DISCUSSION

The mechanisms regulating islet growths under different situations have been studied extensively (Corbett *et al.*, 1997). Most studies have been concerned with intraislet factors that are expressed during islet regeneration or triggering factors thought to be released by the neighboring parenchyma. A large number of growth factors and growth-stimulating peptides are expressed in or have stimulatory effects in the growing islets (Corbett *et al.*, 1997). Biogenic monoamines such as 5-HT and dopamine are present in higher amounts in the islets of young animals (Cegrell, 1968) that also show a larger proliferative capacity. 5-HT has short-term effects on ion channels and other effectors such as adenylyl cyclase but also have growth factor-like effects in developing brain (Lauder, 1993) and mitogenic effects on fibroblasts (Gerhardt & Van Heerikhuizen, 1997). Islet cell neogenesis can be reactivated by applying external stimuli such as partial pancreatectomy (90%) (Bonner-Weir, 1993). Partial pancreatectomy is an established model to study pancreatic regeneration (Pearson *et al.*, 1977).

DNA synthesis in pancreas after partial pancreatectomy

In this study we examined the regeneration of β-cells of the islets of Langerhans of the pancreas in weanling rats using partial pancreatectomy as the stimulus to regenerate. [^{3}H]thymidine incorporation into replicating DNA was used as a biochemical index for quantifying DNA synthesis during pancreatic regeneration after partial pancreatectomy. DNA synthesis was found to be increased 12 hrs after partial pancreatectomy. The maximal rate of [^{3}H]thymidine incorporation was observed at 72 hrs and declined at 7 days after partial pancreatectomy. The peak in the DNA synthesis is concordant with the previous reports (Pearson *et al.*, 1977;

Brockenbrough *et al.*, 1988). Enhanced β-cell function and proliferation maintains the normoglycemic level in rats during pancreatic regeneration (Leahy *et al.*, 1988).

Circulating insulin levels during pancreatic regeneration

Circulating insulin level was found to be increased during pancreatic regeneration. Insulin was reported to increase the cell proliferation of β-cells *in vitro* (Rabinovitch *et al.*, 1982). Previous studies suggest that the increase in the β-cell proliferation is related to the degree to which insulin biosynthesis and/or release is increased (Chick *et al.*, 1975; King & Chick, 1976). Insulin can stimulate β-cell replication directly possibly through a receptor for multiplication stimulating activity or another insulin like growth factor (Rabinovitch *et al.*, 1982). There are also reports about the increase in the insulin secretion after the partial pancreatectomy, besides maintaining the normoglycaemic level, it also helps the remaining β-cell mass to regain its original mass and volume by inducing cell division. The signal for islet cell proliferation is related to a long standing demand for increased insulin secretion (Dubuc, 1976). After major pancreatectomy in dogs, insulin treatment enhances the proliferation of the remnant pancreas and maintains endogenous insulin secretion for a long period, prolonging survival and promoting pancreatic regeneration (Ohashi, 1993).

5-HT content in the brain regions during pancreatic regeneration

Serotonin is involved in regulating cellular functions of central and peripheral nervous systems, endocrine and exocrine organs, as well as vascular and hematopoietic systems (Wilkinson & Dourish, 1991). An increase in 5-HT content was observed during active pancreatic proliferation in brain stem, cerebral cortex and hypothalamus. The increased contents in the cerebral cortex and hypothalamus were reversed to basal level when pancreatic DNA synthesis declined to control level. The

relationship between enhanced monoamine content in the ventromedial hypothalamus (VMH), a characteristic of hyperinsulinemic and insulin-resistant animals and islet dysfunction is already reported (Liang & Cincotta, 1999). The increase in brain 5-HT content may be due to increase in tryptophan uptake through the BBB with other neutral amino acids. Increased insulin in the plasma during pancreatic regeneration tends to release the tryptophan bound to albumin and hence increasing the concentration of free tryptophan in plasma (Trulson & Mackenzie, 1978). Central serotonergic neurons participate in the regulation of sympathetic nerve discharge. Brain serotonergic changes are reported to regulate autonomic nerve function in rats (Kuhn *et al.*, 1980). Thus our result suggests a close relationship between 5-HT level in brain regions and pancreatic islet cell growth. The increased 5-HT content in the brain regions during pancreatic regeneration may inhibit sympathetic nervous system and thus increases insulin secretion from pancreatic β-cells.

5-HT content in the pancreas and plasma during pancreatic regeneration

Pancreas is a rich source of 5-HT (Bird *et al.*, 1980). The 5-HT content in the pancreas decreased at the time of peak DNA synthesis. Low concentration of 5-HT in the pancreatic islets can stimulate insulin synthesis within the pancreatic islets. 5-HT is taken up into the insulin granules and secretes 5-HT/insulin in a pulsatile fashion on stimulation of pancreatic islet β-cells under physiologic conditions (Zhou & Misler, 1996). 5-HT induced a dose dependent hpoglycaemia and an increase in serum insulin levels (Sugimoto *et al*, 1990) due to a decreased uptake of 5-HT at high concentration. This shows that low concentration of 5-HT within the pancreatic islets has a role in the regulation of insulin secretion and islet cell proliferation during pancreatic regeneration

5-HT level in the plasma increased during pancreatic regeneration and it reversed to normal level by 7 days after pancreatectomy. Decreased 5-HT content in the islets may be responsible for the increased serum insulin levels. The increase in serum 5-HT levels during pancreatic regeneration suggests a decreased uptake of 5-HT into pancreas. Higher amounts of 5-HT promote proliferative capacity of the islets of young animals (Cegrell, 1968).

EPI and NE content decreased in plasma and adrenals during pancreatic regeneration

Norepinephrine and epinephrine concentrations decreased in the plasma and adrenals during regeneration of the pancreas. Sympathetic system is inhibitory to insulin secretion. Epinephrine when used in high doses *in vivo* or *in vitro*, reduces the insulin response to stimulators (Malaisse, 1972). EPI and NE have an antagonistic effect on insulin secretion and glucose uptake (Porte & Williams, 1966). Studies from our lab reported a decrease in the adrenergic activity during pancreatic regeneration. The decrease in the NE and EPI stimulate the β-adrenergic receptors which are stimulatory to insulin secretion (Ani, 2000). Activation of the splanchnic nerves innervating the adrenals results in the catecholamine release from chromaffin cells into the circulation.

Brain 5-HT_{1A} receptor alterations

Serotonin containing neurons are concentrated in the raphé nuclei of the brainstem and connect to the cerebral cortex, hypothalamus, and major autonomic nuclei, where they appear to exert broad regulatory control. Central serotonergic activity influences autonomic functions like thermogenesis, cardiovascular control, circadian rhythms and pancreatic function (McCall & Clement, 1990; Ramage, 2000; Liang & Cincotta, 1999). Serotonergic neurons act through the autonomic nervous

system or hypothalamic-pituitary axis to affect BP and key metabolic processes (Fuller, 1990).

Several studies have described the role of 5-HT_{1A} receptors in the neuroendocrine regulation (Fuller, 1990; Van de Kar, 1991). The relationship between the serotonergic and the sympathoadrenal system lead in turn to a control of both plasma glucose levels and insulin release (Chaouloff, 1990a). 5-HT_{1A} receptor agonist, 8-OH DPAT has been shown also to act on the endocrine system. 8-OH DPAT through activation of 5-HT_{1A} receptors decreases plasma insulin and increases basal plasma glucose levels in several rat strains, via a central mechanism of action (Chauloff & Jeanrenaud, 1987).

This effect of insulin release is attributed to regulation of catecholamine-epinephrine and norepinephrine, plasma levels because of the stimulation or inhibition of the adrenal medulla. Pre-ganglionic sympathetic nerve fibres pass from the intermediolateral horn cells of the spinal chord through the sympathetic chains, through the splanchnic nerves and synapse in the adrenal medullae. They synapse on cells that are derived from nervous tissue that secrete nor-adrenaline and adrenaline directly into the circulation. Wallin *et al* (1981) have demonstrated that measurements of peripheral sympathetic nerve activity correlate well with measurements of plasma nor-adrenaline. Epinephrine and norepinephrine have long been known to inhibit insulin secretion *in vivo* (Porte Jr D, 1967b); Brunicardi, 1995) and *in vitro* (Brunicardi, 1995; Sorenson *et al.*, 1979).

In our present study we analysed the receptor binding parameters and expression of the 5-HT receptors in sham operated and pancreatectomised rats. Scatchard analysis was performed for determining the B_{max} and K_d of these receptors (Scatchard, 1949). Receptor mRNA status was analysed by RT-PCR technique using specific primers. 5-HT_{1A} receptor binding parameters were analysed using the receptor specific agonist [^{3}H]8-OH DPAT (Nenonene *et al.*, 1994). 5-HT_{1A}

receptor has two affinity sites and the double affinity status of the receptor was confirmed by displacement analysis using 8-OH DPAT.

Cerebral cortex receives an extensive 5-HT input originating from midbrain raphe 5-HT neurons (Tork, 1990). Scatchard analysis of the cerebral cortex showed that the affinity of the high affinity receptor binding decreased in 72 hrs pancreatectomised rats. The low affinity receptor number and affinity decreased during active pancreatic proliferation. $Ki_{(H)}$ increased in 72 hrs pancreatectomised rats along with an increase in the log (EC_{50})-1 indicating a shift in high affinity towards low affinity. $Ki_{(L)}$ and log (EC_{50})-2 showed an increase in 72 hrs pancreatectomised rats indicating a shift in affinity of the low affinity site towards much lower affinity. We observed an increased 5-HT content in the cerebral cortex during pancreatic regeneration. These results indicate that the increased 5-HT is able to down regulate the $5\text{-}HT_{1A}$ receptors in the cerebral cortex. Adrenocortical secretion in response to intraperitoneal and intracerebroventricular administration of the $5\text{-}HT_{1A}$ receptor agonist 8-OH DPAT involves a sympathomedullary activation (Saphier & Welch, 1994). Since our results indicate a decreased $5\text{-}HT_{1A}$ receptor binding, this reduces the sympathetic stimulation and increases insulin secretion from pancreatic islets mediated by a decreased norepinephrine release from adrenal glands. Our RT-PCR studies were also concordant with the receptor studies. Decreased expression of $5\text{-}HT_{1A}$ mRNA is reported in long-term adrenalectomised rats in the dentate gyrus (Liao *et al.*, 1993). The transduction action of the $5\text{-}HT_{1A}$ receptor is usually associated with a decrease in adenylyl cyclase activity. In cultures of hippocampal neurons, $5\text{-}HT_{1A}$ agonists block the forskolin-induced formation of p-CREB, an important transcription factor increased by cAMP (Nishi & Azmitia, 1999). Receptors that result in alterations in the cAMP or Ca^{2+} pathways would be expected to result in altered CREB phosphorylation and altered transcriptional

activity (Hyman & Nestler, 1993). Regulation of these transcription factors through 5-HT_{1A} receptors regulates gene expression in the brain.

The midbrain raphe nuclei of brain stem are the source of wide spread serotonergic innervation throughout the brain (Azmitia & Gannon, 1986). In the case of brain stem, Scatchard analysis revealed an increased K_d of the high affinity 5-HT_{1A} receptor indicating a decreased affinity of the receptor in 72 hrs pancreatectomised rats. The low affinity receptor number decreased in 72 hrs pancreatectomised rats without any change in the affinity. The decrease in receptor number during pancreatic proliferation may be due to increased 5-HT brain levels in the brain stem of these rats, since the 5-HT_{1A} receptor expression is sensitive to autoinhibition (Nishi & Azmitia, 1999). In 72 hrs pancreatectomised rats $Ki_{(H)}$ and log (EC_{50})-1 increased indicating a shift in high affinity towards lower affinity side. Our RT-PCR studies also revealed that during pancreatic cell proliferation 5-HT_{1A} receptors are getting down regulated in the brain stem.

An increased sympathetic activity due to the activation of 5-HT_{1A} receptors will induce increased EPI output from the adrenal medulla that will inhibit insulin secretion (Bauhelal & Mir, 1990, 1993). This was proved by injecting the specific 5-HT_{1A} receptor agonist 8-OH DPAT to normal rats. The rats showed a very rapid increase in blood glucose level that reached its peak within 30 min. A similar observation was reported from a number of laboratories (Laude *et al.*, 1990; Bauhelal *et al.*, 1990).

The results of the present study indicate that pancreatectomy trigger a regulatory effect on the 5-HT_{1A} receptor system in the brain stem and cerebral cortex. 5-HT_{1A} receptors were down regulated both in the cerebral cortex and brain stem. An increase in local release of 5-HT may be responsible for the decrease in [^{3}H]8-OH DPAT receptor binding 72 hrs after pancreatectomy. The decreased 5-HT_{1A} receptor binding reduces the sympathetic stimulation and epinephrine release

from adrenal glands, thereby increasing insulin secretion from pancreatic islets. The adrenaline releasing effect of 8-OH DPAT antagonised by the mixed 5-HT_{1A}-β adrenoreceptor antagonist pindolol has been already reported (Chauloff *et al.*, 1990 b). Opposite effects occur with the use of 5-HT depending on whether or not cellular cAMP is elevated and this in turn, depends on the activity of cellular phosphodiesterase (Fanburg & Lee, 1997). Thus, the 5-HT_{1A} receptor down regulation in the brain stem and cerebral cortex during pancreatic regeneration increases insulin secretion from pancreatic β-cells which is mediated through epinehrine release from adrenal medulla.

The hypothalamus plays a central role in the integration of neurohormonal function (Oommura & Yoshimatsu, 1984). 5-HT is known to influence a number of hypothalamic associated functions such as sleep, thermoregulation and neuroendocrine function. 5-HT exerts a modulatory effect on the hypothalamic pituitary-adrenal (HPA) system (Fuller & Snoddy, 1990). When we studied the 5-HT_{1A} receptor status in the hypothalamus, the affinity of the high affinity receptor decreased in 72 hrs pancreatectomised rats as evidenced by the K_d of the receptor. The density and affinity of the low affinity 5-HT_{1A} receptor decreased during active regeneration, i.e., in 72 hrs after pancreatectomy. Both affinity sites shifted toward their corresponding lower affinity sites as indicated by the increase in the $Ki_{(H)}$, $Ki_{(L)}$, log (EC_{50})-1 and log (EC_{50})-2 in pancreatectomised rats. RT-PCR analysis also revealed a down regulation of this receptor during active islet cell proliferation.

Serotonergic neurons innervate hypothalamic neurons that regulate the secretion of several hormones. 5-HT containing neurons in the midbrain directly innervate corticotropin-releasing hormone (CRH)-containing cells located in paraventricular nucleus of the hypothalamus (Hanley & Van de Kar, 2003). Direct synaptic connections between serotonergic nerve terminals and CRH neurons in the hypothalamic PVN have been demonstrated at the electron microscopic level

(Liposits *et al.*, 1987). The CRH neurons in turn stimulate the secretion of ACTH from the anterior lobe of the pituitary gland, which in turn stimulates corticosterone secretion from the adrenal cortex. Other evidence also points to direct serotonergic innervation of oxytocin-containing neurons in the hypothalamic PVN (Saphier, 1991). Additionally, several lesion studies have provided evidence that the serotonergic stimulation of the secretion of ACTH, corticosterone, prolactin, oxytocin and renin is mediated by neurons in the hypothalamic PVN (Bagdy & Makara, 1994).

5-HT_{1A} subtype is involved in the neural regulation of hypothalamo-pituitary-adrenocortical (HPA) secretion (Saphier & Zhang, 1993). Stimulation of 5-HT_{1A} receptors activates the HPA axis (Gilbert *et al.*, 1988). Activation of 5-HT_{1A} receptors has been shown to induce corticotropin (ACTH) and corticosteroid release in rodents (Gilbert *et al.*, 1988), an effect antagonised by pindolol, a stereoselective 5-$HT_{1A/1B}$ blocker (Lesch, *et al*, 1990). 5-HT_{1A} receptor agonists elevate plasma corticosterone and EPI concentrations. 5-HT and 8-OH DPAT stimulate the release of corticotropin-releasing factor (CRF) (Jones *et al.*, 1976). Our data suggest that the HPA axis can be inhibited by the down regulation of 5-HT_{1A} receptors. In addition to its well known ACTH releasing activity, CRF is also known to stimulate directly sympathetic tone to the adrenal medulla via a central site of action (Fisher, 1989), manifested by increase in efferent adrenal nerve activity, plasma adrenaline concentration and an increase in BP. (Brown & Fisher, 1983; Brown *et al.*, 1985). Since 5-HT_{1A} receptors inhibit HPA axis its down-regulation leads to a decreased CRF release and sympathetic activity which is stimulatory to insulin secretion. 5-HT_{1A} receptor antagonist pindolol blocked elevation of the plasma ACTH concentration induced by 5-HT_{1A} receptor subtype mediated release of CRH from the paraventricular nuclei of the hypothalamus in rats (Pan & Gilbert, 1992). 5-HT_{1A} receptors are linked through G proteins to second messenger enzymes, each receptor can stimulate the release of multiple molecules of oxytocin and CRH. CRH also is

linked *via* G proteins to effector enzymes. Hence, activation of each CRH receptor on corticotrophs in the pituitary will lead to the release of multiple molecules of ACTH, which can further stimulate the release of even more molecules of corticosterone (Charmers & Watson, 1991)

It is well established that the autonomic fibres supplying the pancreas travel via the vagus and splanchnic nerves (Helman *et al.*, 1982). These nerves are clearly related to the ventral hypothalamus. The ventro-medial hypothalamic nuclei is considered as the sympathetic centre and the stimulation of this area decreases insulin secretion (Helman. *et al.*, 1982). Studies of *in vivo* pancreatic nerve activity after VMH lesions show increased parasympathetic and decreased sympathetic nerve firing rates (Oommura & Yoshimatsu, 1984). Decreased 5-HT_{1A} receptor binding observed in the hypothalamus reduces the sympathetic nerve stimulation thus reducing the inhibitory effect of EPI on insulin secretion

Brain 5-HT_{2C} receptor alterations

The 5-HT_{2C} receptor displays a heterogeneous distribution in the CNS (Pazos & Palacios, 1985). 5-HT_2 receptor stimulation has been shown to trigger numerous biochemical and behavioral effects, including phosphatidyl inositol hydrolysis, platelet aggregation, head twitches, and vasoconstriction (Conn *et al.*, 1987). Stimulation of centrally located 5-HT_2 receptor leads to adrenal epinephrine release that elevates plasma glucose levels and inhibits insulin release (Veronique Baudrie & Chauloff, 1992). Acute administration of the 5-$HT_{2C/2B}$ receptor agonist 1-(3-chlorophenyl) piperazine (mCPP) induced hyperglycaemia in rats and it is mediated by the activation of 5-$HT_{2C/2B}$ receptors. The effects of mCPP are considered to be connected to the activation of the sympathoadrenomedullary system and catecholamine release (Sugimoto *et al.*, 1996). Pretreatment with the 5-HT_{1C}

ceptor antagonist ritanserine dose dependently attenuated EPI and NE responses, suggesting 5-HT_{1C} receptor mediated mechanism (Bagdy *et al.*, 1989)

When we analysed the 5-HT_{2C} receptor status in the cerebral cortex, we found that 5-HT_{2C} receptor number decreased significantly in 72 hrs after pancreatectomy as indicated by a decreased B_{max}. There was no shift in affinity of the receptor in 72 hrs pancreatectomised rats as indicated by the unchanged Ki and log (EC_{50}). RT-PCR analysis also confirmed the receptor data. 5-HT_2 receptor stimulation has neuroendocrinological consequences, as exemplified by the activation of the corticotropic and sympathoexcitation (McCall & Harris, 1988; Bagdy *et al.*, 1989). Our results showed a decreased binding of 5-HT_{2C} receptors in the cerebral cortex, which reduces the sympathoexitatory effect. This decreased expression of 5-HT_{2C} receptors observed in the cerebral cortex decreases the sympathetic effect, which is stimulatory to insulin secretion. Increased plasma levels of insulin and leptin analogous to Type 2 diabetes in mice lacking 5-HT_{2C} suggests that the 5-HT_{2C} receptor has a role in tonic inhibition of neuronal excitability (Tecott *et al.*, 1995)

In the brain stem, Scatchard analysis revealed a decreased B_{max} and increased K_d of the high affinity 5-HT_{2C} receptor indicating a reduction in the receptor density as well as the affinity of the receptor in 72 hrs pancreatectomised rats. The expression of different serotonergic receptor mRNAs appears to change during development and 5- HT receptors can be regulated by a variety of exogenous agents (Roth *et al.*, 1991) Down-regulation of receptor binding sites in choroid plexus cells is seen after agonist treatment (Barker & Sanders-Bush, 1993). A decreased mRNA expression as revealed by RT-PCR analysis confirmed our receptor data. Down-regulation of the receptor by 5-HT is associated with an equivalent decrease in the level of receptor mRNA (Ivins & Molinoff, 1991). Previous studies showed that methysergide, which acts as a partial 5-HT_1 receptor agonist and as a

5-HT_{2C} receptor antagonist, potentiates both insulin and glucagon release (Marco *et al.*, 1976). Thus the down-regulation of the 5-HT_{2C} receptors observed in the brai stem during pancreatic regeneration suggests the stimulatory role of insulin secretio mediated by sympathetic system. This indicates that regulation of receptor activit probably occurs at multiple points in the metabolic cycle of the receptor protein.

5-HT_{2C} receptor number and receptor affinity towards its ligand decreased i hypothalamus of 72 hrs pancreatectomised rats. The analysis of Ki and log (EC_{5} showed an inecreased values in 72 hrs after pancreatectomy indicating a shift i affinity of the receptor towards lower affinity. Our RT-PCR results were als consistent with the receptor data. 5-HT has been shown to control the activity c hypothalamic CRF neurons and pituitary corticotrope cells through activation c 5-$HT_{(2A/2C)}$ receptor subtypes (Contesse *et al.*, 2000). Activation of centrally locate 5-HT_{2C} leading to hypoglycaemia due to increased insulin secretion is alread reported (Sugimoto *et al.*, 1996). Our results showed a decreased 5-HT_{2C} binding i the hypothalamus. This decreases the CRF release which in turn decreases th plasma EPI concentration. CRF mediates EPI responses after several stimu (Brown, 1985). Our data suggest that EPI response might be mediated by CR release. The increase in 5-HT turnover in the rat hypothalamus during insulir induced hypoglycaemia described earlier suggests that 5-HT might be the mediat of CRF release and EPI response (Yehuda & Meyer, 1984).

Thus, central 5-HT_{1A} and 5-HT_{2C} receptors can act as stimulators c pancreatic β-cell proliferation depending upon the NE and EPI release by adren medulla through which its function is mediated. The circulating NE and EPI levels i the experimental groups were also consistent with our receptor data.

5-HT_{1A} and 5-HT_{2C} receptor alterations in pancreatic islets

Neurotransmitter receptors are usually restricted to neuronal cells. However, neurotransmitters have been shown to stimulate or inhibit proliferation of non-neuronal cells by activating receptors coupled to different second messenger pathways (Lauder, 1993). 5-HT has been found to promote cell proliferation in various cell types. In aortic smooth muscle cells, 5-HT induced mitogenesis was comparable with that of human-platelet derived growth factor (Nemeck *et al*, 1986).

Scatchard analysis, displacement analysis and RT-PCR studied 5-HT_{1A} receptor functional status of pancreatic islets. The number of 5-HT_{1A} receptor significantly increased in 72 hrs pancreatectomised rats. The affinity of the receptor decreased in 7 days pancreatectomised rats. $Ki_{(H)}$ and $Ki_{(L)}$ increased in 7 days pancreatectomised rats indicating the shift in affinity of the receptor towards low affinity sites. Our results revealed that pancreatic 5-HT_{1A} receptor status is getting up regulated during pancreatic regeneration. This indicates that up-regulation of 5-HT_{1A} receptor expression is facilitating the islet cell proliferation. In pancreatic cell line, activation of pertussis toxin sensitive $5HT_{1A/1B}$ receptors stimulate proliferation through the activation of PLC and PKC that resulted in the down regulation of cAMP (Ishizuka *et al.*, 1992). Agonists at 5-HT_{1A} receptors inhibit adenylyl cyclase and activate phosphoinositide hydrolysis in HeLa cells (Raymond *et al.*, 1991). Moreover the receptor affinity was found to be decreased by 7 days after partial pancreatectomy, which may be a compensatory mechanism for increased receptor number. These receptor data are well supported by our RT-PCR analysis. The translation of the mRNA results in increased protein synthesis finally leading to either growth or differentiation (Fantl, 1993).

5-HT_{2C} receptor number was increased in the islets of 72 hrs and 7 days pancreatectomised rats. This shows the up regulation of 5-HT_{2C} receptors during active proliferation of islet cells. Our present results are well supported by the fact

that 5-HT_{2C} receptor can function as a protooncogene in NIH-3T3 cells. NIH-3T3 cells that express high levels of 5-HT_{2C} receptor form foci in cell culture (Julius 1989). The 5-HT_2 receptors are coupled to phospho-inositide turnover and diacylglycerol formation, which activates protien kinase C (PKC), an important second messenger for cell division (DeCorcelles *et al.*, 1984). Displacement studies on [^{3}H]5-HT binding to crude membranes from control and regenerating liver tissue using cold ketanserin and spiperone, showed an increased involvement of 5-HT_2 receptors in the regenerating liver during the DNA synthetic phase (Sudha, 1997).

Serotonergic stimulation of insulin synthesis and secretion from pancreatic β-cells *in vitro*

Signal-transduction in the pancreatic β-cell and thereby the insulin secretory process is regulated by a sophisticated interplay between glucose and a plethora of additional factors including other nutrients, neurotransmitters, islet generated factors and systemic growth factors. The coupling of glucose metabolism to electrical activity remains central in all models of β-cell stimulus-secretion coupling. The resting membrane potential of the β-cell is set by the ATP-sensitive potassium (K_{ATP}) channel (Ashcroft & Rorsman, 1990). Incubation of the pancreatic β-cells with stimulatory glucose concentrations leads to the activation of a cascade of reactions, which ends in the exocytosis of stored insulin. This complex of processes starts with the uptake of glucose by the β-cell high-K_m/low affinity glucose transporter GLUT2 and proceeds with the conversion of glucose into glucose-6-phosphate by the β-cell isoform of glucokinase (Randel, 1993; Matschinsky, 1996). Metabolism of glucose in glycolysis and the Krebs cycle results in the generation of ATP. Elevation in the ATP/ADP ratio leads to closure of the K_{ATP}, which in turn results in depolarization of the plasma membrane. The subsequent opening of voltage-gated L-type Ca^{2+} channels leads to an increase in the cytoplasmic free Ca^{2+} concentration, $[Ca^{2+}]_i$ which promotes insulin secretion (Berggren & Larsson, 1994).

Pancreatic islet is considered as a tissue rich in 5-HT (Bird *et al.*, 1980). 5-HT has a direct effect on the insulin secretion from the pancreatic islets (Peschke *et al.*, 1997). Serotonergic receptors play a role in the regulation of blood glucose by facilitating insulin release (Jun Yamada *et al.*, 1990). 5-HT stimulated the output of insulin in the presence of a low concentration of glucose. When the islets were incubated with glucose at a higher concentration there was a lower insulin release in the presence of 5-HT than that obtained with glucose alone at the same concentration (Lechin *et al.*, 1975). High concentrations of NE, DA, and 5-HT in the pancreatic islets can decrease glucose-stimulated insulin secretion (Zern *et al.*, 1980). 5-HT dose dependently inhibited insulin secretion from pancreatic islets in the presence of 20mM glucose. This result indicates that although 5-HT may help in the maintenance of the blood sugar level in normal pancreas by increasing insulin secretion it may also aggravate the hyperglycaemia observed in diabetes mellitus (Adeghate *et al.*, 1999). 5-HT inhibits glucose-induced insulin release by affecting early steps in the β-cell stimulus-secretion coupling (Lindstrom & Sehlin, 1983).

5-HT_{1A} agonist, 8-OH DPAT, at low concentrations (10^{-6}M & 10^{-7}M) stimulated insulin secretion in the presence of 4mM glucose. But at high concentration (10^{-4}M) 8-OH DPAT inhibited the insulin secretion from pancreatic islets. There was a dose dependent inhibition of insulin secretion by 8-OH DPAT in the presence of 20mM glucose. In the isolated perfused pancreas of the rat, 8-OH-DPAT, at 10^{-8}M and 10^{-7}M, concentrations known to activate 5-HT_{1A} receptors *in vitro* (Bouhelal *et al.*, 1990), this induces glucose-stimulated insulin release

The 5-HT_{2C} receptors stimulate phospholipase C, and increases IP_3 and DAG (Conn *et al.*, 1986) which leads to increased intracellular calcium (Watson *et al.*, 1995). IP_3 mediates Ca^{2+} mobilization from intracellular Ca^{2+} stores and plays an important role in insulin secretion from pancreatic β-cells (Laychock, 1990). IP_3 exerts its action through receptors that are ligand-activated, Ca^{2+} selective channels.

IP_3 receptors have been localized to the endoplasmic reticulum, nucleus and insulin granules (Yoo *et al.*, 1990). We examined the effects of 5-HT_{2C} receptor antagonist, mesulergine on insulin secretion. Mesulergine inhibited 5-HT mediated insulin secretion at both 4mM and 20mM glucose concentrations. Our results indicate that mesulergine inhibits 5-HT_{2C} receptor activity and blocked the increase in intracellular calcium and thus inhibited insulin release.

Twenty four hrs islet cell culture was done to study the long-term effect of 5-HT, 5-HT_{1A} and 5-HT_{2C} receptors on insulin synthesis and release from the isolated islets. Long-term insulin secretion studies showed similar changes as in the 1 hour incubations. 5-HT stimulated insulin secretion at lower concentrations and inhibited insulin secretion at higher concentration in the presence of 4mM glucose. There was a dose dependent inhibition of insulin secretion by 5-HT from pancreatic islets in the presence of 20mM glucose. In the long-term studies 8-OH DPAT showed stimulatory effect at lower concentrations and inhibitory effect at higher concentration at 4mM glucose. Similar to 1 hour secretion studies high concentration of 8-OH DPAT abolished the glucose stimulated insulin secretion. Mesulergine inhibited 5-HT mediated insulin secretion at both 4mM and 20mM glucose concentration.

Long-term and 1 hr culture showed stimulatory and inhibitory role of 5-HT, 5-HT_{1A} and 5-HT_{2C} receptors on insulin secretion.

Effect of 5-HT, 8-OH DPAT and mesulergine on islet DNA synthesis

Effect of 5-HT on islet DNA synthesis

Higher concentration of 5-HT (10^{-4}M) when added to primary islet culture did not increase DNA synthesis but was able to increase DNA synthesis at lower concentration. In the absence of growth factors, 5-HT is potent at inducing cell-cycle progression of L cells expressing the 5-HT_{2B} receptor (LM6) (Canan *et al.*, 2000).

Mene *et al* suggetsed activation of a 5-HT_2 receptor in rat mesangial cells to acount for 5-HT induced cell proliferation (Mene *et al.*, 1991). It also stimulated the EGF and TGFβ1 mediated DNA synthesis. EGF and TGF-β are known mitogens for cultured vascular smooth muscle cells (Huang *et al.*, 1992). There are several reports on effects of growth factors in the normal β-cell growth. EGF was shown to stimulate [^{3}H]-thymidine incorporation in islets (Sieradzski *et al.*, 1987). Furthermore, a recent report showed that EGF was an important factor for pancreas precursor cell proliferation *in vitro* (Corentin *et al.*, 2001), and that islet cell migration and differentiation were impaired in the mice lacking EGF receptors (Miettinen *et al.*, 2000). These findings suggest EGF is important in the growth and differentiation of islet cells.

Effect of 8-OH DPAT on islet DNA synthesis

8-OH DPAT is the specific agonist of the 5-HT_{1A} receptor. We used 8-OHDPAT to study the 5-HT_{1A} receptor mediated effect on DNA synthesis of islets kept in primary culture. 8-OH DPAT at a concentration of 10^{-4} M was enough to inhibit the basal DNA synthesis but it induced EGF mediated DNA synthesis in primary islet cultures. In addition to this it enhanced the TGFβ1 mediated DNA synthesis. When we studied the dose dependent effect of 8-OH DPAT in primary islet DNA synthesis, we found a dose dependent inhibition from 10^{-6}M to 10^{-4}M. 8-OH DPAT at a concentration of 10^{-8}M was also found to increase DNA synthesis. This may be due to the activation of the high affinity receptors of 5-HT_{1A} receptor. In addition to increasing the DNA synthesis by itself, it increased the EGF mediated DNA synthesis from 10^{-8}M to 10^{-4}M significantly. DNA synthesis was found to be maximum at 10^{-6}M concentration. 8-OH DPAT also enhanced the TGFβ1 mediated DNA synthesis in a similar trend as that of 8-OH DPAT alone. Thus from our results it is very clear that 5-HT_{1A} receptor mediates stimulation of DNA synthesis *in vitro*.

G-proteins that are coupled to 5-HT_{1A} receptors are PTX sensitive (Raymond *et al.*, 1991). Pertussis toxin inhibited potentiation of EGF effect induced by 8-OH DPAT. In transfected NIH-3T3 cells, transforming and mitogenic effects of 5-HT_{1A} agonists involve a pertussis toxin-sensitive G protein but do not seem to be linked to adenylyl cyclase inhibition (Varrault, *et al.*, 1992). Activation of ERK2 by the 5-HT_{1A} receptor-selective agonist (8-OH-DPAT) was inhibited completely by pertussis toxin (Daniel *et al.*, 1996)

Effect of mesulergine on islet DNA synthesis

We have studied the role of 5-HT_{2C} receptor in mediating the islet DNA synthesis by blocking the 5-HT_{2C} receptor using mesulergine. Addition of mesulergine to the primary islet culture resulted in a decrease in the basal and EGF mediated DNA synthesis and enhanced the TGFβ1 mediated DNA synthesis suppression. It also inhibited the basal DNA synthesis induced by 5-HT from (10^{-8}M to 10^{-4}M) and EGF mediated DNA synthesis of primary islets in culture. Mesulergine enhanced the TGFβ1 mediated DNA synthesis inhibition at a concentration from 10^{-8}M to 10^{-4}M. Thus, mesulergine was able to completely inhibit the EGF mediated DNA synthesis indicating the regulatory role of 5-HT_{2C} receptor in islet cell proliferation. Our results are well supported by the reports that in NIH-3T3 cells 5-HT_{2C} receptor functions as a protooncogene. Moreover the formation of foci is dependent on activation of the 5-HT_{2C} receptor by 5-HT (Julius, 1989).