The pancreas is a complex organ composed of two different cell populations, exocrine and endocrine. The exocrine component includes acinar and ductal cells that secrete and transport digestive enzymes into the intestine. Exocrine cells make up the majority of the pancreas and are grouped together into acini and a highly branched ductal system. The endocrine cells account for approximately 4% of the volume of the pancreas and they are grouped together into islets of Langerhans. The islets are composed of a few hundred to several thousands of cells, of which 65–80% are insulin-secreting β-cells. These cells are mainly located in the center of the islet and are surrounded by a mantel of three other cell types, i.e., glucagon-secreting α-cells, somatostatin secreting - delta cells and pancreatic polypeptide secreting cells (PP-cells).

The endocrine pancreas is derived from progenitor cells in the ducts of the exocrine portion of the pancreas. The pancreatic β-cell is characterized by a limited proliferative potential in man (Hellerstrom et al., 1985). In animals there are, however indications of regenerative phenomena in the β-cell (Logothetopoulos, 1972). Studies showed that the rat pancreatic islet cells in allogeneic grafts are capable of regeneration (Socha et al., 2003). The use of partial pancreatectomy as a research tool in studying pancreatic regeneration is well established and dates back to studies at the turn of the century. Pancreatectomy, or removal of pancreas, is a very useful approach to demonstrate the regenerative potential of β-cells. 60% partial pancreatectomy does not result in glucose intolerance or permanent diabetes. This maintenance of glucose homeostasis is due to regeneration among the remaining pancreatic β-cells (Leahy et al., 1988; Lohr et al., 1989). However, when 85-90% partial pancreatectomy is performed, mild hyperglycaemia ensues which is followed
by increased β-cell replication and a 40% increased β-cell mass (Bonner-Weir, 1983). Regeneration of endocrine cells started immediately after 90% pancreatectomy (Hayashi et al., 2003). Interestingly, 95% pancreatectomy results in severe hyperglycaemia with non-existent or very minor signs of β-cell replication (Clark et al., 1982). Based on the pancreatectomy models, it is evident that β-cells have a certain regenerative capacity.

The degree of regeneration of pancreas is variable, depending on the nature of the stimulus to regenerate (Logothetopoulos et al., 1983). Exocrine cells exhibit significant regenerative potential after incomplete destruction, following the use of the selective pancreatic toxin ethionine or following cerulein induced pancreatitis (Kasai et al., 1982). It appears that endocrine and exocrine regeneration are under different control mechanisms (Gepts et al., 1990). There is much evidence to suggest that prolonged stimulation of insulin secretion in vivo leads to a compensatory increase of the total volume of the pancreatic islets (Martin et al., 1963). Th insulin secretion from the β-cell is the result of a complex interaction between metabolic and neural (Campfield et al., 1980) external inputs acting in concert with other controlling factors.

NEURAL INNERVATION OF THE PANCREAS

The endocrine pancreas is richly innervated, but the abundance and organization of this innervation are highly variable between species (Kobayashi & Fujita, 1969). Most of the nerve fibres enter the pancreas along the arteries (Woods & Porte Jr. D, 1974; Miller, 1981). Well differentiated synapses with islet cells have rarely been observed (Orci et al., 1973; Watanabe & Yasuda, 1977). The innervation of the islet is very plastic, as suggested by the observation that islets transplanted in
The portal vein of diabetic rats became reinnervated by hepatic nerves (Gardemann et al., 1994).

The autonomic innervation of the endocrine pancreas has several origins. The autonomic nervous system uses two interconnected neurons to control effector functions and is divided into two systems, the sympathetic and the parasympathetic nervous systems, according to the location of the preganglionic cell bodies. However, there are indications suggesting that these two systems are not always independent of each other, but display anatomical interactions (Berthoud & Powley, 1993) or share similar neurotransmitters (Sheikh et al., 1988; Verchere et al., 1996). The endocrine pancreas also receives other types of nerves. These nerves are of peptidergic and nonpeptidergic nature (Brunicardi et al., 1995; Ahren, 2000).

The parasympathetic innervation

The preganglionic fibres of the parasympathetic limb originate from the dorsal motor nucleus of the vagus (Luiten et al., 1984 & Ahren, 1986) and possibly also in the nucleus ambiguus (Luiten et al., 1984), which are both under the control of the hypothalamus. They are organized in well separated branches traveling within the vagus nerves (cranial nerve X), and through the hepatic, gastric (Berthoud et al., 1990), and possibly celiac branches of the vagus (Kinami et al., 1997), they reach intrapancreatic ganglia that are dispersed in the exocrine tissue. These ganglia send unmyelinated postganglionic fibres toward the islets (Woods & Porte Jr. D, 1974). Preganglionic vagal fibres release ACh that binds to nicotinic receptors on intraganglionic neurons. Postganglionic vagal fibres release several neurotransmitters: ACh, vaso active intestinal peptide (VIP), gastrin-releasing peptide (GRP), nitric oxide (NO), and pituitary adenylate cyclase-activating polypeptide (PACAP) (Ahren, 2000). Cholinergic terminals are found in the neighborhood of all islet cell types at the periphery and within the islet (Love & Szébeni, 1999).
Cholinergic synapses with endocrine cells have been observed in some species (Voss et al., 1978).

**The sympathetic innervation**

The sympathetic innervation of the pancreas originates from the thoracic and upper lumbar segments of the spinal cord (Furuzawa et al., 1996). The myelinated axons of these cells traverse the ventral roots to form the white communicating rami of the thoracic and lumbar nerves that reach the paravertebral sympathetic chain (Chusid, 1979). Preganglionic fibres either communicate with a nest of ganglion cells within the paravertebral sympathetic chain or pass through the sympathetic chain, travel through the splanchnic nerves, and reach the celiac (Brunicardi et al., 1995; Ahrén, 2000) and mesenteric ganglia (Furuzawa et al., 1996). Ganglia within the paravertebral sympathetic chain, and the celiac and mesenteric ganglia, give off postganglionic fibres that eventually reach the pancreas. The existence of intrapancreatic sympathetic ganglia has also been reported (Liu et al., 1998). The preganglionic fibres release ACh that acts on nicotinic receptors on intraganglionic neurons, whereas the postganglionic fibres release several neurotransmitters: norepinephrine, galanin, and NPY (Ahrén, 2000). A rich supply of adrenergic nerves in close proximity of the islet cells has been observed in several mammalian species (Esterhuizen et al., 1968).

**Sensory fibres**

The sensory nerve fibres report pain information associated with diseases of the exocrine tissue, such as pancreatic cancer and pancreatitis (Rossi et al., 1995; Di Sebastiani et al., 2000), but there are no reports of sensations of pain associated with a destruction of the endocrine pancreas. However, it is possible that sensory fibres play a role in the control of insulin secretion. Thus, neonatal treatment of mice with
capsaicin (to destroy these fibres) results in more glucose-stimulated insulin secretion than in nontreated mice, suggesting that sensory fibres exert a direct, tonic inhibition of insulin secretion (Karlsson et al., 1994).

Other types of nerves

Immunocytochemistry has revealed the presence of neurotransmitters other than those described above in pancreatic nerves: cholecystokinin (Karlsson & Ahrén, 1992), 5-HT (Kirchgessner & Gershon, 1990), and methionine-enkephalin (Ahrén, 2000).

CENTRAL NERVOUS SYSTEM REGULATION OF INSULIN SECRETION

The autonomic nervous system, acting through both its sympathetic and parasympathetic branches, has the potential to modulate the rate of insulin secretion over a wide range at a constant intermediate glucose concentration. Studies conducted have demonstrated that insulin secretion in response to glucose from β-cells of the endocrine pancreas can be modified by the activity of both the sympathetic and parasympathetic branches of the autonomic nervous system (Burr et al., 1976; Campfield et al., 1980). Electrical stimulation of the sympathetic nerves to the pancreas or exposure of the pancreas to exogenous norepinephrine decreased glucose-induced insulin secretion. Sympathetic inhibition was observed at glucose concentrations greater than 5mM (Campfield et al., 1976; Campfield et al., 1980).

The recent demonstration that, central nervous system cell groups projecting into the pancreatic vagal motor neurons received inputs from adrenergic, noradrenergic and serotonergic neurons from the lower brain stem and a dopaminergic input from paraventricular nucleus of hypothalamus (Lowey et al., 1994). Also it 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 noradrenergic pathways (McCaleb & Myers, 1982) by an activation of sympathetic-adrenal system. Plasma glucose appears to be under separate serotonergic and dopaminergic control exerted via 5-HT1A and DA_D receptors respectively (Alster & Hillegaart, 1996).

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 hypothalamus plays a central role in the integration of neurohormonal function (Oommura & Yoshimatsu, 1984). The ventromedial hypothalamic nucleus is considered as the sympathetic centre and the stimulation of this area decreases insulin secretion (Helman et al., 1982). Lesions of the ventromedial hypothalamic nucleus, resulted in behavior alterations and morphological changes in pancreatic islets (Scalfani, 1981). Ventrolateral hypothalamic nucleus is the parasympathetic centre, stimulation of which increases the circulating level of insulin (Helman et al., 1982). Lesions in ventrolateral hypothalamic nucleus results in decrease body weight; food intake, plasma insulin levels and decrease in islet size (Powley & Opsahl, 1976). Hyperactivation of the HPA axis in diabetes is associated with increased expression of hypothalamic corticotrophin-releasing hormone (CRF) mRNA and hippocampal mineralocorticoid receptor (MR) mRNA (Chan et al., 2002).

FACTORS AFFECTING INSULIN SECRETION FROM PANCREATIC β-CELLS

**Glucose**

Insulin is secreted primarily in response to elevated blood glucose concentrations. The mechanism of glucose induced insulin release is not completely understood. Phosphorylation of glucose to glucose-6-phosphate serves as the rate-limiting step in glucose oxidation (Schuit, 1996). Glucokinase acts as a glucose sensor during this process. The entry of glucose into β-cells is followed by
acceleration of metabolism that generates one or several signals that close ATP-sensitive $K^+$ channels in the plasma membrane. The resulting decrease in $K^+$ conductance leads to depolarisation of the membrane with subsequent opening of voltage dependent $Ca^{2+}$ channels. The rise in the cytoplasmic free $Ca^{2+}$ eventually leads to the exocytosis of insulin containing granules (Dunne, 1991). 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). Although intracellular $Ca^{2+}$ activates protein kinases such as $Ca^{2+}$ and calmodulin dependent protein kinase (Breen & Aschoft, 1997), it remains unclear how increases in intracellular $Ca^{2+}$ leads to insulin release. It is suggested that PKC may be tonically active and effective in the maintenance of the phosphorylated 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**

Many amino acids increase insulin secretion. Amino acids like arginine increase insulin secretion from pancreatic β-cells (Holstens et al., 1999). Several in vitro studies have suggested the production of nitric oxides from islet nitric oxide system may have a negative regulation of the L-arginine induced secretion of insulin and glucagon in mice. L-Tryptophan which is the precursor of 5-HT can act as a stimulator of insulin release (Bird et al., 1980)

**Fatty acids**

Free fatty acids act as signaling molecules in various cellular processes, including insulin secretion (Haber et al., 2003). 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 (Laghmich et al., 1997). A monomethyl ester of succinic acid along with D-glucose is required to maintain the β-cell response to D-glucose (Fernandez et al., 1996).

**Glucagon**

Glucagon is secreted by the α-cells of the pancreatic islets. It has been shown that glucagon has a striking stimulation of insulin release in the absence of glucose (Sevi & Lillia, 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. Intra-islet glucagon appears to be a paracrine regulator of cAMP in vitro (Schuit, 1996). Glucagon stimulates insulin release by elevating cAMP. cAMP through activation of protein kinase A, increases Ca^{2+} influx through voltage dependent L-type Ca^{2+} channels, thereby elevating [Ca^{2+}] 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).

**Substrates derived from nutrients**

Substrates like pyruvate (Lisa et al., 1994), citrate, ATP (Tahani, 1979), NADH and NADPH (Iain et al., 1994) may involve indirect reflux stimulation triggered by food intake or local islet stimulation through the production of metabolites. Adenosine diphosphate acts as an intracellular regulator of insulin secretion. Heterotrimeric GTP-binding protein G_{ai} is 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
is accompanied by an increase in the islet content of cAMP (Rabinovitch et al., 1976).

**Somatostatin**

This hormone is secreted by the pancreatic δ-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).

**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 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. Amylin fibril formation in the pancreas may cause islet cell dysfunction and cell death in type II - diabetes.
mellitus (Alfredo et al., 1994). Pancreatic islets amylin play a role in islet enlargement, an important issue in the progression towards overt diabetes (Wook & Cooper, 2001).

**Adrenomedullin**

Adrenomedullin is a novel hypotensive adrenal polypeptide isolated from human phaeochromocytoma and is structurally related to calcitonin gene related peptide and islet amyloid polypeptide. It has been suggested that besides being a 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 α2-adrenergic receptors in activating G-proteins.

**Macrophage migration inhibitory factor (MIF)**

MIF, originally identified as cytokines, secreted by T lymphocytes. It 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 express MIF and its production is regulated by glucose in a time and concentration dependent manner. MIF and insulin were both present 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 Q₁₀ improved insulin release (Conget et al., 1996) and it may also have a blood glucose lowering effect. Inositol hexa bisphosphate stimulates non-Ca⁺ mediated and purine-Ca²⁺ mediated exocytosis of insulin by activation of protein kinase C. (Efanov et al., 1997). Small GTPases 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).

**ROLE OF NEUROTRANSMITTERS IN INSULIN REGULATION**

**Epinephrine and Norepinephrine**

Various neurotransmitters like NE, GABA, 5-HT, DA and ACh have important role in cell proliferation and insulin secretion (Paulose et al., 2004). Epinephrine and Norepinephrine are secreted by the adrenal medulla. Norepinephrine (NE) is a principal neurotransmitter of sympathetic nervous system. These hormones inhibit insulin secretion, both in vivo and in vitro (Renstrom et al., 1996; Porte, 1967). Epinephrine exerts opposite effects on peripheral glucose disposal and glucose stimulated insulin secretion (Avogaro et al., 1996). NE and EPI, the flight and fright hormones are released in all stress conditions and are the main regulators of glucose turnover in strenuous exercise (Simartirkis et al., 1990). In severe insulin-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). It is already known that, when used in high doses in vivo or
in vitro, epinephrine reduces the insulin response to stimulators (Malaisse, 1972). EPI and NE have an antagonistic effect on insulin secretion and glucose uptake (Porte et al., 1966a, b). They also inhibit insulin-stimulated glycogenesis through inactivation of glycogen synthase and activation of phosphorylase with consequent accumulation of glucose-6-phosphate. In addition, it has been reported that epinephrine enhances glycolysis through an increased activation of phosphofructokinase. In humans, adrenaline stimulates lipolysis, ketogenesis, thermogenesis and glycolysis and raises plasma glucose concentrations by stimulating both glycogenolysis and gluconeogenesis. Adrenaline 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 Randle (Coore et al., 1964), who incubated pancreatic tissue from the rabbit. As judged by Malaisse et al., the inhibitory effect of EPI on glucose-induced insulin secretion is mediated through the activation of α-adrenoreceptors (Malaisse et al., 1967). Adrenaline inhibits insulin release through α2A and α2C adrenoreceptors via distinct intracellular signaling pathways (Peterhoff et al., 2003).

Acetylcholine

Acetylcholine is one of the principal neurotransmitters of the parasympathetic system. Acetylcholine increases insulin secretion (Tassava et al., 1992) through vagal muscarinic and non-vagal muscarinic pathways (Greenberg et al., 1994). They function through muscarinic receptors present on pancreatic islet cells (Ostenson et al., 1993). Central muscarinic M1 and M3 receptors are involed in
the regulation of insulin secretion from pancreatic β-cells during pancreatic regeneration (Paulose & Renuka, 2004).

**Dopamine**

High concentrations of dopamine in pancreatic islets can decrease glucose stimulated insulin secretion (Tabeuchi et al., 1990). L-DOPA the precursor of dopamine had similar effect to that of dopamine (Lindstrom et al., 1983). Dopamine D$_1$ receptors are implicated in the control of blood glucose levels (Alster et al., 1996). Dopamine D$_1$ 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.

**γ-Aminobutyric acid**

Gamma aminobutyric acid (GABA) is the main inhibitory neurotransmitter in central nervous system. GABA is reported to 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 et al., 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 et al., 1990). GABA through its receptors has been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic α-cells and δ-cells respectively (Gaskins et al., 1995). GABA which is present in the cytoplasm and in synaptic-like microvesicles (Reetz et al., 1991) 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 GABA_A receptors increases plasma glucose concentration (Lang, 1995). Thus, any impairment in the GABAergic mechanism in central nervous system and/or in the pancreatic islets is important in the pathogenesis of diabetes.

**Serotonin**

Since the early seventies the hypothesis for a control of circulating glucose and insulin levels by 5-HT system has been the matter of numerous works. 5-HT content is increased in the brain regions and hypothalamic nuclei (Chen et al., 1991; Lackovic et al., 1990), but there are reports suggesting a decrease in brain 5-HT content during diabetes (Jackson et al., 1999; Sumiyoshi et al., 1997; Sandrini et al., 1997). Ohtani et al., 1997 have reported a significant decrease in extracellular concentrations of NE, 5-HT and their metabolites in the ventro medial hypothalamus (VMH). 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 control 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., 1993).
5-HT IN THE PANCREATIC ISLETS

In the pancreas, 5-HT is mainly present in β-cells of the islet of Langerhans (Ekhholm et al., 1971; Cetin, 1992). 5-HT containing nerves were also observed in the periacinar and periinsular regions of normal pancreas. 5-HT may help in the maintenance of the blood sugar level in normal pancreas by increasing insulin secretion and decreasing glucagon secretion (Adeghate et al., 1999). In response to 5-HT receptor activation, both decreased and increased insulin levels, respectively with hyperglycaemia and hypoglycaemia, have been reported in animals (Bjorkstrand, 1996; Chaouloff et al., 1987c). Insulin resistance has been reported to vary inversely with brain serotonergic activity (Horacek et al., 1999), and genetic variation in two 5-HT receptors has been associated with abdominal obesity and diabetes (Yuan et al., 2000). Pancreatic islets receive innervation from both divisions of the autonomic nervous system, and pancreatic endocrine secretion is partly controlled by the autonomic nervous system (Holst et al., 1986). Pharmacological manipulations of pancreatic islet 5-HT and dopamine content in vitro and in vivo systems have resulted in evidence for monoaminergic inhibition (Feldman et al., 1972) and stimulation of insulin secretion (Telib et al., 1968). Several amino acids are able to synthesis insulin release in the presence of glucose. 5-HTP is readily taken up into the islet in the presence of glucose and stimulates insulin secretion. But the enzyme 5-hydroxytryptophan decarboxylase readily converts it into 5-HT that inhibits insulin secretion (Sundler et al., 1990). When 5-HTP was tested in conjunction with a decarboxylase inhibitor, the glucose stimulated insulin release from rabbit pancreas was significantly enhanced (Gylfe et al., 1973). Tryptophan a precursor of 5-HTP, has a stimulating effect on insulin release from hamster pancreas. The presence of monoamine oxidase enzyme that catabolises 5-HT within the β-cells shows an effective 5-HT metabolism within the islets (Feldman & Chapman, 1975). 5-HT can also act as a marker for insulin secretion. 5-HT is
taken up into insulin granules and co-released with insulin on stimulation of pancreatic β-cells by glucose (Zhou & Misler, 1996).

PANCREATIC REGENERATION AND β-CELL GROWTH

The adult pancreas has a capacity to respond to changing physiological needs such as the requirement for increased β-cell mass/function during pregnancy, obesity or insulin resistance and an ability to regenerate cells including β-cells that has been convincingly demonstrated in animal models of pancreatic injury and diabetes (Rosenberg, 1995 & 1998). Animal models in which pancreatic endocrine and exocrine regeneration can be observed include chemically induced models of pancreatic injury following administration of alloxan (Davidson et al., 1989; Waguri et al., 1997), streptozotocin (Like & Rossini, 1976) or cerulein (Elsasser et al., 1986) and hemipancreatectomy (Bonner-Weir et al., 1993; Sharma et al., 1999). Although the triggers may differ, in each of these models pancreatic regeneration is thought to occur through the expansion of progenitor cells present either in, or closely associated with, the ductal epithelium. In these models, both endocrine and exocrine cells have been observed to arise from duct cells (Waguri et al., 1997; Bonner-Weir et al., 1993). Supporting this observation, 'transitional' cells have been identified that co-express ductal markers with endocrine or exocrine cell-specific markers, suggesting a reprogramming of duct-like cells (Wang et al., 1985). In the 90% pancreatectomy model, regeneration has been suggested to mimic embryonic pancreogenesis with proliferation occurring initially from expansion of the common pancreatic duct epithelium followed by branching of smaller ductules and subsequent regeneration of exocrine, endocrine and mature duct cells (Bonner-Weir et al., 1993)
Islet cells in regeneration

The endocrine cell mass in the adult pancreas is maintained through a slow turnover of cells involving a balance of replication from existing differentiated cells, apoptosis and neogenesis from less-differentiated progenitor cells. Morphometric analysis, combined with mathematical modelling, has shown that the turnover of adult rat \(\beta\)-cells is 1 to 4% per day (Bonner-Weir, 2000a; Finegood et al., 1995). In situations of increased demand, this rate may be increased through changes in the rate(s) of replication, apoptosis or neogenesis. Although there is little evidence for islet-derived progenitors, mitotic analysis indicates that islet cells contribute to the regeneration observed in animal models of diabetes and pancreas injury. Islet cells may increase their rate of replication in times of stress (Waguri et al., 1997), although this is usually accompanied by neogenesis that appears to derive from the ducts (Waguri et al., 1997). Three-dimensional reconstruction of histological sections has revealed that all cells within rat islets are 'differentiated', inferring that there is not an easily discernible, and discrete progenitor cell population in the islet (Bonner-Weir, 2000b). While this does not necessarily preclude the possibility that a sub-population of 'differentiated' islet cells possesses a more multipotent phenotype or retains the capacity to de-differentiate and assume a new fate, there is presently little data to support this. Some evidence for islet-derived progenitors is provided by three studies in which \(\beta\)-cells apparently reverted to a more primitive insulin-\(Pdx1\)+ phenotype. When cultured as a monolayer (Beattie et al., 1999) adopted a duct-like phenotype in a collagen matrix (Yuan et al., 1996), streptozotocin-treated, normoglycaemic mice, exhibited enhanced neogenesis (Guz et al., 2001).

Mechanism of \(\beta\)-cell growth

Beta-cell growth is a cumulative effect of the following three phenomena during \(\beta\)-cell development (i) differentiation of \(\beta\)-cells from precursors, a process
referred to as neogenesis (ii) changes in the size of individual β-cells and (ii) replication capacity of existing β-cells (Swenne, 1992). The relative contribution of replication, neogenesis or increased β-cell size to the increased β-cell mass is not very clear at this time. The ability of the pancreas to regenerate and the effects of trophic hormones on regeneration of the pancreas after partial pancreatectomy are not completely understood. There is strong evidence to the existence of neogenesis as a plausible mechanism for changes in β-cell mass based on studies in rat models (Swenne, 1982; Swenne & Eriksson, 1982). In contrast, changes in size of individual β-cells is not very well documented, even though, glucose, which is the primary stimulator of β-cell replication, increases β-cell size and apparently leads to increased insulin synthesis (Hakan Borg et al., 1981). Several studies pioneered by Hellerström and Bonner-Weir have lead to an improved understanding of mechanisms associated with β-cell proliferation (Bonner-Weir 1994; Hellerstrom 1984). Swenne performed the initial cell cycle characterization of β-cells and paved the way for further investigations into the replication capacity of β-cells. Islet cell replication has been determined by standard thymidine incorporation assays and more recently using antibody-based bromodeoxyuridine assays.

Upon receiving stimulatory influences from either cytokines or growth factors, mammalian cells undergo a regulated cell cycle progression. Every phase of the cell cycle is under regulatory influences of different cell cycle proteins. Changes in cell cycle progression modulate the rate of proliferation and growth. Moreover, the decision made by a cell to exit the cell cycle to undergo an irreversible post-mitotic differentiation state or a state of irreversible cellular senescence is dictated by changes in the cell cycle. Finally, the decision of putting an end to the cellular lifespan by undergoing apoptosis is also a reflection of decisions made by proteins regulating the cell cycle machinery (Sherr, 1996; Grana et al., 1995). The cell cycle
is typically divided into the following phases, G0 (reversible quiescence), G1 (first gap phase), S (DNA synthesis), G2 (second gap phase) and M (mitosis).

Pancreatic β-cells, similar to other cell types, pass through several distinct phases of the cell cycle. Studies pioneered by Ingemar, Swenne, Claes and Hellerström have elucidated the replication capacity of β-cells. Swenne maintained β-cell enriched fetal rat pancreatic islets in tissue culture at various glucose concentrations (Swenne, 1982). The observations prompted two inferences, (a) glucose stimulated β-cell proliferation by increasing the number of cells entering the cell cycle and (b) only a limited fraction of the total β-cell population is capable of entering the active cell cycle. Furthermore, these studies allowed an estimation of the rate of new β-cell formation per 24 hrs, which indicated that 4.2% new β-cells were formed in the presence of 2.7 mM glucose, whereas, 10.4% new β-cells were formed in the presence of 16.7 mM glucose. Furthermore, an age-dependent study of cell cycle progression of β-cells isolated from fetal, 1-week, 3-week and 3-month old rats revealed that the cell cycle was similar in all age groups (Swenne, 1983).

The growth of β-cells is determined by the number of β-cells entering the cell cycle rather than changes in the rate of the cycle. The β-cell passes through the cell cycle at a relatively high rate but the fraction of proliferating cells is low. During fetal life, the β-cell exhibits a poor insulin response to glucose. In late fetal life, glucose is a strong stimulus to β-cell replication and the metabolism of glucose is a pre-requisite for this process. Glucose stimulates proliferation by recruiting β-cells from a resting G0 state, into the proliferative compartment composed of cells in an active cell cycle. The drastic reduction of β-cell proliferation with increasing age is, most likely, due to a gradual withdrawal of cells from the active cell cycle into an irreversible G0 state. However, the observations that a very small fraction of β-cells are capable of entering the cell cycle argues that β-cells have replication potential.
This fraction can be potentially increased by recruitment of β-cells, which are in the quiescent G0 phase to re-enter the cell cycle and undergo replication.

Brelje et al (1994) studied the regulation of islet β-cell proliferation in response to prolactin (PRL). Insulin secretion and β-cell proliferation increased significantly in neonatal rat islets in response to prolactin. Initial PRL mitogenic stimulus occurred by a limited procurement of non-dividing β-cells into the cell cycle followed by majority of the daughter cells proceeding directly into additional cell division cycles. The maximal PRL stimulatory affect was maintained by a continued high rate of recruitment of β-cells into the cell cycle with only about one-fourth of the daughter cells continuing to divide. This study suggested that instead of a limited pool of β-cells capable of cell division, β-cells are transiently entering the cell cycle and dividing infrequently in response to PRL, indicating that the majority of β-cells are not in an irreversible G0-phase. This observation partly contradicts the initial islet cell cycle studies and prompts a careful analysis of the cell cycle machinery active in β-cells.

The re-entry of resting β-cells into the active cell cycle requires the knowledge of proteins involved in regulation of cell cycle progression of β-cells. At this time, we have very little knowledge of the molecules which determine the cell cycle kinetics of β-cells. The low proliferative capacity of β-cells has also been proposed to result from a low expression of p34CDC2 Serine/threonine kinase and cyclin B1 which are necessary for normal progression of the cell cycle (Mares et al., 1993). Several other studies highlight the role of cell cycle proteins in controlling the replication capacity of β-cells. Expression of growth promoting genes such as SV40 large T antigen and the oncogenes v-src, myc and ras have been altered either in transgenic mice or in islet cells in culture. Transgenic mice with insulin promoter driven β-cell specific expression of SV40 large T antigen developed insulinomas (Hanahan, 1985). However, the observation that expression of large T antigen was
not sufficient to form β-cell tumors indicated that transformation of β-cells is a rare event requiring multiple co-operating mutations. β-cells derived from the SV40 large T antigen transgenic mice maintained elevated DNA synthetic rates compared with control islets in which the DNA synthesis gradually decreased with age (Teitelman et al., 1988). Beta cells from transgenic mice harbored elevated levels of p53 protein, which can bind to SV40 large T antigen. The interaction is thought to inactivate the anti-proliferative activity of p53 (Marshall, 1991).

Transfection of activated v-src oncogene, a cytoplasmic tyrosine kinase, into β-cells stimulated DNA synthesis and substrate phosphorylation (Welsh et al., 1988). Similarly, transfection of activated myc and ras oncogenes also led to increased rates of DNA synthesis (Welsh et al., 1988). DNA synthesis in β-cells was also stimulated by overexpression of growth factor receptors such as the platelet-derived growth factor receptor (PDGF-R) and fibroblast growth factor receptor (FGF-R). Growth factor mediated signal transduction pathways lead to changes in expression of cell cycle proteins, eventually, resulting in the increased proliferation effects. Identification of cell cycle modulators of β-cell proliferation will provide insights into the replication potential of fetal, young and adult islet cells.

FACTORS REGULATING β-CELL GROWTH

Development of pancreatic endocrine cells in the rat fetus reveals the presence of insulin-positive β-cells by gestational day 13 (Fujii, 1979). Measurement of changes in the alpha, β- and delta-endocrine cell population in post-natal rodent islets indicates a continuous increase of β-cell mass throughout post-natal life (McEvoy, 1981). Morphological quantification of endocrine cells in human fetal pancreas reveals the presence of insulin-positive β-cells by the eighth fetal week (Clark & Grant, 1983) with almost a 130-fold increase in β-cell mass between the 12th week in utero and the fifth post-natal month (Stefan et al., 1983). New
pancreatic exocrine and islet cells are formed by differentiation of pre-exist embryonic ductal cells, which is referred to as neogenesis, or by replication of cells. While neogenesis is the primary mode of increase in β-cell mass during gestation, after birth most of the β-cells are formed by replication.

Studies with rodent islets have been the basis of much of our information on factors influencing β-cell replication. Among the various factors, glucose is a primary regulator of β-cell replication and is known to stimulate replication in both fetal and adult rodent islets (Hellerstrom, 1984). In addition, glucose leads to an increased cell proliferative compartment (Swenne, 1982). Insulin and IGF-1 stimulate islet cell replication in neonatal rodent pancreatic cells in culture providing evidence that insulin itself can regulate the replication capacity of β-cells in an autocrine fashion (Rabinovitch et al., 1982).

This study prompted the examination of several other growth factors for their role in regulating β-cell replication (Hill et al., 1998). Thus, growth hormone (GH) prolactin and the related placental lactogen, IGF-1, IGF-2 and platelet-derived growth factor (PDGF) have been recognized as stimulators of β-cell replication (Brelje & Sorenson, 1991). Growth hormone has been reported to stimulate the in vitro replication of foetal, neonatal and adult rat β-cells. The stimulation of replication activity resulted in an increased insulin content and secretion where the effects of GH were mimicked by prolactin and its related peptide, placental lactogen.

Growth hormone elicits many of its actions by inducing local production of IGFs in target cells. Studies aimed at investigating a similar paracrine pathway operative in islet cells have yielded confusing results. GH, but not glucose, stimulated the release of IGF-1 from fetal and adult rat islets leading to mitogenesis which could be partially negated by addition of monoclonal antibodies to IGF (Swenne et al., 1987). The presence of high-affinity IGF-1 receptors on β-cells and the finding that exogenous IGF-1 stimulates β-cell replication (Van Schravendi
et al., 1987), supported a concept that GH mitogenic activities might be mediated, at least in part, by a paracrine regulation involving IGF-I. This theory has been challenged by several studies, which failed to demonstrate an intermediary role for IGF-I in mitogenic activities of GH in β-cells (Romanus et al., 1985). Romanus et al. failed to detect increased IGF-I secretion from islets after GH stimulation. Other factors which lead to a stimulation of β-cell replication include, amino acids (Swenne et al., 1980), lithium (Sjoholm et al., 1992), the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA) (Sjoholm, 1991a), nicotinamide (Sandler & Andersson, 1986), amniotic fluid (Dunger et al., 1990) and serum (Hellerstrom & Swenne, 1985).

Inhibitors of β-cell proliferation include transforming growth factor β (TGF-β), the cytokine interleukin 1-β (IL1-β), pancreastatin and the diazepam binding inhibitor, all of which inhibit fetal rodent β-cell proliferation. TGF-β inhibits glucose stimulated β-cell replication (Sjoholm & Hellerstrom, 1991b). IL1-β suppresses islet cell proliferation in adult mice and rats (Southern et al., 1990). However, the role of IL1-β in fetal islet cell proliferation is slightly complex with the first 24 hrs of stimulation leading to a suppression of β-cell proliferation followed by a potent mitogenic stimulus after 3 days of cytokine exposure. Sjoholm et al., (1991c) identified pancreastatin and diazepam-binding inhibitor (acyl-CoA binding protein) as inhibitors of β-cell replication. Both pancreastatin and diazepam-binding inhibitor are produced by islet cells (Chen et al., 1988) and inhibit insulin secretion and may function as inhibitors of β-cell replication in vivo.

NEUROTRANSMITTERS AS GROWTH SIGNALS

Neurotransmitters act as growth regulatory signals in primitive organisms, embryos and the developing nervous system. They exert these effects by activating receptors and signal transduction mechanisms similar to those used in
neurotransmission. Neurotransmitters and their receptors linked to second messengers mediate growth responses in neuronal and non-neuronal cells. Stimulation of proliferation is most often associated with activation of G-proteins negatively coupled to adenylate cyclase (Gi), or positively coupled to phospholipase C (Gq) or to pertussis toxin-sensitive pathways (Go, Gi) (Lauder, 1993).

Norepinephrine

Norepinephrine is reported to amplify the mitogenic signals of both EGF and HGF by acting through the $\alpha_1$ adrenergic receptors. 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 PH (Knopp et al., 1999). NE also enhances the mito-inhibitory effects of TGF-β1 on cultured hepatocytes isolated from the early stages of regeneration (Michalopouloue & DeFrancis, 1997). Prazosin, a specific antagonist of $\alpha_1$ adrenergic receptor, as well as sympathetic denervation greatly decreases DNA synthesis at 24 hrs after PH (Cruise et al., 1989). Addition of NE to hepatocytes stimulates $\text{Ca}^{2+}$ mobilisation or PI turnover and either or both of these processes were proposed to be involved in the mitogenicity of NE (Exton, 1981 & 1988). Hepatic neoplasm are characterised by an increase in $\alpha_2$- and $\beta$-adrenergic receptors and a concomitent decline in $\alpha_1$-receptors (Sanae, 1989). Studies from our lab have shown that $\alpha_1$ receptors expressed altered affinity in hypothalamus and brain stem of diabetic rats (Pius, 1996). $\alpha_1$-adrenergic receptors are inhibitory $\beta$-adrenergic receptors are stimulatory to islet DNA synthesis (Ani Das, 2000).

Gamma amino butyric acid

Gamma amino butyric acid (GABA) is the principal inhibitory neurotransmitter of the mammalian brain. GABA inhibits the growth of murine squamous cell carcinoma and HeLa cell lines (Boggust & Al-Nakib, 1986). Gliomas
with high proliferation rate lack the expression of functional GABA binding sites (Labrakakis et al., 1988). GABA also plays an important role in terminating the growth of rapidly developing tissues in utero (Gilon et al., 1987). Studies from our lab have shown that hypothalamic GABAergic system plays an important role in the neoplastic transformation of rat liver. GABA
A receptor agonist muscimol, dose dependently inhibited EGF induced DNA synthesis and enhanced the TGFβ1 mediated suppressed DNA synthesis in rat primary hepatocyte culture (Biju et al., 2001). Increased GABA
A receptor activity inhibits proliferation of HepG2, human hepatocyte carcinoma cell line. The inhibition is prolonged in the cell line co-transfected with GABA
A receptor β2 and γ1 subunit genes (Zhang et al., 2000).

Acetylcholine

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 et al., 1989). Acetylcholine esterase kinetic parameters in brain stem during pancreatic regeneration in pancreatectomised rats showed a decrease in the cholinergic activity (Renuka et al., 2004). Acetylcholine is reported to induce proliferation of rat astrocytes and human astrocytoma cells (Guzzetti et al., 1996). Muscarinic M1 and M3 receptors were up regulated in the pancreas at the time of pancreatic regeneration (Renuka, 2003).

SERO TONIN

Serotonin has been known for the last half century to influence vasoactivity and to participate in neurotransmission. More recently this compound has been recognized to cause proliferation of a variety of cells in culture, including those of vascular smooth muscle. Furthermore, the proliferative effect is synergistic with that of more conventional growth-producing polypeptides. A hypertrophic, as well as a proliferative response, has been shown to occur in some smooth muscle cells. There
is a synergistic effect of urotensin II with 5-HT on vascular smooth muscle proliferation (Watanabe et al., 2001).

5-HT is synthesised in situ from tryptophan through the actions of enzymes tryptophan hydroxylase and aromatic L-amino acid decarboxylase. Both dietary and endogenous 5-HT are rapidly metabolized and inactivated by monoamine oxidase and aldehyde dehydrogenase to the major metabolite, 5-hydroxyindoleacetic acid (5-HIAA). 5-HT is produced in and released from neurons that originate with discrete regions, or nuclei, in the brain (Cooper et al., 1991). Many serotonergic neurons are located at the base of the brain in an area known as the raphe nucleus, which influences brain functions related to attention, emotion, and motivation. The axons of the neurons in the raphe nucleus extend, or project, throughout the brain to numerous regions with diverse functions. These brain regions include the amygdala, an area that plays an important role in the control of emotions, and the nucleus accumbens, a brain area involved in controlling the motivation to perform certain behaviors, including the abuse of alcohol and other drugs. In these brain regions, the axon endings of the serotonergic neurons secrete 5-HT when activated. The neurotransmitter then traverses the small space separating the neurons from each other (i.e., the synaptic cleft) and binds to specialized docking molecules (i.e., receptors) on the recipient cell. The binding of 5-HT to its receptors initiates a series of biochemical events that converts the extracellular, chemical signal into an intracellular signal in the recipient cell. 5-HT can influence mood states; thinking patterns; and even behaviors, such as alcohol drinking.

The actions of 5-HT are terminated by three major mechanisms: diffusion, metabolism; and uptake back into the synaptic cleft through the actions of specific amine membrane transporter systems.
CLASSIFICATION OF 5-HT RECEPTORS

The various effects of 5-HT on the central nervous system and peripheral organs are mediated through activation of multiple types of receptors (Hoyer & Martin, 1997). 5-HT receptors can be classified into seven classes from 5-HT\textsubscript{1} to 5-HT\textsubscript{7}, based upon their pharmacological profiles, cDNA-deduced primary sequences and signal transduction mechanisms of receptors (Bradley et al., 1986; Zifa & Fillion, 1992). All 5-HT receptors belong to the superfamily of G-protein coupled receptors containing a seven transmembrane domain structure except 5-HT\textsubscript{3} receptor, which forms a ligand-gated ion channel.

5-HT\textsubscript{1} Receptor

At least five 5-HT\textsubscript{1} receptor subtypes have been recognised, 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B}, 5-HT\textsubscript{1D}, 5-HT\textsubscript{1E} and 5-HT\textsubscript{1F}. All are seven transmembrane, G-protein coupled receptors (via Gi or Go), encoded by intronless genes, between 365 and 422 amino acids with an overall sequence homology of 40%. 5-HT\textsubscript{1A} receptor subtype, which is located on human chromosome 5cenq11, is widely distributed in the CNS, particularly hippocampus (Hoyer et al., 1994). The 5-HT\textsubscript{1B} receptor is located on human chromosome 6q13 and is concentrated in the basal ganglia, striatum and frontal cortex. The receptor is negatively coupled to adenylyl cyclase. The 5-HT\textsubscript{1D} receptor has 63% overall structural homology to 5-HT\textsubscript{1B} receptor and 77% amino acid sequence homology in the seven transmembrane domains. The receptor is located on human gene 1p36.3-p34.3 and is negatively linked to adenylyl cyclase. 5HT\textsubscript{1D} receptor mRNA is found in the rat brain, predominantly in the caudate putamen, nucleus accumbens, hippocampus, cortex, dorsal raphe and locus coeruleus (Hoyer et al., 1994). The 5-HT\textsubscript{1E} receptor was first characterised in man as a \textsuperscript{[\textit{3}H]}5-HT binding site in the presence of 5-carboxamidotryptamine (5-CT) to block binding to the 5-HT\textsubscript{1A} and 5-HT\textsubscript{1D} receptors. Human brain binding studies have reported that 5-HT\textsubscript{1E} receptors are concentrated in the caudate putamen with lower levels in the amygdala,
frontal cortex and globus pallidus (Hoyer et al., 1994). This is consistent with the observed distribution of 5-HT

mRNA (Hoyer et al., 1994). The receptor has been mapped to human chromosome 6q14-q15, is negatively linked to adenylyl cyclase and consists of a 365 amino acid protein with seven transmembrane domains. 5-HT

receptor subtype is most closely related to the 5-HT

receptor with 70% sequence homology across the 7 transmembrane domains. mRNA coding for the receptor is concentrated in the dorsal raphe, hippocampus and cortex of the rat and also in the striatum, thalamus and hypothalamus of the mouse (Hoyer et al., 1994). The receptor is negatively linked to adenylyl cyclase.

5-HT2 Receptor

The 5-HT2 receptor family consists of three subtypes namely 5-HT2A, 5-HT2B and 5-HT2C. 5-HT2C was previously termed as 5-HT1C before its structural similarity to the 5-HT2 family members was recognised. All three are single protein molecules of 458-471 amino acids with an overall homology of approximately 50% rising to between 70-80% in the seven transmembrane domains. All three are thought to be linked to the phosphoinositol hydrolysis signal transduction system via the \( \alpha \) subunit of Gq protein. In human pulmonary artery endothelial cells, 5-HT2C receptor stimulation causes intracellular calcium release via a mechanism independent of phosphatidylinositol hydrolysis (Hagan et al., 1995). 5-HT2A receptor previously termed as 5HT2 receptor is located on human chromosome 13q14-q21 and is widely distributed in peripheral tissues. It mediates contractile responses of vascular, urinary, gastrointestinal and uterine smooth muscle preparations, platelet aggregation and increased capillary permeability in both rodent and human tissue (Hoyer et al., 1994). The 5-HT2B receptor located on chromosome 2q36-2q37.1 mediates contraction of the rat stomach fundus and endothelium dependent relaxation of the rat and cat jugular veins and possibly of the pig pulmonary artery, via nitric oxide release (Choi & Maroteaux, 1996). 5-HT2B receptor mRNA has been detected
Throughout the mouse, rat and guinea pig colon and small intestine. 5-HT$_2C$ specific antibodies have recently used to show the presence of the receptor protein in the choroid plexus (highest density), and at a lower level in the cerebral cortex, hippocampus, striatum, and substantia nigra of rat and a similar distribution in man. The receptor has been mapped to human chromosome Xq24. No splice variants have been reported but the receptor is capable of post translational modification whereby adenosine residues can be represented as guanosine in the second loop to yield 4 variants.

**5-HT$_3$ Receptor**

The 5-HT$_3$ receptor binding site is widely distributed both centrally and peripherally and has been detected in a number of neuronally derived cells. The highest densities are found in the area postrema, nucleus tractus solitarius, substantia gelatinosa and nuclei of the lower brainstem. It is also found in higher brain areas such as the cortex, hippocampus, amygdala and medial habenula, but at lower densities. Unlike other 5-HT receptors, 5-HT$_3$ receptor subunits form a pentameric cation channel that is selectively permeable to Na$^+$, K$^+$ and Ca$^{++}$ ions causing depolarisation. The 5-HT$_3$ receptor is a member of a superfamily of ligand-gated ion channels, which includes the muscle and neuronal nicotinic acetylcholine receptor (AchR), the glycine receptor, and the γ-aminobutyric acid type A receptor (Karlin & Akabas, 1995; Ortells & Lunt, 1995). Like the other members of this gene superfamily, the 5HT$_3$ receptor exhibits a large degree of sequence similarity and thus presumably structural homology with the AchR (Maricq et al., 1991).

**5-HT$_4$ Receptor**

Receptor binding studies have established that the 5-HT$_4$ receptor is highly concentrated in areas of the rat brain associated with dopamine function such as the striatum, basal ganglia and nucleus accumbens. These receptors are also located on
GABAergic or cholinergic interneurons and/or on GABAergic projections to substantia nigra (Patel et al., 1995). The receptor is functionally coupled to the protein.

**5-HT₅ Receptor**

Two 5-HT receptors identified from rat cDNA and cloned were found to have 88% overall sequence homology, yet were not closely related to any other 5-HT receptor family (Erlander et al., 1993). These receptors have thus been classified 5-HT₅₅ and 5-HT₅₆ and their mRNAs have been located in man (Grailhe et al., 1994). In cells expressing the cloned rat 5-HT₅₅ site, the receptor was negatively linked to adenylyl cyclase and may act as terminal autoreceptors in the mouse frontal cortex (Wisden et al., 1993).

**5-HT₆ Receptor**

Like the 5-HT₅ receptor, the 5-HT₆ receptor has been cloned from rat cDNA based on its homology to previously cloned G protein coupled receptors. The receptor consists of 438 amino acids with seven transmembrane domains and is positively coupled to adenylyl cyclase via the Gₛ G protein. The human gene has been cloned and has 89% sequence homology with its rat equivalent and is coupled to adenylyl cyclase (Kohen et al., 1996). Rat and human 5-HT₆ mRNA is located in the striatum, amygdala, nucleus accumbens, hippocampus, cortex and olfactory tubercle, but has not been found in peripheral organs studied (Kohen et al., 1996).

**5-HT₇ Receptor**

5-HT₇ receptor has been cloned from rat, mouse, guinea pig and human cDNA and is located on human chromosome 10q23.3-q24.4. Despite a high degree of interspecies homology (95%) the receptor has low homology (<40%) with other
HT receptor subtypes. The human receptor has a sequence of 445 amino acids and appears to form a receptor with seven transmembrane domains.

5-HT AND CELL PROLIFERATION

Growth-regulatory effects of 5-HT mediated by specific 5-HT receptor subtypes have been linked to a number of signal transduction pathways that either promote or inhibit cell proliferation (Lauder, 1993). 5-Hydroxytryptamine has been implicated as a potential mitogen (Seuwen & Poussegur, 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). In pancreatic cell line, activation of pertussis toxin sensitive 5HT1A/B receptors stimulate proliferation through the activation of PLC and PKC that resulted in the down regulation of cAMP (Ishizuka et al., 1992). 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). Studies from our lab reported the involvement of 5-HT, S2 receptors in the DNA synthesis of primary culture of rat hepatocytes (Sudha et al., 1997). 5HT1A receptor agonist 8-OHDPAT inhibited the DNA synthesis in rat hepatocytes in vitro. Studies using mesulergine, 5HT2C antagonist revealed that 5HT2C receptors are stimulatory to hepatocyte cell division (Pyroja, 2002).

5-HT1A Receptor and Cell Proliferation

Signal transduction cascades following the activation of 5-HT1A receptors were found to involve activation of the adenylyl cyclase/PKA pathway (Lambert
et al., 2001). Treatment of cells with either the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, or forskolin (which directly activates adenylyl cyclase) increased cellular IGF-I. These treatments increased cAMP synthesis and caused phosphorylation of the cAMP response element binding protein, CREB, an effect that was blocked by a PKA inhibitor, Rp-cAMPS. Rp-cAMPS also blocked the increases in IGF-I caused by 8-OH-DPAT or forskolin, consistent with the presence of a cAMP response element (CRE) in the IGF-I promoter (Thomas et al., 1996). Taken together, these findings suggest that the activation of 5-HT receptors positively coupled to the adenylyl cyclase/PKA pathway directly promote transcription of IGF-I, an important growth factor in craniofacial development.

5-HT$_{1A}$ is a "transiently expressed" intronless receptor, i.e., at specific times in development or during stress, very high amounts are expressed quickly. 5-HT$_{1A}$ receptor develops early and the receptor levels can then be reduced as the cells or animal ages. The decrease in receptor number is probably due to increased 5-HT brain levels, since the 5-HT$_{1A}$ receptor expression is sensitive to autoinhibition (Nishi & Azmitia, 1999). The transduction action of the 5-HT$_{1A}$ receptor is usually associated with a decrease in adenylyl cyclase activity. In cultures of hippocampal neurons, 5-HT$_{1A}$ agonists block the forskolin-induced formation of p-CREB, an important transcription factor increased by cAMP (Nishi & Azmitia, 1999). In adult neurons, the 5-HT$_{1A}$ receptor also is associated with a hyperpolarisation of the membrane potential, attributed to opening a K$^+$ current (Baskys et al., 1989). The 5-HT$_{1A}$ receptor uses these cellular mechanisms to differentiate its target cells. The 5-HT$_{1A}$ receptor is found on serotonergic neurons and nonserotonergic neurons (Hamon, 1997). In the presence of a phosphodiesterase inhibitor 5-HT elevates bovine smooth muscle cellular cAMP and this elevation correlates with an inhibition of cellular proliferation (Assender et al., 1992). Similarly other agents such as forskolin, histamine, isoproterenol and cholera toxin, which elevate cellular cAMP,
inhibit the proliferation. This activity of 5-HT is mimicked by 8-OH DPAT an 
reputed, 5-HT₁₅ agonist (Fanburg & Lee, 1997). 5-HT inhibits cellular growth of 
pulmonary artery smooth muscle cells (SMC) through its action on 5-HT₁₅ or 5-HT₄ 
receptors (Lee et al., 1997).

The involvement of the 5-HT₁₅ receptor in cell proliferation is assumed to be 
inhibitory given its stimulatory effects on cell differentiation (Lauder et al., 1983). 
However, some studies indicate a direct and indirect role for 5-HT₁₅ receptors in cell 
proliferation. 5-HT₁₅ agonists given in culture accelerate cell division, generate cell 
foci, and increase DNA synthesis in transfected NIH-3T3 cells (Varrault et al., 1992). 
The early studies of 5-HT and cell proliferation in culture appear to argue that 5-HT 
may be important for cell differentiation and the inhibition of cell division in the 
CNS. The 5-HT₁₅ receptor is uniquely positioned during the early development of 
the brain to influence neuronal mitosis, in the maturation and the assembly of the 
spindle apparatus in the cell body which promotes cell division. In the 5-HT cell 
line, RN46A, the 5-HT₁₅ receptor is 20-fold higher in the undifferentiated cell than in 
the differentiated cell. It is suggested that the cell body of 5-HT₁₅ receptors may 
mediate autoregulation of serotonergic neuron development (Eaton et al., 1995).

The 5-HT₁₅ receptors in the adult brain have clearly been shown to be 
involved in maintaining the mature state of neurons in the mammalian brain 
(Azmitia, 1999). Liu and Albert (1991) have demonstrated with transfection of the 
rat 5HT₁₅ receptor into a variety of cells that the receptor, acting through pertussis 
toxin (PTX) sensitive G proteins, can change its inhibitory signalled phenotype into a 
stimulatory one, depending on cell type, differentiation and culture medium.

5-HT₁₅ receptor is prominently expressed in neuronal cells (e.g. hippocampal 
CA-1, dorsal raphe nuclei) where it also opens potassium channels via activation of a 
PTX-sensitive G protein (Colino & Halliwell, 1987) and closes calcium channels 
(Penington & Kelly, 1990 & Ropert, 1988). This results in hyperpolarisation of the
membrane potential, closing of voltage-dependent calcium channels, and decrease \( \text{Ca}^{2+} \). The expression of the rat 5-HT\(_{1A}\) receptor in pituitary GH4C1 cells (GH4ZD cells) resulted in a 5-HT-induced inhibition of \( \text{Ca}^{2+} \); and cAMP accumulation similar to that observed in neurons (Liu et al., 1991). Thus, 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).

The cells exposed to apoptotic-inducing conditions may actually up-regulate 5-HT\(_{1A}\) receptors. Neuronal cell lines stably transfected with a promoter-less segment (G-21) of the human 5-HT\(_{1A}\) receptor gene (Singh et al., 1996) show a 10- to 15-fold increase in the receptor when deprived of nutrient. 5-HT\(_2\) receptor drugs are not effective in these models of apoptosis. Conversely, reduced 5-HT levels in the hippocampus potentiate ischemic-induced neuronal damage (Nakata et al., 1997).

**5-HT\(_{2C}\) Receptor and Cell Proliferation**

5-HT\(_{2C}\) receptors [formerly termed 5-HT\(_{1C}\)] are widely expressed in the brain and spinal cord, are particularly enriched in the choroid plexus, and appear to mediate many important effects of 5-HT (Blier et al., 1990). Previous studies have shown that 5-HT\(_{2C}\) receptor undergoes RNA editing with the potential for producing 14 different receptor isoforms (Niswender et al., 1998; Burns et al., 1997). The rat 5-HT\(_{2C}\) receptor is one of the three 5-HT\(_2\) subtype receptors linked to phospholipase C via G-protein coupling and is regulated by RNA editing (Burns et al., 1997). Parr et al. (1991) and Pakala et al. (1994) proposed a 5-HT\(_2\) receptor to be responsible for 5-HT-induced proliferation of porcine smooth aortic muscle cells and canine aortic endothelial cells. Similarly, Pitt et al. (1994) and Corson et al. (1994) suggested that a 5-HT\(_2\) receptor is responsible for proliferation of rat vascular smooth muscle cells caused by 5-HT through an increase in intracellular \( \text{Ca}^{2+} \). Crowley et al. (1994) also concluded that stimulation of proliferation of bovine aortic smooth muscle cells by 5-HT occurs through a 5-HT\(_2\) receptor.
5-HT$_2$ receptor can be referred to as a programmable receptor i.e., events during development may affect the number, affinity, or function of these receptors in the adult brain (Meaney et al., 1994). For example, both prenatal and postnatal stress to the mother significantly increases the number of 5-HT$_2$ receptors in the offspring, which leads to activation of protein kinase C (PKC) and the activation of several important transcription factors including c-Fos, Jak, and STAT. 5-HT stimulates the turnover of phosphoinositide in primary cultures of astroglia from the cerebral cortex, striatum, hippocampus, and brain stem. 5-HT$_2$ receptors in glioma cells appear to regulate proliferation, migration, and invasion. 5-HT was found to positively modulate these three processes in vitro (Merzak et al., 1996).

5-HT$_2$ receptor antagonists, ketanserin (10$^{-4}$M) and spiperone (10$^{-6}$M), blocked stimulation of DNA synthesis by 5-HT. Displacement studies on $[^3]$H5-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 of 5-HT in the regenerating liver during the DNA-synthetic phase. 5-HT enhanced the phosphorylation of a 40-kd substrate protein of protein kinase C (PKC) in the regenerating liver during the DNA synthetic phase of the hepatocyte cell cycle. This was blocked by ketanserin, indicating that 5-HT$_2$ receptor activates PKC, an important second messenger in cell growth and division, during rat liver regeneration. 5-HT can act as a potent hepatocyte co-mitogen and induce DNA synthesis in primary cultures of rat hepatocytes, which is suggested to be mediated through the 5-HT$_2$ receptors of hepatocytes (Sudha & Paulose, 1997).

The predominant 5-HT$_2$ receptor in the neonatal period is the 5-HT$_{2C}$ receptor (Ike et al., 1995). The 5-HT$_{2C}$ receptors activate phospholipase C (Conn et al., 1986), whereas 5-HT$_{1A}$ receptors modulate adenylyl cyclase activity (Siegelbaum et al., 1982). In neurons that express the 5-HT$_{1C}$ receptor activation by 5-HT is likely to generate inositol polyphosphates that release intracellular Ca$^{2+}$ (Conn et al., 1986).
NIH-3T3 cells that express high levels of 5-HT\textsubscript{2C} receptor form foci in cell culture. Moreover the formation of foci is dependent on activation of the 5-HT\textsubscript{1C} receptor by 5-HT. In addition the introduction of transformed foci into nude mice results in the rapid appearance of tumours. In fibroblasts this receptor alters the growth properties of cells and results in malignant transformation (Julius et al., 1989).

In the present study the role of brain and pancreatic 5-HT\textsubscript{1A} and 5-HT\textsubscript{2C} receptors on regulation of pancreatic \(\beta\)-cell proliferation and insulin release during pancreatic regeneration have been investigated using rat models. \textit{In vitro} studies were conducted to confirm the involvement of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2C} receptors in the regulation of pancreatic \(\beta\)-cell proliferation using specific ligands in primary cultures.