched controls (Garris, 1990).

Our results show that there is marked difference in the alterations of brain monoamines in diabetes as a function of age. Though, we have observed an age-related increase in NE content, in control rats, this was not the case in STZ-diabetic rats. STZ-diabetic young rats, showed a significant increase in NE content in hypothalamus, brainstem, corpus striatum and cerebellum and no change in NE content was noted in the cerebral cortex. This is concordant with the previously published reports (Lackovic *et al* , 1990; Chen & Yang, 1991, Tasaka *et al*, 1992). STZ-diabetic adult rats showed significant increase in NE in brain stem and cerebellum while in the hypothalamus and corpus striatum there was a significant decrease in NE content and cerebral cortex did not show any significant change. STZ-diabetic old rats showed significant decrease in the NE

content in hypothalamus and brain stem, while in other regions, no significant change was noted. A decrease in hypothalamic NE content have been reported in diabetic state (Chu *et al*, 1986; Shimizu, 1991). NE levels were also decreased in pons and medulla (Ramakrishna & Namasivayam, 1995) neocortex and caudal segment of the brain stem (Kulikov *et al.*, 1986). However, none of these studies have addressed the role of ageing in these changes.

Thus, we see a differential regulation of NE in young and old diabetic rats as a function of age. A differential regulation of glutamate dehydrogenase activity in the brain of young and adult STZ-diabetic rats have been reported (Biju & Paulose, 1998). The mechanism behind this differential regulation of NE in the different brain regions of young and old rats is unclear. Several possibilities can be considered. Elevation of NE could be due to inhibition of presynaptic release of NE or an increased reuptake of released NE (Bitar, *et al*, 1986), and a decrease in the metabolic degradation of NE (Bitar & De'Souza, 1990, Ramakrishna & Namasivayam, 1995, Trulson & Himmel, 1985). An elevation in tyrosine hydroxylase enzyme activity in response to both short and long term hyperglycaemia, in all the brain regions have been reported (Gupta, *et al* 1992). This increase in tyrosine hydroxylase activity could also be responsible for the high levels of NE reported in diabetes. The decrease in NE content in adult and old diabetic rats could be due to either decreased uptake, synthesis or an increased rate of degradation in old diabetic rats. An age related decrease in the uptake of NE and DA in the synaptosomes of hypothalamus and striatum of mouse has been reported (Sun, 1976; Jonec & Finch, 1975). Monoamine oxidase have been reported to be higher during short term diabetes (Mayanil *et al*, 1982). Besides, a significant lack of responsiveness of noradrenergic system in old rats (Greenberg & Weiss, 1979) could also play a role in the differential regulation observed.

Dopamine also showed differential regulation in diabetes as a function of age. Our results are concordant with those of others (Chu *et al.*, 1986; Tasaka *et al* .,1992; Shimizu, 1991) with respect to young and adult diabetic rats. Of all the brain regions studied, only corpus striatum showed significant increase in DA content in diabetic state in young, rats. However, in adult diabetic rats, there was a significant increase in DA

content in corpus striatum , cerebral cortex and cerebellum. Diabetic old rats on the other hand, showed a decrease in DA content in hypothalamus with no significant change in all other regions. This could be due to failure of the dopaminergic system which we have already discussed with reference to ageing. The increase in DA content in young and adult rats could be due to a decrease in its degradation (Trulson & Himmel, 1983; Bellush *et al.*, 1991) as evidenced by decrease in its metabolite homovanillic acid. Elevation in tyrosine hydroxylase enzyme activity (Gupta *et al.*, 1992) could also contribute to increased DA. A decrease in the release of dopamine from synaptosome in hyperglycaemic state (Lim & Lee, 1995) could also account for the elevated DA in young and adult diabetic rats. Thus, we see a differential regulation of DA and its metabolite, HVA in different regions of the brain as a function of age.

Serotonin showed differential metabolic regulation in diabetes as a function of age. From our results it is evident that 5-HT content decreased in the hypothalamus of young diabetic rats due to increased metabolic degradation. In cerebral cortex and corpus striatum, the decreased 5-HT content was due to decreased synthesis and increased degradation. In adult diabetic rats, brain stem and corpus striatum showed significant reduction in 5-HT content due to decreased synthesis and also increased metabolic degradation. However, hypothalamus and cerebellum showed significant increase in 5-HT levels. , This was due to increased synthesis and decreased degradation. In diabetic old rats, 5-HT synthesis decreased significantly in brain stem, while in the cerebellum, there is decrease in 5-HT synthesis and degradation. Though alterations in 5-HT levels in diabetic state are reported in literature, we find there no report on the effect of ageing on 5-HT metabolism in diabetic state. Our work throws light on the differential metabolism of 5-HT occurring in different regions of the diabetic brain as a function of age.

Serotonin levels have been reported to be lower in neocortex and central segment of brain stem (Kulikov *et al* , 1986) hypothalamus and brain stem (Chu *et al* , 1986; Shimizu, 1991) and no change in 5-HT were reported in corpus striatum (Chu *et al* , 1986) in diabetic state. However, we report that there is reduction of 5-HT levels in corpus striatum of young and adult rats, though no change was evident in old rats. Though no reports of 5-HT content in cerebellum is available, we found that there was

differential regulation of 5-HT in cerebellum of adult and old diabetic rats, for 5-HT levels increased in adult and decreased in old. The discrepancies in the literature could be because of the differences in age groups studied and the animal models chosen. Studies in diabetic mice, revealed an increase in 5-HT in the hypothalamus, hippocampus, pons-medulla and cortex (Chen & Yang, 1991).

Changes in monoamines have profound effect on mood alterations. Quite moderate change of brain tryptophan concentration can lead to altered behaviour (Lytle *et al* , 1975). The mood swings commonly observed in diabetic patients may be attributable, at least in part to alteration in forebrain NE metabolism (Trulson & Himmel, 1985) . Altered dopaminergic function in the CNS may mediate certain endogenous mood disorders as well as the emotive changes that accompany diabetes mellitus (Trulson & Himmel, 1983). Hyperthermia, hyperlocomotor activity, hyperphagia, associated with diabetes mellitus is attributed to changed noradrenergic activity in the brain (Bitar *et al.*, 1987). Also altered response to painful stimuli observed in diabetics have been attributed to the dopaminergic and serotonergic system (Chu *et al* , 1986).

Among the various neuroendocrine alterations, increased adrenal activity have been recognised in diabetic patients (Hudson *et al,* 1984). The hypothalamo-pituitary-adrenal axis might play a very significant role in the pathophysiology of diabetes mellitus. Central catecholamines play a stimulating role on the secretion of ACTH through an action at the hypothalamic level (Giullaume *et al.*, 1987). ACTH from the brain influence the adrenal cortex to secrete corticosteriods. Corticosteroids play an important role in carbohydrate metabolism, excessive corticosteriods can result in hyperglycaemia. It is therefore reasonable to think that if the adrenal activity can be suppressed, many metabolic alterations in diabetes could be prevented.

In young diabetic rats, both adrenalectomy and metyrapone treatment significantly reduced the increased NE levels in all the brain regions studied and the extent of recovery was as good as insulin therapy. In diabetic old rats, where a decrease in NE was noted, adrenalectomy and metyrapone treatment, further decreased the NE content. DA content was also affected by these treatments in the corpus striatum, where a significant increase in DA was noted. Adrenalectomy and more efficiently, metyrapone treatment reversed the

DA content to control values. Metyrapone treatment significantly increased the 5-HT values in young diabetics. In old rats too, metyrapone treatment increased 5-HT values wherever it decreased. Dopaminergic system was unresponsive to metyrapone treatment in the old.

From our results, it is quite clear, that adrenal hormones does have a profound effect on regulating the levels of monoamines in the brain. These effects may be acting via the hypothalamo-pituitary-adrenal axis-because the blood-brain-barrier prevents the entry of these hormones directly into the brain. Moreover, we find that increased levels of NE in brain has positive correlation to hyperglycaemia and that this is reversed by the reduction of adrenal medullary as well as cortical hormones.

Adrenalectomy increases pro-opio-melanocorticotropin (POMC) transcription in the brain (Robert *et al.*, 1993). The POMC family consists of peptides that act as hormones and neurotransmitters or neuromodulators. They are ACTH, β-lipotropin, α-MSH, corticotrophin like intermediate lobe peptide, γ-lipotropin, β- endotropin, β- MSH, γ-endorphin and α-endorphin. β-endorphin has been reported to stimulate insulin secretion in diabetes (Giugliano *et al.*, 1987). Besides, Guellan, *et al.*, (1978) have found an enhancement of the number of beta-receptor sites after adrenelectomy in hepatic plasma membranes. Perhaps, this increase in beta-receptor sites might occur in pancreas as well. It is well documented that β-adrenergic receptor stimulation enhances insulin secretion. Also, ACTH in supra physiological concentrations stimulate insulin release (Robert *et al.*, 1993). Adrenalectomy and metyrapone administration results in enhanced ACTH release, under these conditions sufficient corticosteriods are absent to impose a feed back suppression of ACTH and thus promote insulin secretion. This explains why adrenalectomy and metyrapone were effective in controlling the hyperglycaemia and monoamine alterations in diabetic state.

Adrenergic receptor function in the brain stem of diabetic rats as a function of age

Brain stem region has direct connection with the pancreatic islets through the vagus nerve. Therefore we decided to study the alterations of adrenergic receptors in this

region. NE can act on distinct receptor subtypes α_1, α_2, β_1, β_2 and β_3. It is generally accepted that β-adrenergic receptors are positively coupled to adenylate cyclase (Dohlman *et al* , 1991), α_1-adrenergic receptors receptors are coupled to phospholipase C and PI turn over (Dean *et al* , 1997), while α_2-adrenergic receptors are negatively coupled to adenylate cyclase (Yamazaki, *et al.*, 1982). Adenylate cyclase is involved in the generation of cAMP which is an important second messenger in signal transduction. cAMP system may be regulated by various other hormones, neurotransmitters and neuromodulators in addition to adrenergic receptors.

From our results it appears that the increase in NE in old rats may be leading to the adrenergic receptor down-regulation. The affinity for the ligand increased in all subtypes of receptors, however, the strength of binding decreased. Down-regulation of biological response despite adequate concentration of the ligand, may be due to phosphorylation of the adrenergic receptors (Sibley *et al.*, 1984). However, cAMP levels were high, indicating that there was activation of adenylate cyclase. This could be due to either the signal transducted through β_1-adrenergic receptor subtype in old rats which also displayed high affinity for the ligand,(β_1-adrenergic receptors were not detected in control young rats) or functional alteration of G-protein could occur. Incidentally, β_1-adrenergic receptors are the ones primarily involved in neuronal function (Nicoll *et al* , 1990). From our results, it appears that in the old rats there is increased neuronal transmission in the old compared to young. In young diabetic rats, there was an up-regulation of adrenergic receptors with an increase in affinity for the ligand. α-adrenergic receptors displayed higher affinity for the ligand and but the strength of binding was low as in old controls. However, total β-adrenergic receptors and β_1-adrenergic receptors showed enhanced activity. β_1-adrenergic receptors were not expressed in control young. This partly explains the increase in cAMP levels noted in young diabetics compared to control. Basal adenylate cyclase activity was increased in the retina of diabetic rats, consistent with the observed reduction of G_i/G_o inhibitory proteins. Such functional alterations of cAMP producing system were reversed by insulin therapy (Abbracchio *et al.*, 1991). Perhaps similar mechanisms might be operating in this case also. A functional imbalance between

G_s-protein and G_i/G_o protein mediated transduction mechanism with an increased efficiency for G_s activity, probably as a result of the loss of G_i/G_o inhibitory function has been found in the striatum, hepatocytes and adipocytes of diabetic animals (Abbracchio *et al.*, 1989; Hadjiconstantinou *et al.*, 1988; Gawler *et al.*, 1988). Diabetic old rats displayed an up-regulation of adrenergic receptors in response to lowered NE, with no net change in affinity for the ligand. Also cAMP levels were unaltered in spite of the adrenergic up-regulation. α-adrenergic receptors remained in the high affinity state with decreased strength of binding just as in control. However, significant changes in β-adrenergic receptors were noted. There was a decrease in function of total β-adrenergic receptors and β_1-adrenergic receptors. This relative desensitisation of β-adrenergic receptors must have prevented further enhancement of adenylate cyclase activity. Thus, it appears that there is a lack of responsiveness in noradrenergic receptor system in old diabetic rats. An impairment of adenylate cyclase activity or G-protein function cannot be ruled out. Impairment of adenylate cyclase activity has been reported in the sciatic nerve of diabetic rats (Shindo *et al.*, 1993). Our results also show that there is only reversal of the number of receptors by insulin therapy but not in the affinity status. Thus, from our studies it is clear that a differential regulation of adrenergic receptor function is occurring in diabetic state as a function of age.

These functional alterations in adrenergic receptor subtypes many lead to various neuroendocrine disturbances. NE through the mediation of α_1-adrenergic and β-adrenergic receptors could induce ACTH surges (Szafarezyk *et al.*, 1987) in rats resulting in excess corticosteroid production. An age-related increase of corticosteroids with age has been reported (Slotkin *et al.*, 1996). The enhancement of NE, observed as a function of age could thus contribute to increase in glucocorticoids . This in turn, could influence pancreatic endocrine function, for it has been reported that glucocorticoids up-regulate α_{2R}-adrenergic receptor expression and signalling in pancreatic β-cells (Hamamdzic *et al.*, 1995).

Systematic literature review has not revealed any reports on the alterations of total adrenergic receptors in the brain stem in STZ-diabetics as a function of age. However, Bitar & De'Douza (1990) have reported an increase in the density of β_1-receptors in

hypothalamus, thalamus and amydala in diabetic rats. Garris (1990) has reported an increase in NE accompanied by increase in α_1 and α_2 and a decrease in β-adrenergic receptors in olfactory bulbs, cortex, hypothalamus, midbrain, cerebellum and pons, in genetically obese diabetic mice as a function of age. The discrepancies in observations could be due to the differences in the animal model chosen and the age groups studied.

Monoamines and their metabolites in whole pancreas

Very few laboratories have reported on the effect of diabetes on pancreatic catecholamine concentration. Ostenson *et al.*, (1993) have reported that in NIDDM rat model, pancreatic contents of NE and EPI, were similar in diabetic and non diabetic rats. In Chinese hamsters too, there was no significant increase in NE in the pancreatic islets in diabetes (Feldman and Gerritsen, 1988)

We observed a differential regulation of NE content in the pancreas in diabetic state. An age-related decrease in NE content was noted in the pancreas of control of rats. NE content increased significantly in old diabetics, while it was unaltered in the young. This shows that there is a reciprocal relationship in the NE metabolism occurring in the brain and pancreas. Metyrapone treatment was successful in reversing the metabolic derangement, perhaps by stimulating insulin release via increased ACTH, which we discussed in context of brain monoamines.

DA content also showed a differential regulation in diabetic rats as a function of age. In old rats, DA content decreased significantly compared to young. Young diabetic rats had significant decrease in DA content, while DA content was unaltered in old diabetics rats. Metyrapone was effective in reversing the changes. Here also reciprocal relationship existed in the DA content in corpus striatum and pancreas of young rats. The general failure of dopaminergic system as a function of age noted in the brain is also reflected in the pancreas of old rats. No reports are available on the alterations of DA content in the pancreas in the diabetic state as a function of age. However, DA is reported to reduce the glucose induced insulin secretion in mice (Lindstrom, *et al.*, 1983) and golden hamsters (Zern *et al.*, 1980). These studies and our study shows that DA has a

role in regulating insulin secretion, though very often DA is implicated with altered behaviour.

Serotonin content did not show any significant change in pancreas in all the experimental groups studied.

Catecholamines in isolated pancreatic islets

Increased NE in the islets of control old and diabetic young and old rats could be as a result of increased uptake and decreased degradation. Elevated levels of plasma catecholamines in diabetes have been reported (Jackson *et al.*, 1997). Most released NE, is efficiently removed by neuronal and extraneuronal uptake (Eisenhofer *et al.*, 1992). NE is metabolised by catechol-o-methyl transferase to normetanephrine (NMN) extraneuronally (Eisenhofer *et al.*, 1995). Our results have shown an age related decrease in NMN content in the whole pancreas of old and diabetic young rats. Perhaps an impairment of catechol-o-methyl transferase during ageing and diabetes might be occurring. Catechol-o-methyl transferase activity in the brain has been reported to decrease with age (Stramentioli, 1977). Decrease in the degradation of adrenal medullary hormones as a function of age has been reported (Hruza, 1973). Beta-adrenergic stimulation appears to increase catecholamine clearance (Cryer *et al.*, 1980). Ostenson *et al.*, (1989) have found that the β-cells of STZ diabetic islets are more sensitive to α_2-agonism than normal islets. The failure in the degradation system perhaps due to β-adrenergic receptor damage might be operating in the old as well as diabetic pancreatic islets alike. Incidentally α_2-adrenergic receptor stimulation inhibits insulin secretion (Niddam *et al.*, 1990; Lacey *et al.*, 1993) and β-adrenergic stimulation (Lacey *et al.*, 1996) enhances insulin secretion. It also appears that the EPI in old diabetics is degraded more slowly when compared to young diabetics implying an age related receptor damage in the pancreas.

Besides, inhibiting insulin secretion NE and EPI may have a role in triggering a regenerative response in the pancreas, especially in the young diabetics. A limited β-cell regeneration has been observed in STZ-treated neonatal rats (Weir *et al.*, 1981).

Neurotransmitters like NE (Cruise *et al.*, 1985) and 5-HT (Sudha & Paulose, 1998) have been shown to enhance hepatocyte DNA synthesis. This could be a possible explanation of the increased recovery trend observed in young STZ-diabetic rats.

Adrenergic Receptor function in isolated pancreatic islets of diabetic rats as a function of age

Pancreatic islets were isolated and the adrenergic receptors in the islets cell suspension was studied as a function of age. Previous studies indicated that there was no age-related differences in [^{3}H]norepinephrine binding to pancreatic islets isolated from neonatal and adult rats (Gembal & Wojcikowski, 1993). However, our results show that there was significant differences in noradrenergic receptor binding parameters in young and old rats and that a differential regulation occurred in diabetic state as a function of age. The receptor data is concordant with the pancreatic content of NE. In old rats there is receptor up-regulation in response to decrease in NE. The receptor analysis revealed that there is enhancement of α_2-adrenergic receptor function compared to young. Lacombe *et al.*, (1993) has reported that pancreatic islets of adult hamsters had higher number of α_2-adrenergic receptors with no change in affinity towards the ligand compared to young. There was also a desensitisation of total β-adrenergic and β_1-adrenergic while, α_1-adrenergic receptor function was not altered. Under these circumstances, we would expect a reduction in cAMP. However, there was no significant change in cAMP content as a function of age. This implies that there may be an impairment in the receptor-effector coupling as a function of age. The low NE content observed in young diabetics might also be due to increased clearance rate compared to diabetic old rats. The adrenergic receptors demonstrated an up-regulation with increased affinity towards the ligand accompanied with increase in cAMP levels. There was a functional enhancement of β_1-and α_1-adrenergic receptors accompanied by desensitisation of α_2-adrenergic receptors. The β-adrenergic receptors generally increase cAMP levels by activating adenylate cyclase and this results in potentiation of insulin release. α_1-receptors have been implicated in triggering regenerative response in hepatocytes when stimulated by NE (Cruise *et al.*,

1985). Therefore, during diabetic state in young rats. the increased noradrenergic activity might trigger DNA synthesis and this may be the cause for observed recovery trend and β-cell regeneration in neonatal STZ-rats (Weir *et al.*, 1981). Neurotransmitters like 5-HT has also been reported to trigger hepatocyte proliferation (Sudha & Paulose, 1998). In diabetic old rats, the pancreatic islet, adrenergic receptors showed an up-regulation with decrease in affinity for the ligand accompanied with decrease in cAMP content. There was a functional decrease of α_1-receptors implying that the regenerative capacity is low for diabetic old rats. The cAMP levels were lowered in spite of the down-regulation of α_2-adrenergic system and enhancement of β_1-adrenergic function. This means that in old diabetic rats, there is a an impairment of receptor-effector coupling, or an impairment in adenylate cyclase activity. No major alteration in the function of adenylate cyclase in the pancreatic β-cells of diabetic rats have been reported. However, a defective glucose-induced cAMP generation, due to a block in the activation of adenylate cyclase has been reported in diabetic state (Dachicourt, *et al* , 1996). A decrease in the functional responses of cAMP increasing agents like β-adrenergic agonists has been reported in diabetic hearts and this change has been attributed to phosphorylation of cardiac regulatory phosphoproteins including phospholamban (Gando, 1994).

Thus the progressive increase in the noradrenergic transmission from the brain accompanied by enhancement of α_2-adrenergic and decrease in β-adrenergic function in the pancreatic islets, could eventually lead to adult-onset diabetes mellitus. Also, other neurotransmitters and their receptors could also play a role insulin function and cell proliferation (Paulose *et al.*, 1998). A study of differential regulation of adrenergic receptors at the molecular level in young and old pancreatic islets as well as in the brain of diabetic rats, will enlighten the exact mechanisms involved. If we can modify the functional expression of the adrenergic receptors subtypes to trigger β-cell function, it will be of clinical significance.

Glucose induced insulin secretion - In vitro studies

The effect of increasing glucose on insulin secretion and cAMP production within the pancreatic islets was studied. There is a significant decrease in the amount of insulin

secreted by the islets of the old rats compared to young at both low and high glucose concentration. However, both young and old islets responded positively towards increasing concentrations of glucose, by increasing the insulin secretion. Our results are concordant with the findings of Bombara *et al.*, (1995) and Castro *et al.*, (1992). They have reported an impaired glucose induced insulin release during ageing in Sprague Dawley and Wistar rats. cAMP system was responsive to increasing glucose concentration in both age groups of rats. However, during ageing there appears to be an impairment in stimulus /coupling of cAMP accumulation. No reports in age-related studies on cAMP accumulation on glucose induced insulin secretion is available. Perhaps age-related adrenergic receptor alterations and/or G-protein function may play a regulatory role.

We also studied the effect of DA on glucose induced insulin secretion as a function of age *in vitro*. We found that DA had an inhibitory effect on glucose induced insulin secretion from old pancreatic islets at high concentration of glucose, while in young rats, the DA had a stimulatory effect. We found that the inhibitory effect of DA in old rats is mediated via DA-D_2 receptors. DA-D_2 receptors are negatively linked to adenylate cyclase (Susan, 1994). This could result in decrease in cAMP and thus bring about inhibition of insulin secretion. Though high concentrations of DA have been reported to decrease glucose induced insulin secretion (Zern *et al.*, 1980; Tabeuchi *et al.*, 1990). No reports on the effect of DA on insulin secretion as a function of age or the mechanism involved is available. We conclude that DA exerts a differential influence on age-related glucose induced secretion, and that it is partly linked to the adenylate cyclase-cAMP system. Addition of forskolin an activator of cAMP resulted in overcoming the DA induced inhibition in insulin secretion to certain extent. These findings along with our results in the brain regions points that DA does have a role in the pathophysiology of diabetes.

In our earlier discussion we have shown that corticosteroids may contribute substantially towards pathogenesis of diabetes. We also conducted *in vitro* experiments to see the effect of corticosteroids on insulin secretion. We found that at low concentrations these hormones stimulated insulin release and at high concentrations inhibited insulin

release in both young and old rats. We conclude that during diabetes there might be corticosteroid excess, which also exerts an additional inhibitory influence along with adrenal medullary hormones. Though the exact mechanism is not clear, it could be because of the up-regulation of α_2-adrenergic receptors and signalling in pancreatic islets by corticosteroids (Hamamdzic *et al.*, 1995). Moreover, corticosteroids produces insulin resistance in tissues in terms of peripheral glucose uptake (Brindley *et al.*, 1996).

CONCLUSION

We conclude from our studies that the alterations in the monoamine metabolism and adrenergic receptor function during ageing, could be a pre-disposing factor in the development of adult-onset diabetes mellitus. Our *in vitro* studies show that there is significant decrease in the amount of glucose induced insulin secretion by the islets of old rats compared to young. Though cAMP system in pancreatic islets was responsive to increasing glucose concentration, there appears to be an impairment in stimulus/coupling of cAMP production with age. There was progressive increase in NE content with age in different regions of the brain and this seems to be positively correlated to blood glucose concentrations, implying its role in insulin function. The functional expression of the different adrenergic subtypes played a significant role in ageing and in the pathogenesis of diabetes mellitus. Our results showed that there is increased neuronal transmission in the brain stem of old compared to young. There was an enhancement of β-adrenergic receptor function in the brain stem of diabetic young rats, while there was a decrease in β-adrenergic function in the diabetic old rats. In pancreatic islets too there was enhancement of total adrenergic function during ageing. This was accompanied by decrease of β-adrenergic receptor function and enhancement of α_2-adrenergic function. These conditions have an inhibitory influence on pancreatic insulin secretion. In diabetic young rats, pancreatic islets demonstrated an enhancement of β_1-and α_1-adrenergic receptor function, while in the old, there was a functional decrease in α_1-receptors accompanied by impairment of adrenergic receptor-cAMP system coupling. Thus, we see that there is differential regulation of adrenergic receptor function in diabetes as a function of age.

Adrenal hormones do have a role to play in the pathophysiology of diabetes. This was confirmed by blocking corticosteroid biosynthesis and adrenalectomy in diabetic rats. Both these manipulations resulted in reversal of alterations in monoamine content and hyperglycaemia during diabetes. *In vitro* experiments confirmed that at low concentrations of corticosteriods there was stimulated insulin secretion, while at high concentrations, insulin release was inhibited. Manipulation of the adrenal hormonal function by modifying the expression of adrenergic receptors at the molecular level may have far reaching clinical significance in the control and cure of diabetes mellitus.

SUMMARY

1) Streptozotocin diabetic rats of three age groups-young, adult and old were used as an *in vivo* model to study the effect of ageing on monoamines and adrenergic receptors.

2) Monoamines, adrenergic receptor function and second messenger cAMP were studied in brain stem and pancreatic islets as a function of age in diabetes.

3) Significant differences in the regulation of brain and pancreatic monoamines were noted in diabetes as a function of age. Monoamine changes in brain and pancreas were contributory towards the decrease in insulin function during ageing.

4) The role of adrenal hormones in diabetes mellitus was studied *in vivo* with emphasis on brain and pancreatic monoamines, blood glucose parameters. Our findings show that adrenal hormones do play a role in bringing about monoamine alterations in brain and pancreas as well as hyperglycaemia during diabetes mellitus.

5) There was increased noradrenergic function in the brain stem and pancreatic islets of the old compared to the young accompanied by an enhanced α_2-adrenergic function and a decrease in β-adrenergic function in the islets. These conditions could be a pre-disposing factor towards the development of diabetes mellitus.

6) There was differential regulation of adrenergic receptor function in the brain stem and pancreatic islets of young and old diabetic rats. The brain stem of young diabetic rats showed enhancement of β-adrenergic function while the old showed a decrease. In the pancreatic islets of young diabetic rats there was an enhancement of β_1-and α_1-adrenergic receptor function while in the old there was a functional decrease in the α_1-adrenergic receptor.

7) cAMP determination has revealed that there is an activation of adenylate cyclase in the brain stem of old rats indicating an increased neuronal activity or functional alteration of G-protein. cAMP content was higher in the brain stem of young diabetic rats, while it was unaltered in the old. In pancreatic islets of old rats, an impairment of receptor-effector coupling was noted. In diabetic state, cAMP content increased in the young whereas there was a decrease in the old, implying an impairment of adenylate cyclase activity in islets of diabetic old rats.

8) Isolated pancreatic islets were used as the *in vitro* system to study glucose induced insulin secretion, cAMP synthesis and the involvement of dopamine and corticosteroids in glucose-insulin secretion as a function of age.

9) Significant decrease in glucose induced insulin secretion was observed as a function of age, accompanied by impairment of stimulus/cAMP generation coupling. Corticosteroids at low concentrations, stimulated insulin secretion, while at high concentrations, it inhibited insulin secretion. DA exerted opposite effects on young and old pancreatic islets. In old rats, DA inhibited insulin secretion while in the young it stimulated insulin secretion. Also, the inhibitory action of DA in old rats is mediated via DA-D_2 receptors which are negatively coupled to adenylate cyclase. Forskolin,which is an inhibitor of Gi protein was effective in overcoming this inhibitory effect of DA.

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