Literature Review

Diabetes mellitus is one of the diseases familiar since the ancient times, ‘Ayurveda’ the 3000-5000 year old traditional system of Indian herbal medicine describes it as ‘Meha’ or ‘Madhumeha’ meaning ‘Honey urine’ (Shashtri & Chaturvedi, 1977) and one among Ashtamaharogaa: (the eight major diseases as described in Ashtangahrihaya, the Ayurvedic text written in Sanskrit). In diabetic state the body does not produce or properly use insulin. Type 1 diabetes results from the body's failure to produce insulin, which is allowing glucose to enter and fuel the cells. It is caused by autoimmune destruction of pancreatic islet β-cells. The presence of healthy β-cells mass in the pancreas is an important factor in maintaining the body homeostasis.

Pancreas

Pancreas is a complex organ consisting of both endocrine and exocrine cells. Approximately 5 percent of the total pancreatic mass is comprised of endocrine cells. These endocrine cells are clustered in groups within the pancreas, which look like little islands of cells when examined under a microscope. This appearance led to these groups of pancreatic endocrine cells being called "pancreatic islets". Within pancreatic islets are cells, which make specific pancreatic endocrine hormones, of which there are only a few, the most famous of course being insulin. Pancreatic islets are scattered throughout the pancreas. Like all endocrine glands, they secrete their hormones into the bloodstream and not into tubes or ducts like the digestive pancreas. Because of this need to secrete their hormones into the blood stream, pancreatic islets are surrounded by small blood vessels. 65-80% of the islets are insulin- secreting β-cells.
The destruction of β-cell mass will lead to impaired insulin secretion and thereby hyperglycemia. Management of diabetes is burdensome both to the individual and society, costing over 100 billion US dollars annually. Transplantation of the pancreatic β-cells to the patient body is suggested as one of the treatment methods. Shortage of pancreatic tissue, together with a lifetime requirement of immunosuppressive drugs to prevent rejection and recurrent disease, remain as major hurdles yet to be overcome prior to widespread applicability. In this context newer techniques such as use of stem cells and regeneration of the remaining healthy β-islet cells have been proposed more interesting alternatives in diabetic therapy. Development of stem cells into potential pancreatic β-cells and the regeneration of existing islets by down-regulation of autoimmunity were recommended for future research to cure this ailment (Ramiya et al., 2004). Indeed, islet-regeneration research will soon match the level of interest.

Age related changes in the capacity of β-cell for proliferation affect the insulin production and contribute to a decrease in glucose tolerance with advance in age (Hellerstrom, 1984). Cell cycle analysis of rat islets maintained in tissue culture indicates that proliferating β-cells proceed through the cell cycle at similar rates irrespective of the postnatal age (Swenne, 1983). The sensitivity to glucose in terms of DNA synthesis by the β-cells is also similar, but the proliferative capacity seems to be restricted by a decreasing number of cells capable of entering the cycle. The decrease in the capacity to proliferate with age may signify a gradual withdrawal of cells from the active cell cycle into an irreversible G0 state. Therefore, the capacity of β-cells to respond with proliferation to diabetogenic stimulus decreases with age (Hellerstrom, 1984).

Light and electron microscopic studies have demonstrated that there are three different types of nerve endings in the pancreas: sympathetic, parasympathetic and peptidergic nerves (Miller, 1981). The neurotransmitters found in the first two nerve
terminals are catecholamines and acetylcholine. The peptidergic nerve terminals contain various peptides as neurotransmitters. The nerve fibres enter the pancreas in association with the vascular supply and they are distributed to blood vessels, acinar tissue and islets. Adrenergic fibres innervate vessels, acini and islets. Cholinergic fibres are found mainly in islets. Peptidergic nerves are found in both exocrine and endocrine tissue (Ahren et al., 1986).

Partial pancreatectomy and pancreatic regeneration

The natural source for new pancreatic β-cells is an important issue both for understanding the pathogenesis of diabetes, and for possibly curing diabetes by increasing the number of β-cells. Transplantation of pancreatic islets can now be applied successfully to treat diabetes, but its widespread use is hampered by a shortage of donor organs. Since insulin-producing β-cells cannot be expanded significantly in vitro, efforts are under way to identify stem or progenitor cells that potentially could be grown and differentiated into β-cells in vitro. Such cells could provide an ample supply of transplantable tissue. Current research in this field focuses mainly on pluripotential embryonic stem cells and on pancreas-specific adult progenitor cells. β-cell replication is the only source for new β-cells without contributions from stem cells or other non-β-cells. The pancreatic gland has an enormous potential for growth and regeneration, mainly in rodents. Animal models of pancreatic regeneration can be easily established in weanling rats.

The pancreatic gland shows a tendency for growth and regeneration, mainly in rodents. The mammalian pancreas has a strong regenerative potential, but the origin of organ restoration is not clear, and it is not known to what degree such a process reflects pancreatic development (Jensen et al., 2005). The human pancreas however does not show proliferative properties after partial pancreatectomy, but research in this field has been scarce (Morisset, 2003).
Streptozotocin (STZ)-induced diabetic mice can be cured by injection of the regenerating pancreatic extract (RPE) of the partially pancreatectomized Wistar-Kyoto rats (Shin et al., 2005). Pancreatitis-associated protein (PAP) and regenerating protein 1a (Reg1a) are up-regulated during the pancreas regeneration. Transplantation of pancreas has beneficial effects on impaired islet, inducing regeneration in the spontaneously diabetic Torii rat (25-week-old) (Miao et al., 2005). Pancreatic regeneration following chemically induced pancreatitis in the mouse occurs predominantly through acinar cell dedifferentiation, whereby a genetic program resembling embryonic pancreatic precursors is reinstated (Jensen et al., 2005).

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 an 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 Ca²⁺ channels. The rise in the cytoplasmic free Ca²⁺ 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 Ca²⁺ activates protein kinases such as Ca²⁺ and calmodulin dependent
protein kinase (Breen & Aschroft, 1997), it remains unclear how increases in intracellular Ca\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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 (Wookey & Cooper, 2001).
**Adrenomedullin**

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

**Galanin**

Galanin is a 29 amino acid neuropeptide localised in the intrinsic nervous system of the entire gastrointestinal tract and the pancreas of man and several animal species (Scheurink et al., 1992). Among other functions galanin inhibits insulin release (Ahren et al., 1991), probably via activation of G-proteins (Renstrom et al., 1996) by the mediation of activated galanin receptors. However, galanin receptors are not as effective as α2-adrrenergic receptors in activating G-proteins.

**Macrophage migration inhibitory factor (MIF)**

MIF, originally identified as cytokines, is 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 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., 1966). EPI and NE 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 phospho-fructokinase. 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 & Randle (1964). As judged by Malaisse et al., (1967), the inhibitory effect of EPI on glucose-induced insulin secretion is mediated through the activation of α-adrenoreceptors.

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).

**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 D3 receptors are implicated in the control of blood glucose levels (Alster et al., 1996).
Dopamine D₁ 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.

**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 content of 5-HIAA and accelerated the cerebral 5-HT turnover (Juszkiewicz, 1985). The 5-HIAA content was reported to be approximately twice as high as the controls regardless of duration of treatment. Brain tryptophan, the precursor of 5-HT, was also reduced in brain regions during diabetes (Jammicky et al., 1991). Insulin treatment was reported to reverse this reduced tryptophan content to normal (Jammicky et al., 1993). Studies suggest that the brain 5-HT through 5-HT₁₅ receptor has a functional role in the pancreatic regeneration through the sympathetic regulation (Mohanan et al., 2005).
**γ-Aminobutyric acid**

Gamma aminobutyric acid 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 (GAD), 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.

**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 (Bonner-Weir et al., 1993; Waguri
et al., 1997). 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., 1995). 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 β-cells is 1 to 4% per day (Finegood et al., 1995; Bonner-Weir et al., 2000).
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, 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 islets (Bonner-Weir, 2000). 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 β-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 β-CELL GROWTH

β-cell growth is a cumulative effect of the following three phenomena during β-cell development (i) differentiation of β-cells from precursors, a process referred to as neogenesis (ii) changes in the size of individual β-cells and (iii) 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 prime 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 Hellerstrom and Bonner-Weir have lead to an improved understanding of mechanisms associated with β-cell proliferation (Hellerstrom, 1984; Bonner-Weir 1994). Swenne performed the initial cell cycle characterization of β-cells and paved the way for further investigations into the replication capacity of β-cells. Standard thymidine incorporation assays and more recently using antibody-based bromodeoxyuridine assays have determined islet cell replication.

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 (Grana et al., 1995; Sherr, 1996). 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 elucidated the replication capacity of β-cells (Swenne, 1982; Hellerstrom, 1984). 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. 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 G_0 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 G_0 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 G_0 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 molecular mechanisms that 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 that 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). β-cells from transgenic mice harbored elevated levels of p53 protein, which can bind to SV40 large T antigen. The interaction is thought to inactive 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. 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 over
expression 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 α, β and δ- 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-existing embryonic ductal cells, which is referred to as neogenesis, or by replication of β-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 of factors influencing β-cell replication. Among the various factors, glucose is a prime 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 β-cell proliferative compartment (Swenne, 1982). Insulin and IGF-1 stimulate islet β-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 fetal, 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-1 (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 Schravendijk et al., 1987), supported a concept that GH mitogenic activities might be mediated, at least in part, by a paracrine regulation involving IGF-1. This theory has been challenged by several studies, which failed to demonstrate an intermediary role for IGF-1 in mitogenic activities of GH in β-cells (Romanus et al., 1985). They failed to detect increased IGF-1 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 Gα, or positively coupled to phospholipase C (Gq) or to pertussis toxin–sensitive pathways (Gα, Gi) (Lauder, 1993).

Norepinephrine

Norepinephrine is reported to amplify the mitogenic signals of both EGF and HGF by acting through the α1 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 (Michalopoulos & DeFrancis, 1997). Prazosin, a specific antagonist of α1 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 Ca²⁺ 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 α2- and β-adrenergic receptors and a concomitant decline in α1-receptors (Sanac, 1989). Studies from our lab have shown that α1-receptors expressed altered affinity in hypothalamus and brain stem of diabetic rats (Pius, 1996). α1-adrenergic receptors are inhibitory β-adrenergic receptors are stimulatory to islet DNA synthesis (Ani Das, 2000)

**Gamma amino butyric acid**

Gamma amino butyric acid 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. GABAₐ 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., 2002a). Increased GABAₐ receptor activity inhibits proliferation of HepG2, human hepatocyte carcinoma cell line. The inhibition is prolonged in the cell line co-
transfected with GABA<sub>A</sub> receptor β<sub>2</sub> and γ<sub>2</sub> subunit genes (Zhang et al., 2000). During the regeneration of liver, GABA<sub>A</sub> receptor acts as an inhibitory signal for hepatic cell proliferation (Biju et al., 2001b).

**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 pancratectomised 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).

**Serotonin**

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 cell proliferation (Watanabe et al., 2001). Serotonin is supposed to act as a potent hepatocyte comitogen and induce DNA synthesis in primary cultures of rat hepatocytes, which is suggested to mediate through the serotonin S<sub>2</sub> receptors of hepatocytes (Balasubrahmanian & Paulose, 1998). During the regeneration
of pancreas 5-HT1A and 5-HT2C receptors get downregulated in brain stem and cerebral cortex and brain stem, which the changes are mediated through the sympathetic system (Mohanan et al., 2005a, b).

**GABA**

GABA is an inhibitory neurotransmitter present in large quantities the inhibitory neurons of the central nervous system (Csillag, 1991). GABA is formed by the decarboxylation of L-glutamic acid by glutamate decarboxylase (GAD). All interneurons are GABAergic neurons and they comprise a great number in the body. In the central nervous system, GABA acts at two distinct types of receptors, ligand-gated ionotropic GABA$_A$ receptors and GABA$_C$ receptors and G-protein linked metabotropic GABA$_B$ receptors, thus mediating both fast and slow inhibition of excitability at central synapses (Vicini, 1999; Marshall et al., 1999). GABA$_A$ receptor is a ligand-gated chloride ion channel playing an important part in polarizing the cell membrane and reducing neuronal excitability in the neuron. GABA$_A$ and GABA$_B$ receptors are abundant but GABA$_C$ receptors are very much localized in areas such as retina etc.

**GABA$_A$ receptors**

In developing neurons by contrast, GABA, acting at the GABA$_A$ receptor, can be excitatory. As an excitatory transmitter in the developing brain, GABA can depolarize the membrane potential and increase the frequency of action potentials (Chen et al., 1996; Owens et al., 1996). Functional GABA$_A$ receptors are expressed at the earliest development time studied, embryonic day 15$^{th}$ in rats (Chen et al., 1995, van den Pol et al., 1995). GABA immunoreactivity is found in axonal growth cones (van den Pol, 1997).
**GABA** B receptors

GABA**B** receptors are expressed both presynaptically (Howe *et al.*, 1987; Dutar & Nicoll 1988; Yoon & Rothman 1991) and postsynaptically (Dutar & Nicoll 1989; Solis & Nicoll 1992). Presynaptic GABA**B** receptors have been shown to decrease neurotransmitter release by increasing K⁺ conductance or decreasing Ca**2⁺** conductance or through a mechanism independent of changes in membrane conductance (Misgeld *et al.*, 1995). Additionally, GABA**B** receptors have been shown to play a role as autoreceptors, providing a negative feedback control for synaptic GABA secretion (Anderson & Mitchell 1985; Pittaluga *et al.*, 1987). Multiple neurochemical studies at a variety of CNS preparations in which transmitter release has been monitored suggest that subtypes of GABA**B** receptors are present on various nerve terminals. Molecular biological cloning of GABA**B** receptors has provided additional support to the concept of multiple GABA**B** receptors. The initial cloning of a GABA**B** receptor that demonstrated the presence of two splice variants (GABA**B**R1a and GABA**B**R1b). This as recently been expanded by the demonstration of a second and different GABA**B** clone: GABA**B**R2 (Kaupman *et al.*, 1997). A major function of GABA**B** receptors is to modulate transmitter release (Bowery *et al.*, 1980; Alford & Grillner 1991; Davies *et al.*, 1991; Thompson *et al.*, 1993; Kombian *et al.*, 1996; Mouginot & Gähwiler 1996).

During this early stage of development, activation of the GABA**A** receptor usually leads to depolarization and the resultant opening of plasmalemmal Ca**2⁺** channels that raise intracellular Ca**2⁺** (Obrietan & van den Pol, 1995). In contrast, activation of the GABA**B** receptor tends to reduce the GABA-mediated elevations in Ca**2⁺**, at both presynaptic and postsynaptic sites of action. Thus GABA would generate two opposing actions, one at the GABA**A** receptor that initially depolarizes the cell, raising Ca**2⁺**, and a slightly later effect at the GABA**B** receptor that would reduce the Ca**2⁺** rise (Karl Obrietan *et al.*, 1998). Baclofen, the GABA**B** agonist,
dose-dependently induced EGF mediated DNA synthesis in primary hepatocyte cultures and it significantly reduced the TGF_β1 suppression of EGF induced DNA synthesis (Biju et al., 2002a).

**GABA in pancreas**

In addition to its presence in the central nervous system, GABA and GAD have been demonstrated in the pancreatic β-cells of normal rat (Garry et al., 1986). GABA is present in large number in the islet cells in the pancreas. The concentration of GABA in the endocrine pancreas is comparable to that measured in the in the central nervous system (Rorsman et al., 1989). In addition, the GABA metabolizing enzyme, GABA-transaminase (GABA-T), was localized in the pancreatic β-cells (Vincent et al., 1983). It is known that the β-cells can produce and release GABA in response to glucose (Okada et al., 1976; Gilon et al., 1991; Satin et al., 1998). It is possible that GABA and Glutamate mediate a paracrine signaling pathway whereby α and β-cells communicate within the islets (Rorsman et al., 1989; Satin et al., 1998; Skerry et al., 2001; Gill et al., 2001).

In the present study we examined the GABA receptor functional regulation and gene expression in an animal model of regulated cell proliferation *in vivo* to elucidate their role in pancreatic cell proliferation. *In vitro* studies were conducted to confirm the involvement of GABA_A and GABA_B receptors in the regulation of pancreatic β-cell proliferation using specific ligands in primary cultures.