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# Neurotransmitter Receptor Gene Expression: Insulin Secretion and Cell Proliferation

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**Abstract**

Brain neurotransmitters and their receptors play an important role in regulating various cellular activities of an organism. The consequences of the neurotransmitter-receptor function can influence the regulation of metabolic manifestations in hypothyroidism, hypertension, diabetes and cell proliferation directly by central nervous system function or through the hypothalamic-pituitary-end organ axis. The functional difference of neurotransmitters and hormones through their receptor subtypes can lead to differential gene expression. Hormones such as insulin, glucagon; thyroxine, tri-iodothyronine, glucocorticoids function as growth regulators. These hormonal functions can be dictated by the neurotransmitters and their receptors. Neurotransmitters- Norepinephrine, Dopamine, Serotonin, Gamma aminobutyric acid, Acetylcholine and their receptor subtypes studies at the molecular level have shown that the functional difference of these neurotransmitters through their receptor subtypes can control insulin synthesis and release, cell proliferation and aging. Thus during developmental periods, neurotransmitter - hormonal function is critical for the cell proliferation, cell differentiation and functional integration of tissues and organs.

## Introduction

Neurotransmitters like norepinephrine (NE), serotonin (5-HT), dopamine (DA), gamma aminobutyric acid (GABA) and acetylcholine (ACh) are involved in the regulation of endocrine function through the mediation of hypothalamus and pituitary. The hypothalamus is the main endocrine centre in the brain where the hormones of the target glands interact with the neurotransmitters (Beilezikian, 1987; Paulose et al 1984, 1999). Neurotransmitter acts by binding to specific membrane bound receptors and neurotransmitter receptor studies revealed that the functional regulation of various neurotransmitters likes NE, GABA, 5-HT, DA and ACh have important role in cell proliferation and insulin secretion (Fig. 1).

## Neurotransmitters and Insulin Secretion

The pancreatic islets are innervated by parasympathetic, sympathetic and sensory nerves. Several neurotransmitters are stored within the terminals of these nerves. The preganglionic fibres of the parasympathetic system originate from perikarya located in the dorsal motor nucleus of the vagus and possibly also in the nucleus ambigus (Ionescu et al 1983; Burkey & Williams, 1983; Ahren, 1986; Louis-Sylvestre, 1987; Chen et al 1990). The sympathetic innervation of the pancreas originates from preganglionic perikarya located in the thoracic and upper lumbar segments of the spinal cord (Furuzawa et al 1996; Gilon & Henquin, 2001). Stimulation of autonomic nerves and treatment with neurotransmitters affect islet hormone secretion (Ahren, 2000). Glucose is an important regulator of various β-cell processes including insulin biosynthesis and release. Glucose, over short intervals stimulates insulin biosynthesis at the level of translation (Permut et al





1972). Studies of insulin gene expression in primary cultures of rat islets transfect Insulin I gene 5'-flanking sequence suggested that metabolic signal from gluc influx is transmitted through the insulin enhancer (German et al 1990). Gluco induced insulin secretion is modulated by neural, hormonal and paracrine facto (Porte et al 1975). Catecholamines (CA) exert a direct and dual effect on the β-c to induce either inhibition or stimulation of insulin secretion through their interacti with their receptors. The pancreatic islet β-cells have more $\alpha_2$ than $\alpha_1$ adrenerg receptors (Ahren et al 1984, 1986, 2000). Although the effect of the sympathet nervous system (SNS) and of circulating CA on islet physiology has been studied both normal and pathological states, little is known about the possible partic ipati of endogenously-generated CA in the control of islet function. Islet cells have be shown to contain enzymes involved both in the synthesis of CA – tyrosi hydroxylase (TH) and dihydroxyphenylalanine (DOPA) decarboxylase and in the inactivation – monoamine oxidase (Alpert et al 1987; Hanahan, et al 1993; Thibau et al 1993; Gagliardino et al 1997)

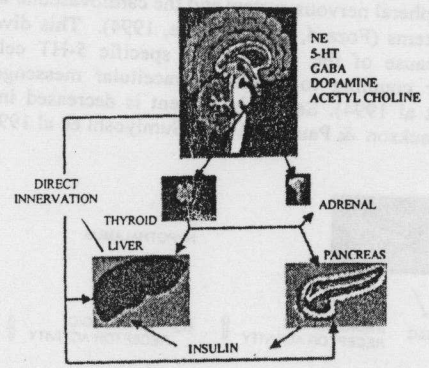


**Fig. 1. Neurotransmitter receptor regulation of cell proliferation and insulin secretion.**

Norepinephrine is the principal neurotransmitter of sympathetic nervous system. inhibits insulin secretion, both *in vivo* and *in vitro* (Renstrom et al 1996; Porte, 196 and exerts opposite effects on peripheral glucose disposal and glucose stir ulate insulin secretion (Avogaro et al 1996). Norepinephrine and epinephrine (EPI), th flight and fright hormones, are released in all stress conditions and are the mai regulators of glucose turnover in strenuous exercise (Simartirkis et al 1990 Neurotransmitter receptor studies (Dakshinamurti, et al 1985, 1988; Viswanathan al 1988, 1990; Paulose & Dakshinamurti 1985) and its regulation of hypothyroidis (Dakshinamurti et al 1986; Tessy et al 1997) leading to sympathetic stimulation an hypertension in pyridoxine deficient rats (Paulose et al 1988 & Dakshinamurti et 1990a, b) which in turn lead to diabetes have been reported. In severe insuli

induced hypoglycaemia, a 15 to 40 fold increase of epinephrine plays a pivotal role in increasing glucose production independently of glucagon (Gauthier et al 1980).

In addition, it has been reported that epinephrine enhances glycolysis through an increased activation of phospho-fructokinase. In humans, EPI stimulates lipolysis, ketogenesis, thermogenesis and glycolysis and raises plasma glucose concentrations by stimulating both glycogenolysis and gluconeogenesis. EPI is, however, known to play a secondary role in the physiology of glucose counter-regulation. Indeed, it has been shown to play a critical role in one pathophysiological state, the altered glucose counter-regulation in patients with established insulin-dependent diabetes mellitus (Cryer, 1993). The inhibitory effect of EPI upon insulin secretion induced by glucose was reported by Coore and Randl (1964), who incubated pancreatic tissue from the rabbit. As judged by Malaisse, et al (1967), the inhibitory effect of EPI on glucose-induced insulin secretion is mediated through the activation of α-adrenoreceptors. Studies reported (Ani, 2000) that central $\alpha_1$ adrenergic receptor gene expression increased and $\alpha_2$ adrenergic receptor gene expression decreased in partially pancreatectomised rats during pancreatic regeneration when the insulin secretion is maximum (Fig. 2).

Serotonin is an endogenous amine involved in diverse biological processes within the central and peripheral nervous system and the cardiovascular and gastrointestinal and respiratory systems (Fozard, 1989; Hindle, 1994). This diversity of actions is made possible because of the existence of specific 5-HT cell surface receptor subtypes and their coupling to distinct intracellular messenger systems or ion channels (Hoyer et al 1994). Serotonin content is decreased in the brain regions during diabetes (Jackson & Paulose, 1999; Sumiyoshi et al 1997; Sandrini et al

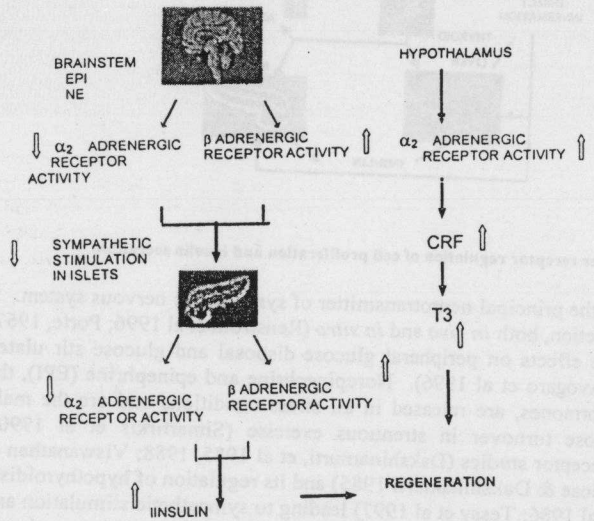


**Fig 2. Adrenergic regulation of pancreatic regeneration and insulin secretion.**



1997) but there are reports suggesting an increase in brain 5-HT content during diabetes (Chen & Yang, 1991; Lackovic et al 1990). Ohtani et al (1997) have reported a significant decrease in extracellular concentrations of NE, 5-HT and their metabolites in the ventro medial hypothalamus (VHM). The ratio of 5-HIAA/5-HT was increased. A similar observation was reported by Ding, et al (1992) with a decrease in 5-HT in cortex (19%) and 5-HT turnover (5-HIAA/5-HT) that increased by 48%. Chu et al (1986) has reported lower 5-HT levels in both hypothalamus and brain stem but not in corpus striatum. Insulin treatment brought about an increase in the cerebral concentration of 5-HIAA and accelerated the cerebral 5-HT turnover (Juszkiewicz, 1985). The 5-HIAA concentration was reported to be approximately twice as high as the controls regardless of duration of treatment. Brain tryptophan, the precursor of 5-HT, was also reduced in brain regions during diabetes (Jamnicky et al 1991).

Insulin treatment was reported to reverse this reduced tryptophan content to normal (Jamnicky, et al 1999). $5\text{-HT}_{2A}$ receptors are upregulated in the brain stem of streptozotocin (STZ) induced diabetic rats. In the cerebral cortex the affinity of these receptors increased (Jackson & Paulose, 1999, 2001). There was a significant increase in 5-HIAA observed at 2-6 hours after insulin administration (Kwok & Juorio, 1987). Affinity of the serotonergic receptors was reported as decreased in the STZ induced diabetic rats (Pius & Paulose, 1999).

Gamma aminobutyric acid is the main inhibitory neurotransmitter in the central nervous system. GABA is reported to be present in the endocrine pancreas at concentrations comparable with those found in central nervous system. The highest concentration of GABA within the pancreatic islet is confined to β-cells (Sorenson, 1991). Glutamate decarboxylase, the primary enzyme that is involved in the synthesis of GABA, has been identified as an early target antigen of the T-lymphocyte mediated destruction of pancreatic β-cells causing insulin-dependent diabetes mellitus (Baekkeskov, 1990). Glutamate dehydrogenase activity is reported to be increased in the brain of diabetic rats (Biju & Paulose, 1998) and the combined administration of pyridoxine and insulin found to be more effective in the control of diabetes (Aswathy, et al 1998). GABA, through its receptors, has been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic α-cells and δ-cells respectively (Gaskins, 1995). It is present in the cytoplasm and in synaptic-like microvesicles (Reetz, 1991) and is co-released with insulin from β-cells in response to glucose. The released GABA inhibits islet α and δ-cell hormonal secretion in a paracrine manner. During diabetes the destruction of β-cells will lead to decrease in GABA release resulting in the enhancement of glucagon secretion from α-cells leading to hyperglycaemia. The brain GABAergic mechanisms also play an important role in glucose homeostasis. Inhibition of central $\text{GABA}_A$ receptors increases plasma glucose concentration (Lang, 1995). Thus, any impairment in the GABAergic mechanism in the central nervous system and/or in the pancreatic islets is important in the pathogenesis of diabetes.

Cholinergic system plays an important role in physiological and behavioural functions. ACh acts by binding to specific membrane receptors and can be divided into muscarinic and nicotinic receptors. Cholinergic stimulation of pancreatic β-cells increases insulin secretion (Kaneto et al 1967). This effect is mediated by muscarinic receptors (Grill & Ostenson, 1983; Henquin & Nenquin, 1988) and is

dependent on extracellular glucose concentration (Henquin et al 1988). Acetylcholine stimulated insulin secretion coupling is mediated by complex mechanisms of signal transduction. It has been proposed that ACh activates phospholipid turnover and thereby increases the intracellular calcium level. Normal β-cells voltage-dependent sodium channels are important for membrane depolarisation. ACh increases sodium influx in to the cells (Henquin et al 1988). ACh hyperpolarises the cell by increasing potassium permeability. Quist (1982) reported that cholinergic agonist, carbachol, causes $Ca^{2+}$-dependent stimulation of phosphate incorporation into phosphatidyl inositol phosphates in the canine heart. Muscarinic $M_1$ and $M_3$ receptors were found to be stimulatory to insulin secretion in rat pancreatic islets *in vitro* (Renuka, 2003).

The muscarinic acetylcholine receptors are widely distributed throughout the body and subserve numerous vital functions in both the brain and autonomic nervous system (Hassal et al 1993). Central muscarinic acetylcholine receptors regulate secretion of insulin from pancretatic islets and maintain normal glucose level. When carbachol, muscarine, bethanechol, methacholine or neostigmine was injected into the third cerebral ventricle, it caused a dose-dependent increase in the hepatic venous plasma glucose concentration. However, in the case of 1, 1-dimethylphenyl-4-piperazinium iodide (DMPP) or nicotine, the level of hepatic venous glucose did not differ from that of the saline-treated control rats. The increase in glucose level caused by neostigmine was dose-dependently suppressed by co-administration of atropine. These facts suggest that cholinergic activation of muscarinic receptors in the central nervous system plays a role in increasing hepatic glucose output. Neostigmine-induced increments in glucose did not occur in adrenalectomized rats. This suggests that the secreted epinephrine acts directly on the liver to increase hepatic glucose output (Iguchi et al 1986). The injection of adrenaline and carbachol into the third cerebral ventricle resulted in a marked hyperglycaemia associated with increased immunoreactive glucagon. Adrenaline-induced hyperglycaemia was not affected by bilateral adrenalectomy, while carbachol-induced hyperglycaemia was completely inhibited by adrenalectomy (Iguchi et al 1985). eostigmine-induced epinephrine and glucagon secretion and increased hepatic glucose output stimulated by direct neural innervation to liver is mediated by central muscarinic receptor in fed rats (Iguchi et al 1990).

The ventro medial hypothalamus, lateral hypothalamus, paraventricular hypothalamus and median site of the lateral-preoptic area were involved in increasing the plasma levels of glucose and epinephrine by cholinergic stimulation (Honmura et al 1992). Studies by Iguchi et al (1992) suggest that the glucoregulatory hippocampal activity evoked by the acetylcholine esterase inhibitor, neostigmine transmitted to peripheral organs *via* the ventromedial hypothalamus. Takahashi et al (1993) reported that neostigmine induced hyperglycaemia affects not only the cholinergic system but also the noradrenergic and dopaminergic systems in the hypothalamus (Takahashi et al 1993). Muscarinic cholinergic system is reported to participate in the $HgCl_2$-induced central hyperglycaemic effect through the function of the adrenal medulla. Norepinephrine and dopamine content were found to be decreased suggesting that their neurons may also be related to hypothalamic glycoregulation (Takahashi et al 1994).

Cholinergic synapses in the ventromedial hypoythalamus participate in a central glucoregulatory system that increases hepatic glucose production mainly through a



stimulation of adrenal medulla epinephrine secretion (Brito et al 1993). Ins
induced hepatic glucose uptake depends on the sensing by muscarinic, intra-he
nerves of a glucose concentration gradient between portal vein and hepatic a
The function of these intrahepatic nerves is impaired in diabetic animals (Stum
al 1998). Insulin partly reversed the changes observed in the STZ-treated
There was a decrease in the muscarinic receptor number and axonal transpo
receptor-bound opiate in STZ induced hyperglycaemia suggesting that imp
axonal transport of receptors partly involved in the neurological disturbance wh
seen in diabetic patients (Laduron & Janssen, 1986). Muscarinic receptor nu
increased in the pancreatic islets of diabetic rats. Cholinergic-induced insulin re
was also higher in STZ induced diabetes than in normal islets (Ostenson &
1987).

Dopamine is a major neurotransmitter in the central nervous system, an
receptors are associated with a number of neuropathological disorders su
Parkinson's disease and Schizophrenia. It also plays a major role in the regulati
appetite and growth hormone. Dopamine and diabetes mellitus are reported to
close link between them. Studies on the effect of dopamine has revealed tha
administration of dopamine $D_1$ and $D_2$ agonists additively inhibits the feeding
and body weight as a result of their combined activation mediated by the acti
hypothalamic neuropeptide (NPY). This reveals the efficiency in which I
agonist combination improves hyperphagia in diabetic animals (Kuo, 2002).
allelic variants of dopamine receptor $D_2$ locus is reported to be associated
weight and height with a linkage disequilibrium with allelic variants of the I
gene that play a major role in the regulation of weight (obesity) and height
serves as a risk factor in late-onset non-insulin-dependent diabetes me
(NIDDM) (Gysin et al 1993).

Diabetes is reported to damage dopaminergic function as a resul
hyperglycemia. Insulin pathways in the brain may play an important ro
regulating dopamine transporter (DAT) activity (France et al 2003). The
regulates extracellular DA levels and during diabetes there is a significant dec
in their number as a result of hypoinsulinemia, which damages the dopamin
activity (Figlewicz et al 1996). It is reported that midbrain dopamine neu
implicated to be critical in the mediation of motivational and reward aspec
stimuli, are affected by alterations in insulin levels. As an approach to evaluat
hypothesis, double-labeling fluorescence immunohistochemistry was use
determine whether the midbrain dopamine neurons express insulin recep
Extensive co-expression of tyrosine hydroxylase (a marker for dopamine neu
with insulin receptor was observed in the ventral tegmentum and substantia
These findings suggest that midbrain dopamine neurons are direct targets of in
and they participate in mediating the effects of these hormones on reward-se
behaviour (Figlewicz et al 2003).

Experiments carried out in the striata of diabetic rats on the behavioural resp
dopamine metabolism and characteristics of dopamine subtypes revealed
diabetes caused an increase in the on-set and duration of cataleptic beha
Dopamine metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic
(HVA), were significantly reduced in the striata of hyperglycaemic rats while
of DA was significantly increased. The ratio of DOPAC and HVA to DA

decreased, suggesting decreased turnover of DA. The affinity of striatal $D_1$ receptors was significantly increased without changes in the number of binding sites, while the maximum binding number of $D_2$ receptors was significantly increased without affecting its affinity in the diabetic rats (Ho et al 1995). Diabetes causes a significant increase in locomotary activities as a result of decreased dopamine $D_1$ receptors. This is believed to be the major cause of hyporesponsiveness leading to dopamine-related locomotor impairment (Kamei et al 1998). Dopamine receptor changes have been established in various neuroleptic disorders (Chiu et al 1981 a, b).

The CNS monoamine cell groups that project to the pancreatic parasympathetic preganglionic neurons have been identified with the use of that originate exclusively from the dorsal vagal motor nucleus and some of these are found to be dopamine neurons because as they were tyrosine hydroxylase immunopositive. Transneuronally labelled aminergic neurons are also found throughout the medulla oblongata (Loewy et al 1994). Electron microscopic autoradiography studies using tritiated compounds have revealed that [$^3$H] DA formed from administered [$^3$H] DOPA are present in the beta cells of the islet. In the β-cells the [$^3$H] DA-grains were observed to be associated with the secretory granules.

The pancreatic β-cells have ability to store substantial amounts of calcium dopamine and serotonin apart from epinephrine and norepinephrine (Ahren et al 1985, 1989). Dopamine accumulation is also observed to inhibit the insulin secretion in the pancreatic islets, which involves interference with a calcium translocation. This is a consequence of a complex interaction between the accumulated dopamine and a pool of $Ca^{2+}$ mainly confined to the secretory granules. Dopamine accumulation initially causes a transient increase in cytosolic $Ca^{2+}$ accompanied by insulin release. The increasing cytosolic $Ca^{2+}$ as a result of dopamine accumulation makes the cell more sensitive to a concomitant stimulation with glucose and the release of insulin is triggered. A long-term dopamine accumulation on the other hand decreases the granular $Ca^{2+}$ pool inhibiting the insulin release. Thus, studies also suggest an extra-neuronal source of dopamine in addition to its occurrence in adrenergic nerves, which effects insulin secretion.

Dysfunction of pancreatic islets plays a crucial role in the etiology of diabetes. Chronic hyperglycaemia or hyperlipidaemia impairs islet function. Studies have demonstrated that dopaminergic agonists ameliorated hyperglycaemia and hyperlipidaemia in obese and diabetic rodents. The effect of dopamine $D_2/D_1$ receptor agonists (bromocriptine/SKF38393, BC/SKF) on islet dysfunction in diabetic mice markedly reduced hyperglycaemia and hyperlipidaemia, and significantly improved isle dysfunction demonstrated by an increase of secretagogue-stimulated insulin release from islets of diabetic mice (Cincotta et al 1998, 2001; Boiadzhieva. 1990). These actions are mediated via alterations in the hypothalamic-neuroendocrine axis, which drives metabolic changes in peripheral tissues leading to a marked reduction in hyperglycemia and hyperlipidemia and corrects autonomic control of islet function. Thus the systemic sympatholytic dopaminergic therapy that attenuates hyperglycemia and hyperlipidemia improves islet function in ob/ob mice by improving aberrations in the α-cell glucose-sensing apparatus, enhancing insulin storage and/or retention, and stabilizing hyperplasia, thus reducing basal insulin levels reducing the β-cell hyperplasia. These studies underline the importance of dopamine as a neuromediator in the regulation of pancreatic insulin secretion.



In the exocrine pancreas studies have sited dopamine receptors involved in the stimulation of cellular cyclic AMP. Dopamine is reported to elicit concentration-dependent stimulation of cellular cyclic AMP with a maximal increase occurring at a concentration of 0.1 mM ($EC_{50}$=1μM). Studies with various agents of dopamine depended on their affinities to stimulate cellular cyclic AMP formation or to inhibit dopamine-stimulated cellular cyclic AMP formation by inhibiting the binding of [$^3$H] dopamine to pancreatic acini, providing evidence that dopamine binding sites are receptors that mediate the action of dopamine on cAMP accumulation (Ribet et al 1982, 1986). Thus, he central dopamine inhibits pancreatic exocrine secretion via D1-like receptors and that the inhibitory effect is mediated via sympathetic nerves, especially α-adrenoceptors. The dopamine $D_2$ receptors increased in the corpus striatum and cerebral cortex but decreased in the hypothalamus and brain stem indicating their involvement in regulating insulin secretion (Eswar, 2003).

The dopamine $D_2$ receptors increased in the corpus striatum and cerebral cortex but decreased in the hypothalamus and brain stem indicating their involvement in regulating insulin secretion. The mechanism of stimulation of insulin at low concentrations and the inhibition has not been well established. Further studies in this aspect with a focus on the second messenger system involved would help in the elucidating the role of dopamine and its various agonists on insulin secretion. Role of dopamine through it $D_2$ receptors is reported to mediate mitogenesis. Dopamine $D_2$ receptors are members of the G protein-coupled receptor superfamily and are expressed on both neurons and astrocytes. It has been observed that in rat C6 glioma cells stably expressing the rat D2L receptor, dopamine (DA) can activate both the extracellular signal-regulated kinase (ERK) and c-Jun NH2-terminal kinase (JNK) pathways through a mechanism involving $D_2$ receptor-G protein complexes and the Ras GTP-binding protein. Agonist binding to $D_2$ receptors rapidly activated both kinases within a short time demonstrating the role of $D_2$ receptor-stimulated MAPK pathways (Roth et al 1998). Similarly a thorough study and research in the pancreatic islets on the involvement of dopamine and its receptors in cell proliferation will be of immense clinical significance in the treatment of diabetes.

**Neurotransmitters and Cell Proliferation**

Neurotransmitters stimulate or inhibit cell proliferation in non neuronal cells by activating receptors coupled to various second messenger pathways (Kluess et al 1991). Norepinephrine is reported to amplify the mitogenic signals of both EGF and hepatocyte growth factor (HGF) by acting through the $\alpha_1$ adrenergic receptor. It induces the production of EGF and HGF at distal sites and also enhances the response to HGF at target tissues (Broten et al 1999). Norepinephrine rises rapidly in the plasma within one hour after partial hepatectomy (Knopp et al 1999). It also suppresses the mito-inhibitory effects of transforming growth factor $\beta_1$ (TGF-β1) on cultured hepatocytes isolated from the early stages of regeneration (Michalopoulose & DeFrancis, 1997). Prazosin, a specific antagonist of $\alpha_1$-adrenergic receptor, as well as sympathetic denervation greatly decrease DNA synthesis at 24 hours after partial hepatectomy (Cruise et al 1989). Addition of NE to hepatocytes stimulates $Ca^{2+}$ mobilisation or phosphoinositol turnover and either or both of these processes was proposed to be involved in the mitogenicity of NE (Exton, et al 1981 1988; Nagano et al 1999). Rat hepatomas lacked the $\alpha_{1A}$ and $\alpha_{1B}$ mRNA and receptor

binding, while in the human hepato-cellular carcinoma cell line, HepG2, their expression is high but they lack receptor binding (Kost et al 1992). Hepatic neoplasm are characterised by an increase in $\alpha_2$ and $\beta$ adrenergic receptors and a concomitant decline in $\alpha_1$ receptors (Sanae et al 1989). Studies have shown that proliferation and insulin secretion of foetal rat $\beta$-cells could be significantly suppressed by $\alpha$-adrenergic stimulation. When $\alpha$-adrenergic agonists were given together with Sp-cAMP[S] or to pertussis toxin-pretreated islets, the suppressed $\beta$-cell proliferation and insulin secretion were partially prevented, suggesting that $\alpha$ adrenergic stimulation represses $\beta$-cell growth and hormone release in part by interfering with GTP binding proteins that connect cell surface receptors to adenylate cyclase (Sjoholm, 1991).

Serotonin has been implicated as a potential mitogen (Seuwen & Pouyssegur, 1990) and was shown to have effects on morphogenesis and neuronal development (Lauder, 1990). 5-Hydroxytryptamine has been recognised to cause proliferation of a variety of cells in culture including vascular smooth muscle cells and hepatocytes (Fanburg & Lee, 1997).

5-Hydroxytryptamine mediates mitogenic effects in many cell types (Garnovskaya, et al 1996 & Cowen et al 1996). The mitogenic action of 5-HT, first identified in bovine aortic smooth muscle cells by Nemeek et al (1986), may bear a relationship to the stimulatory effect of 5-HT on neuroembryogenesis. In cultured rat pulmonary artery smooth muscle cells (SMC), 5-HT induces DNA synthesis and potentiates the mitogenic effect of platelet-derived growth factor (Eddahibi, et al 1999). 5-Hydroxytryptamine's effects on cell proliferation may involve the phosphorylation of GTPase-activating protein (GAP), an intermediate signal in 5-HT-induced mitogenesis of SMC (Lee et al 1997).

In pancreatic cell line, activation of pertussis toxin insensitive $5\text{-HT}_{1A/1B}$ receptors stimulate proliferation through the activation of PLC and PKC that resulted in the down regulation of cAMP (Ishizuka et al 1992). $5\text{-HT}_{1A}$ receptor agonist 8-OHDPAT inhibited the DNA synthesis in rat hepatocytes *in vitro*. Studies using mesulergine, $5\text{-HT}_{2C}$ antagonist revealed that $5\text{-HT}_{2C}$ receptors are stimulatory to hepatocyte cell division. RT-PCR studies revealed that $5\text{-HT}_{1A}$ receptor mRNA decreased in the brain regions and liver during liver regeneration (Pyroja, 2002).

There is a synergistic effect of 5-HT with more traditional protein growth factors, such as platelet derived growth factor, fibroblast growth factor, and insulin like growth factor and with ADP, ATP, thromboxaneA2 (Crowley et al 1994, Stroebel & Groppelt-Struebe, 1994). 5-HT in concentrations as low as 0.1–1 mM stimulates both proliferation and hypertrophy of SMC in culture. Furthermore, the mitogenic action of 5-HT is synergistic with that of conventional peptide growth factors. All agents that block transport of 5-HT block the proliferative response (Lee et al 1991, 1994).

The biological mechanism used by 5-HT to change cell morphology and induce proliferation may directly target the cytoskeleton. The main component of the cytoskeleton is microtubules which gives cells their shape. These microtubules consist of long polymers of tubulin, which spontaneously depolymerise if they are not actively polymerising (Mitchison & Kirschner, 1984). Tan and Lagnado (1975) found effects of 5-HT and related indole alkaloids on brain microtubular proteins. Several years later, it was found that 5-HT is taken up by endothelial cells and binds



to stress fibers (Alexander, et al 1987). Here 5-HT induces actin polymerisation a affects changes in the cytoskeleton. Thus, there is evidence that 5-HT ha: a dir role in regulating and maintaining microtubules and microfilaments. The chan reported in 5-HT induced cytoskeletal stability may be partially mediated microtubule-associated proteins (MAPs). MAPs serve to stabilize the cytoskele by binding to tubulin polymers and inhibiting their depolymerisation. undifferentiated human neuroblastoma cells (LAN-5), high levels of 5-HT (50 µ induce a decrease while low levels of 5-HT (50 nM) induce an increase in cytoplasmic tau protein, a MAP found in high concentrations in the axon.

The effects of 5-HT as a mitogen and/or growth factor have been documented a part of neuromodulator substances acting via G protein coupled receptor signali pathway (Seuwen et al 1988, 1990; Julius, 1991). Specifically, 5-HT has been sho to increase DNA synthesis in rat pulmonary vascular smooth-muscle cells in cult (Pitt, et al 1994). Introduction of functional $5\text{-HT}_{2A}$ receptor and $5\text{-HT}_{2C}$ recep into NIH 3T3 cells results in generation of transformed foci at high frequency (Jul et al 1989). The long-term maintenance of the transformed state requires continu activation of these 5-HT receptors, indicating that they may represent conditio proto-oncogenes (Julius, 1991). 5-HT is mitogenic for bovine pulmonary arte SMC producing both hyperplasia and hypertrophy through its action on a 5-H membrane transporter, with a rapid elevation in tyrosine phosphorylation (Tyr-P) GTPase-activating protein (GAP) (Lee et al 1997) and early inductions of c-m (Lee et al 1994). 5-HT, acting via the $5\text{-HT}_{2A}$ receptor, is a known activator of t ERK pathway in vascular smooth muscle cells (Watts, 1996; Banes et al 1999).

Mobilisation of 5-HT in intestine and its accumulation in liver and spleen tissu were observed at the initial periods after partial hepatectomy (Kulinskii, et al 198 5-HT caused a dose-dependent increase in DNA synthesis in primary cultures of r hepatocytes in the EGF and insulin, as measured by [$^3$H]thymidine incorporati (Sudha & Paulose, 1998). 5-HT and monoamine oxidase inhibitor o-chlorpargyli injected alone or combined increase the endogenous 5-HT level in the regenerati liver and stimulates mitotic activity. The tryptophan hydroxylase inhibitor chlorophenylalanine and reserpine decrease both the endogenous 5-HT level and t mitotic index. There is a close correlation between the endogenous 5-HT level a the mitotic index (Kulinskii et al 1983).

Gamma aminobutyric acid is the principal inhibitory neurotransmitter of t mammalian brain. GABA inhibits the growth of murine squamous cell carcinon and HeLa cell lines (Boggust & Al-Nakib, 1986). Gliomas with high proliferati rate lack the expression of functional GABA binding sites (Labrakakis et al 1998 GABA also plays an important role in terminating the growth of rapidly developi tissues *in utero* (Gilon et al 1987). Studies from lab have shown that hypothalam GABergic system plays an important role in the neoplastic transformation of r liver. $GABA_A$ receptor agonist muscimol, dose dependently inhibited EGF induc DNA synthesis and enhanced the TGF $\beta_1$ mediated suppressed DNA synthesis in r primary hepatocyte culture (Biju et al 2001, 2002). Increased $GABA_A$ recept activity inhibits proliferation of HepG2, human hepatocyte carcinoma cell line. Th inhibition is prolonged in the cell line co-transfected with $GABA_A$ receptor $\beta_2$ and subunit genes (Zhang et al 2000). $GABA_B$ receptors were increased in neoplastic r liver (Biju et al 2002). $GABA_A$ receptor agonist, muscimol, dose dependent

inhibited EGF induced DNA synthesis and enhanced the TGF$\beta$1 mediated DNA synthesis suppression in primary hepatocyte cultures. $GABA_A$ receptor acts as an inhibitory signal for hepatic cell proliferation (Biju et al 2001). Serum GABA levels were increased in partial hepatectomy (PH), lead nitrate (LN) treated and N-nitrosodiethylamine (NDEA) treated rats. GABA content decreased in the hypothalamus of PH and NDEA treated rats, while it increased in LN treated rats. $GABA_A$ receptor number and affinity in hypothalamic membrane preparations of rats showed a significant decrease in PH and NDEA treated rats, while in LN treated rats the affinity increased without any change in the receptor number. The $GABA_B$ receptor number increased in PH and NDEA treated rats, while it decreased in LN treated rats. The affinity of the receptor also increased in NDEA treated rats. Liver cell proliferation is influencing the hypothalamic GABAergic neurotransmission (Biju et al 2001) and these changes in turn regulate the hepatic proliferation (Figure 3). The mitogenic effect of acetylcholine has been studied in different cell types. Acetylcholine analogue carbachol stimulated DNA synthesis in primary astrocytes derived from perinatal rat brain (Ashkenazi, 1989). Acetylcholine is reported to induce proliferation of rat astrocytes and human astrocytoma cells (Guzzetti et al 1996).

Muscarinic $M_1$ and $M_3$ receptors were differentially expressed in the brain regions during pancreatic regeneration in partially pancreatectomised young rats. RT-PCR analysis revealed that central muscarinic $M_1$ receptor mRNA was decreased at the time of regeneration while muscarinic $M_3$ receptor mRNA was increased. It is also

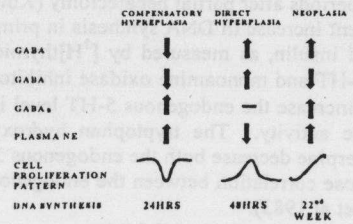


**Fig 3. Liver cell proliferation GABAergic regulation in brain during hepatic proliferation**

found that in the pancreatic islets, both $M_1$ and $M_3$ receptors were increased at the time of regeneration. Muscarinic $M_1$ receptor antagonist pirenzepine and $M_3$ receptor antagonist 4-DAMP mustard inhibited EGF induced DNA synthesis in primary culture of rat pancreatic islets (Renuka, 2003). Muscarinic acetylcholine receptors activate many downstream signalling pathways, some of which can lead to mitogen activated protein kinase (MAPK) phosphorylation and activation. MAPKs play a major role in regulating cell growth, differentiation and synaptic plasticity. Both Gi and Gq coupled muscarinic receptors have been shown to activate MAPK in various systems. Muscarinic $M_3$ receptors activate MAPK in the



oligodendrocyteprogenitors (Ragheb et al 2001). Berkeley et al (2000) reported the involvement of $M_1$ receptors in activation of MAPK in PC12 cells. Acetylcholine analogue carbachol stimulated DNA synthesis *via* muscarinic receptors in primary astrocytes derived from perinatal rat brain. Carbachol is also mitogenic in certain brain derived astrocytoma and neuroblastoma, as well as in Chinese hamster ovary (CHO) cells expressing recombinant muscarinic receptors (Ashkenazi 1989). Proliferation experiments with subtype specific antagonists in astrocytes suggest that cell proliferation is induced by the activation of $M_3$ receptors (Guizette, 1996). Proliferative signalling has been generally associated with polypeptide growth factor receptors which possess an intrinsic protein tyrosine kinase activity (Yarden et al 1986). In NIH 3T3 cells transfected with human muscarinic m1 receptor gene carbachol stimulate DNA synthesis. The effect of carbachol was blocked by atropine further demonstrating the role of muscarinic receptors. The MAPK activity by muscarinic receptors is dependent on PKC and EGF receptor mediated signalling pathways. PKC inhibitors, or down regulation of PKC by long term exposure to phorbol esters, completely inhibited MAPK activation in response to carbachol in SH-SY5Y and SK-N-BE2(C) human neuroblastoma cells which express endogenous $M_3$ receptors (Offermanns et al 1993, Kim, et al 1999). The MAPK activation by $M_3$ receptor stimulation is inhibited by two pathways: one dependent on PKC and the other mediated *via* the EGF receptor and Src (Slack, 2000).

## Conclusion

Neurotransmitters - norepinephrine, serotonin, dopamine, gamma aminobutyric acid and acetylcholine are involved in the regulation of endocrine function through the mediation of hypothalamus and pituitary. Neurotransmitter acts by binding to specific membrane bound receptors. Neurotransmitter receptor studies revealed that the functional balance of various neurotransmitters likes NE, GABA, 5-HT, DA and ACh through their receptor sub types have important role in cell proliferation and insulin secretion. These results have immense clinical significance in the diagnosis and management of diseases like hypothyroidism, hypertension, diabetes and cancer.

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