2. LITERATURE REVIEW

Diabetes, a disease documented in ancient Greek and Hindu writings, is among the top-tent causes of deaths in the world (Rane & Reddy, 2000; Rotter et al., 1990; Ashcroft & Ashcroft, 1992). It is a disease that can arrive during the budding years (juvenile diabetes) or later (maturity or late-onset diabetes) in life. In either case, the life threatening complications associated with the disease remain the same. Despite being one of the oldest documented diseases, complete cure for the disease is still elusive which is primarily due to the lack of a complete understanding of the disease.

Diabetes results due to the inability of the body to effectively regulate the sugar balance leading to severe complications such as hyperglycemia (high blood glucose), obesity, neuropathy, nephropathy, retinopathy, limb disorders, bone disorders such as osteoporosis, coma and sometimes untimely death. The β-cells of the pancreas produce a protein, insulin, which monitors glucose level in the body. Normally, the extra-cellular concentration of glucose is restricted within a very narrow range, irrespective of variations in glucose availability and utilization. Homeostatic control of normal glucose level is achieved by co-ordinate secretion of insulin and glucagon. The basal rate of glucose utilization is approximately 10 grams per hour and to prevent hypoglycemia due to this utilization of glucose, the liver, the only source of endogenous glucose production, synthesizes glucose at a rate of 10 grams per hour. Approximately 75% of the hepatic glucose production is regulated by levels of glucagon, a product of pancreatic islet α-cells. Dysfunction of the α- and β-cells results in a disordered glucose homeostasis. If the β-cells do not respond to increased levels of glucose, hyperglycemia ensues where glucose levels exceed 10mM, a diagnostic feature of diabetes mellitus. Conversely, β-cell overactivity, observed in the case of insulinomas or β-cell tumors, leads to hypoglycemia with a possibility of brain cell injury and death (Rane & Reddy, 2000). In diabetic individuals, the regulation of glucose levels by insulin is defective, either due to defective insulin production (Type I diabetes) or due to insulin resistance (Type II diabetes).

2.1 Type 1 Diabetes or IDDM

Although, diabetes mellitus is defined simply on the basis of the ensuing hyperglycemia, it is a highly heterogeneous disease. The two forms of diabetes, IDDM
and NIDDM were distinguished in the late 1960s. This was followed by a realization that IDDM, presumably, had an autoimmune origin (Bach, 1994; Tisch & McDevitt, 1996). IDDM is a multifactorial disease with a polygenic inheritance. The genotype of the major histocompatibility complex (MHC) is the strongest genetic determinant. Several aspects of the etiology of IDDM, including the origin and pathogenesis, importance of genetic predisposition, interactions of environmental factors and characterization of the anti-β-cell immune response have been reviewed extensively (Ashcroft & Ashcroft, 1992; Bach, 1994; Tisch & McDevitt, 1996). Much of the current understanding of IDDM is based on studies using animal models, which serve as excellent tools for genetic and immunological manipulations that are impossible to carry out in human beings.

2.1.1 Experimentally induced models

Several experimental models have been described which provide clues to the etiology of IDDM. Streptozotocin (STZ) induced IDDM has been reported, wherein, the β-cell destruction is achieved by administration of high doses of selective β-cell toxic agent STZ (Like & Rossini, 1976; Kolb, 1987; Paik et al., 1980). Repeated doses of STZ at sub-diabetogenic doses result in insulitis followed by diabetes which is immunologically mediated. Also, insulitis and diabetes can be induced in normal non-autoimmune adult rats by a combination of thymectomy and sublethal irradiation or in athymic rats by transfer of normal spleen cells (Fowell & Mason, 1993; Stumbles & Penhale, 1993; McKeever et al., 1990). Transgenic mice with genetic manipulations have also provided good animal models for the study of IDDM. Selective β-cell specific expression of various transgenes can be induced, by coupling the transgenes to the insulin gene promoter. Insulitis, the primary characteristic of immunologically mediated diabetes can be induced upon transfer of the SV40 large T antigen in β-cells (Adams et al., 1987). Similar results have been obtained upon transfer of the interferon alpha gene (IFNα), tumor necrosis factor (TNF) alpha and interleukin-10 genes (Stewart et al., 1993; Higuchi et al., 1992) (Picarella et al., 1993; Wogensen et al., 1993). Mice expressing the major histocompatibility complex (MHC) class I or class II genes and non-MHC molecules such as calmodulin can induce IDDM (Allison et al., 1988; Markmann et al., 1988; Gotz et al., 1990; Epstein et al., 1989). IDDM has always been recognized as a hereditary disease and familial transmission of the disease in humans, along with the data from animal models,
indicate that IDDM is both polygenic and multifactorial. This has lead to the identification of IDDM susceptibility loci in humans and the NOD mouse model. The studies provide evidence implicating both MHC-linked as well as non-MHC linked genes in the pathogenesis of IDDM (Ashcroft & Ashcroft, 1992; Bach, 1994; Tisch & McDevitt, 1996).

2.2 Type II Diabetes or NIDDM

Analogous to IDDM, pathogenesis of NIDDM is an equally complex manifestation of defects in several distinct metabolic functions of insulin and accounts for >90% of patients with diabetes (Ashcroft & Ashcroft, 1992; Taylor, 1999; Kahn, 1998). The main characteristics of NIDDM pathology being (a) peripheral insulin resistance in tissues such as skeletal muscle and adipocytes, leading to inefficient glucose uptake by these organs in response to insulin, (b) impaired insulin action to inhibit glucose production by the liver in the face of hyperglycemia and (c) aberrant insulin secretion leading to a decreased insulin output (Kahn et al., 1996). NIDDM is a polygenic disease with a complex inheritance pattern. Moreover, like cancer, the incidence and degree of severity of NIDDM can be exacerbated by the presence of risk factors such as improper diet, lack of physical activity and age. Genetic factors determine the risk of developing NIDDM and susceptibility to insulin resistance and defects in insulin secretion appear to be genetically determined. The evidence of a genetic predisposition in the evolution of a diabetic phenotype is demonstrated by rare mutations in genes encoding glucokinase and transcription factors such as the hepatic nuclear factors (HNFs)-1alpha, -1beta and -4alpha, or IPF1, causing maturity onset diabetes in the young (MODY) (Yamagata et al., 1996; Yamagata et al., 1996; Stoffers et al., 1997; Horikawa et al., 1997).

2.3 FACTORS AFFECTING INSULIN REGULATION FROM PANCREATIC β-CELLS

2.3.1 Glucose

D-Glucose is the major physiological stimulus for insulin secretion. 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. Glucokinase is also linked to the phosphate potential, [ATP]/([ADP][Pi]) (Sweet et al., 1996). An increased ATP/ADP ratio is believed to close $K^+$-ATP channel at the plasma membrane, resulting in decreased $K^+$ efflux and subsequent depolarisation of the $\beta$-cell (Dunne, 1991). Depolarisation, activates voltage-dependent $Ca^{2+}$ channels, causing an influx of extracellular $Ca^{2+}$ (Liu et al., 1996). Although intracellular $Ca^{2+}$ activates protein kinases such as $Ca^{2+}$- and calmodulin dependent protein kinase (Breen & Aschroft, 1997), it remains unclear how increases in intracellular $Ca^{2+}$ leads to insulin release. Intracellular $Ca^{2+}$ stores appears to regulate a novel plasma membrane current $[Ca^{2+}\text{ release activated non-selective cation current }I_{CRAN}]$, whose activity may control glucose activated secretion. Lesions in these pathways leads to the pathogenesis of diabetes mellitus (Dukes et al., 1997). Glucose induced insulin secretion is also partly dependent upon the activation of typical isoforms of protein kinase C (PKC) within the $\beta$-cell (Harris et al., 1996). It is suggested that PKC may be tonically active and effective in the maintenance of the phosphorylation state of the voltage-gated $L$-type $Ca^{2+}$ channel, enabling an appropriate function of this channel in the insulin secretory process (Arkhammar et al., 1994).

2.3.2 Fatty acids

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

2.3.3 Amino acids

Amino acids also act as potent stimulators of insulin release. L-Tryptophan which is the precursor of 5-HT can act as a stimulator of insulin release (Bird et al., 1980). L-Arginine also causes insulin release from pancreatic $\beta$-cells. 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.
2.3.4 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 Ga2 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 in accompanied by an increase in the islet content of cAMP (Rabinovitch et al., 1976).

2.3.5 Glucagon

Glucagon is the hormone secreted by pancreatic α-cells. 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<sup>2+</sup> influx through voltage dependent L-type Ca<sup>2+</sup> channels, thereby elevating [Ca<sup>2+</sup>]<sub>i</sub> and accelerating exocytosis (Carina et al., 1993). Protein phosphorylation by Ca<sup>2+</sup>/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).

2.3.6 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<sup>2+</sup> currents or adenylate cyclase activity (Renstrom et al., 1996).

2.3.7 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).

### 2.3.8 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).

### 2.3.9 Adrenomedullin

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

### 2.3.10 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 \(\alpha_2\)-adrenergic receptors in activating G-proteins.

### 2.3.11 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).

2.3.12 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-Ca\(^{2+}\) 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).

2.4 ROLE OF NEUROTRANSMITTERS IN INSULIN REGULATION

2.4.1 Acetylcholine

Acetylcholine is one of the principal neurotransmitters of the parasympathetic system. Acetylcholine, through vagal muscarinic and non-vagal muscarinic pathways (Greenberg & Pokol, 1994) increases insulin secretion (Tassava et al., 1992). They function through muscarinic receptors present on pancreatic islet cells (Ostenson et al., 1993).

2.4.2 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 & Sehlin, 1983). Dopamine D3 receptors are implicated in the control of blood glucose levels (Alster & Hillegaart, 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.
2.4.3 \textit{\(\gamma\)-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 \(\beta\)-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 \(\beta\)-cells causing insulin-dependent diabetes mellitus (Baekkeskov et al., 1990). GABA through its receptors have been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic \(\alpha\)-cells and \(\delta\)-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 \(\beta\)-cells in response to glucose. The released GABA inhibits islet \(\alpha\)-and \(\delta\)-cell hormonal secretion in a paracrine manner. During diabetes the destruction of \(\beta\)-cells will lead to decrease in GABA release resulting in the enhancement of glucagon secretion from \(\alpha\)-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.

2.4.4 \textit{Serotonin}

Serotonin (5-HT) content is increased in the brain regions and hypothalamic nuclei (Chen & Yang, 1991); (Lackovic et al., 1990), but there are reports suggesting a decrease in brain 5-HT content during diabetes (Jackson & Paulose, 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 (VHM). The ratio of 5-HIAA/5-HT was increased. A similar observation was reported by (Ding et al., 1992) with a decrease in 5-HT in cortex (19%) and 5-HT turnover (5-HIAA/5-HT) that increased by 48%. Chu et al., (1986) has reported lower 5-HT levels in both hypothalamus and brain stem but not in corpus striatum. Insulin treatment brought about an increase in the cerebral concentration of 5-HIAA and
accelerated the cerebral 5-HT turnover (Juszkiewicz, 1985). The 5-HIAA concentration was reported to be approximately twice as high as the controls regardless of duration of treatment. Brain tryptophan, the precursor of 5-HT, was also reduced in brain regions during diabetes (Jamnicky et al., 1991). Insulin treatment was reported to reverse this reduced tryptophan content to normal (Jamnicky et al., 1993). There was a significant increase in 5-HIAA observed at 2-6 hours after insulin administration (Kwok & Juorio, 1987).

2.4.5 Epinephrine and Norepinephrine

These are secreted by the adrenal medulla. Norepinephrine (NE) is a principal neurotransmitter of the 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). They also inhibit insulin-stimulated glycogen synthesis 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 and Randle (Coore & Randle, 1964), who incubated pancreatic tissue from the rabbit. As judged by Malaisse et
the inhibitory effect of EPI on glucose-induced insulin secretion is mediated through the activation of α-adrenoreceptors.

2.5 BRAIN NEUROTRANSMITTER CHANGES DURING DIABETES

A significant increase in the catecholamine contents and activity of metabolising enzymes has been reported in experimental diabetes (Gupta et al., 1992). Norepinephrine has been reported to increase in several brain regions during diabetes (Tassava et al., 1992; Fushimi et al., 1984; Chu et al., 1986; Oreland & Shasken, 1983; Wesselmann et al., 1988; Chen & Yang, 1991), but a significant decrease in NE has been reported in hypothalamus (Ohtani et al., 1997), pons and medulla (Ramakrishna & Namasivayam, 1995). EPI levels were significantly increased in the striatum, hippocampus and hypothalamus of diabetic rats and these changes were reversed to normal by insulin treatment (Ramakrishna & Namasivayam, 1995). Streptozotocin-induced diabetes and acute insulin deficiency were demonstrated to result in increased content of EPI in the supra chiasmatic nucleus. In addition to this, a decreased turnover of dopamine in the ventromedial nucleus in diabetes was found to be reversed by insulin treatment (Oliver et al., 1989). These data indicate that experimental diabetes and acute insulin deficiency result in the rapid onset of detectable alterations in EPI and DA activity in specific hypothalamic nuclei. This can lead to the development of secondary neuroendocrine abnormalities known to occur in the diabetic condition. The DA content was increased in whole brain, (Lackovic et al., 1990; Chen & Yang, 1991) corpus striatum (Chu et al., 1986), cerebral cortex and hypothalamus of diabetic rats (Ohtani et al., 1997; Tassava et al., 1992). Serotonin (5-HT) content is increased in the brain regions and hypothalamic nuclei (Chen & Yang, 1991; Lackovic et al., 1990), but there are reports suggesting a decrease in brain 5-HT content during diabetes (Jackson & Paulose, 1999; Sumiyoshi et al., 1997; Sandrini et al., 1997). Brain tryptophan was also reduced during diabetes (Jamnicky et al., 1991). Insulin treatment was reported to reverse this reduced tryptophan content to normal (Jamnicky et al., 1993).
2.6 ADRENERGIC RECEPTORS

Adrenergic receptors belong to the large family of G-protein coupled receptors. These receptors form the interface between the sympathetic nervous system as well as many endocrine and parenchymal tissues (Hein & Kobilka, 1995). The most striking feature of adrenergic receptors is that they contain seven stretches of 20-28 hydrophobic amino acids that likely represent membrane-spanning regions. Adrenergic receptors are mainly classified into α and β-adrenergic receptors. α-adrenergic receptors are then subdivided into α₁ and α₂ (Lefkowitz & Caron, 1988). α₁ has three subclasses- α₁A, α₁B, α₁C (Price et al., 1994) and α₂ has α₂A, α₂B and α₂C (Hamamdzic et al., 1995). β-adrenergic receptors are subclassified into β₁, β₂ and β₃ (Dohlman et al., 1991). The regulation of adrenergic receptors is associated with G-proteins. Distinct, heterotrimeric G proteins, termed Gs and Gi, mediate the actions of the stimulatory and the inhibitory pathways, respectively (Gilman, 1984). EPI and NE bind to these receptors in a concentration dependant manner. At low concentration EPI and NE can bind and activate β-adrenergic receptors, which in turn stimulate the insulin secretion from pancreatic islets by activating adenylate cyclase through stimulatory G (Gs)-proteins. At high concentration they can bind to α₂A receptors and inhibit insulin secretion through inhibitory G₁ proteins (Lacey et al., 1993). α₁ receptors have also been observed to activate phospholipase A₂ and stimulate calcium influx through plasma membrane calcium channels.

2.6.1 α-adrenergic receptors

Based on the pharmacological and functional criteria, α-adrenergic receptors is further subdivided into two subclasses termed α₁ and α₂ adrenergic receptors (Langer, 1974; Hoffman & Lefkowitz, 1980).

2.6.1.1 α₁ adrenergic receptors

The concept of α₁-adrenoceptor subtypes was first suggested in the mid 1980s on the basis of the affinities of certain α₁-adrenoceptor agonists such as oxymetazoline, and the antagonists, WB4101 and phentolamine. α₁ adrenergic receptors have been implicated
in a variety of functions including smooth muscle contraction, regulation of hepatic
glycogen metabolism, and also, in mitogenesis in certain tissues (Lomasney et al., 1986).
$\alpha_1$-adrenergic receptors appear to be coupled to processes which lead to
phosphatidylinositol 4,5-biphosphate hydrolysis (Berridge et al., 1982). These receptors
are found to increase the renal gluconeogenesis (Saggerson et al., 1980). $\alpha_1$ adrenergic
receptors also play an important role in control of blood pressure via induction of vascular
smooth muscle contraction (Minneman & Esbenshade, 1994). It is generally accepted that
activation of $\alpha_1$-adrenergic receptors stimulates phospholipase C, leading to increased
hydrolysis of phosphatidylinositol 4,5-biphosphate to inositol 1,4,5-triphosphate and 1,2-
diacylglycerol. Both these components play important roles as intracellular second
messengers that increase intracellular $\text{Ca}^{2+}$ concentrations and activate various isoforms of
protein kinase respectively. These coupling mechanisms are typically mediated by
pertussis toxin-insensitive G-proteins, likely in the Gq/11 family. (Perez et al., 1993).
(Schwinn et al., 1995). Additional stimulation of $\alpha_1$-receptors activates phospholipase
D and phospholipase A2 via pertussis toxin-insensitive/ sensitive G-proteins (Minneman &
Esbenshade, 1994). Although this predominant view of $\alpha_1$ receptors signaling provides
substantial insight into $\alpha_1$-receptor-mediated responses in various cells, there are clear
indications that these mechanisms may not explain all aspects of $\alpha_1$-receptor signalling.
Recent evidence demonstrates that $\alpha_1$-receptor stimulated mitogenic responses
in myocytes may due to activation of tyrosine protein kinases (TPKs) and MAP kinases
(Thorburn et al., 1994), suggesting that $\alpha_1$-adrenergic receptors may share common signal
pathways with tyrosine kinase receptors in the stimulation of mitogenesis.

[^H]Prazosin, a hypertensive drug, which blocks these receptors, is widely used to
study the characteristics of $\alpha_1$ adrenergic receptors. Morrow et al (Morrow & Creese,
1986) suggest that[^H]Prazosin labels subtypes of $\alpha_1$-adrenergic receptor binding sites
that are discriminated by the antagonists, phentolamine and WB4101, and the agonist
phylephrine. Apparently this drug has high affinity for post-synaptic $\alpha$-receptors and
relatively low affinity for pre-synaptic $\alpha$-receptors (autoreceptors) (Cambridge et al.,
1977). Three $\alpha_1$ adrenoreceptor subtypes have recently been identified by molecular
cloning techniques: the $\alpha_{1A}$ (Stewart et al., 1994), the $\alpha_{1B}$ (Voight et al., 1990) and $\alpha_{1D}$
(Lomasney et al., 1991) - adrenoreceptor subtypes. At the RNA level all three subtypes
appear to be present in the heart (Rokosh et al., 1996). At the protein level, both the $\alpha_{1A}$ and $\alpha_{1D}$ adrenoreceptor subtypes have been reported to present in cardiac tissue, using selective receptor antagonists (Lazou et al., 1994).

### 2.6.1.2. $\alpha_2$-adrenergic receptors

$\alpha_2$-adrenergic receptors mediate many of the physiological actions of the endogenous catecholamines, adrenaline and noradrenaline, and are targets of several therapeutic agents. $\alpha_2$-adrenergic receptors are coupled by pertussis-toxin sensitive G proteins to various effectors, including adenylate cyclase and ion channels. The $\alpha_2$-adrenergic receptors respond to endogenous NE and EPI to elicit a variety of physiological responses, including inhibition of neurotransmitter release, suppression of insulin release from pancreatic $\beta$-cells, activation of platelet aggregation, and contraction of arteriolar smooth muscle. The $\alpha_{2A}$-adrenergic receptors subtype appears to mediate reduction in blood pressure following $\alpha_{2A}$ agonist administration (MacMillan et al., 1998). Three human $\alpha_2$-adrenoceptor subtype genes have been cloned and designated as $\alpha_{2-C10}$, $\alpha_{2-C4}$, and $\alpha_{2-C2}$, according to their location on human chromosomes 10, 4 and 2. They correspond to the previously identified pharmacological receptor subtypes $\alpha_{2A}$, $\alpha_{2C}$ and $\alpha_{2B}$. The receptor proteins share only about 50% identity in their amino acid sequence, but some structurally and functionally important domains are very well conserved. The most obvious functionally important differences between the receptor subtypes are based on their different tissue distributions; e.g. the $\alpha_{2A}$ subtype appears to be an important modulator of noradrenergic neurotransmission in the brain. The three receptors bind most $\alpha_2$-adrenergic drugs with similar affinities, but some compounds (e.g. oxymetazoline) are capable of discriminating between the subtypes. All $\alpha_2$-adrenoceptors couple to the pertussis-toxin sensitive inhibitory G proteins Gi and G(o), but recent evidence indicates that also other G proteins may interact with $\alpha_2$-adrenoceptors, including Gs and Gq/11. Inhibition of adenylate cyclase activity, which results in decreased formation of cAMP, is an important consequence of $\alpha_2$-adrenergic receptor activation (Aantaa et al., 1995).

EPI and NE inhibit insulin secretion by $\alpha_2$-adrenergic receptor activation. $\alpha_2$-adrenergic receptor activation leads to inhibition of insulin release by a mechanism distal
to those regulating β-cell cyclic AMP production and [Ca\(^{2+}\)] (Ullrich & Wollheim, 1985). α\(_2\)-adrenergic receptor agonists are potent inhibitors of insulin release in the isolated islet preparation from rats (Morgan & Montague, 1985), as well as in mice in vivo (Skoglund et al., 1986) and in man (Porte & Williams, 1966). The mechanism of action of α\(_2\)-adrenergic receptor agonists in mediation of the hyperglycaemic response is of peripheral origin and involves pancreatic β-cell post synaptic α\(_2\)-adrenergic receptors, possibly through the inhibition of insulin release (Angel & Langer, 1988). α\(_2\)-adrenergic receptors are known to have a critical role in regulating neurotransmitter release from sympathetic nerves and from adrenergic neurons in the central nervous system (Miller, 1998; Langer, 1997). Hein et al. (1999) have now studied neurotransmitter release in mice in which the genes encoding the three α\(_2\)-adrenergic receptor subtypes were disrupted. They found that both the α\(_{2A}\) and α\(_{2C}\)-subtypes are required for normal presynaptic control of the transmitter release from sympathetic nerves in the heart and from central noradrenergic neurons. α\(_{2A}\)-adrenergic receptors inhibit transmitter release at high stimulation frequencies, whereas the α\(_{2C}\)-subtype modulates neurotransmission at lower levels of nerve activity (Hein et al., 1999).

EPI and other adrenoceptor agonists are perviously shown to induce a hyperglycaemic response following in vivo administration. Clonidine was used as a potent agonist for inducing hyperglycaemia by activating α\(_2\)-adrenoceptors (DiTullio et al., 1984). DPI (3,4-dihydroxyphenylimino)-2-imidazolidine, a peripherally active adrenoceptor agonist, and UK 14.304, a highly selective α\(_2\)-adrenoceptor agonist, also could induce hyperglycaemia similar to clonidine (Angel & Langer, 1988). In as much as α\(_1\) agonists have been available exclusively selective probes for α\(_2\) adrenoceptors for several years, previous autoradiographic studies have used \(^{[3H]}p\)-Aminoclonidine and \(^{[3H]}\)Clonidine for the visualization of α\(_2\)-adrenoceptors (Unnerstall et al., 1984). However, the recent development of the potent and selective radiolabelled α\(_2\)-antagonists, \(^{[3H]}\)Rauwolscine and \(^{[3H]}\)Idazoxan, has allowed to localise their respective binding sites in the rat brain (Boyagian et al., 1987). The pharmacological characterisation of α-adrenoceptors has been facilitated by the introduction of \(^{[3H]}\)Yohimbine. Its use has shown that the absolute affinities and rank order of potency of a number of antagonists for
the $\alpha_2$-adrenoeceptor binding sites on human platelets differ from those on rat cerebral cortex membranes (Cheung et al., 1982).

2.6.2 $\beta$-adrenergic receptors

Adrenergic receptor is a member of the large family of $G$ protein-coupled receptors and is subjected to a complex regulation by hormones and other signalling molecules. The catecholamines, EPI and NE evoke specific responses in a variety of tissues. The $\beta$-adrenergic system has in many ways served as the premier model in which to investigate the processes by which external stimuli regulate cellular behavior. Stimulation of $\beta$-adrenergic receptor normally results in signalling by the heterotrimeric $G_5$ protein, leading to the activation of adenylate cyclase, production of cAMP, and activation of cAMP-dependent PKA. Gu et al., (2000) reported that cell death of thymocytes can be induced after stimulation of $\beta$-adrenergic receptor, or by addition of exogenous cAMP.

The concept of two subtypes of $\beta$-adrenergic receptors termed $\beta_1$ and $\beta_2$ developed by Lands et al. in 1967 was initially interpreted in terms of absolute organ specificity such that the heart contained exclusively $\beta_1$ and the bronchial system contained $\beta_2$ adrenergic receptors (Lands et al., 1967; Lands et al., 1967). Later in 1983 Tan and Curtis-Prior (Tan & Curtis-Prior, 1983) proposed the presence of another subtype of $\beta$-adrenergic receptor in the rat adipocytes which was then termed as $\beta_3$-adrenergic receptors. Isoprenaline, CGP 12177, BRL 37344 and NE are used as potent agonists of $\beta$-adrenergic receptors. The antagonists of $\beta$-adrenergic receptors widely used are propranolol, atenolol, betaxolol and practalol (Arch & Kaumann, 1993).

2.7 ADRENERGIC RECEPTORS IN DIABETES

Previous studies have shown that in diabetic condition $\alpha_2A$-receptors are more activated which brought out the insulin inhibition and in turn hyperglycaemia (Lacey et al., 1993). Rat islet cell membrane is equipped with $\alpha_2A$ (Filipponi et al., 1986) which are linked to adenylate cyclase and inhibits insulin secretion. Studies conducted in C57BL/KsJ mice revealed that all of the $\alpha_1$- and $\alpha_2$-adrenergic receptor population were
elevated in the regional brain samples of diabetic compared with controls. However, β-adrenergic receptor populations were depressed in diabetes compared with age-matched controls (Garris, 1990). Studies from our lab, have shown that α1-adrenoceptors expressed altered affinity in hypothalamus and brain stem of STZ-diabetic rats (Pius, 1996).

### 2.8 Pancreatic β-Cell Mass and Diabetes

Pancreatic 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 differentiation or neogenesis of β-cells has been extrapolated upon detection of insulin-positive β-cells in pancreatic ducts. 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 (Eriksson & 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 (Borg & Andersson, 1981).

The number of functionally intact β-cells in the islet organ is of decisive importance for the development, course and outcome of diabetes mellitus. Generally speaking, the total β-cell mass reflects the balance between the renewal and loss of these cells. While factors resulting in damage and degeneration of the β-cells have attracted much attention there has been relatively little interest in the kinetics and regulation of β-cell proliferation. In early fetal life both the endocrine and exocrine pancreatic cells probably arise from a common “protodifferentiated” cell type. The renewal of β-cells in diabetes has been studied in several animal models. Both in experimental diabetes, such as that induced by alloxan, and in hereditary diabetes, exemplified by the diabetic mutant mouse (gene symbol db), there is initial stimulation of the mitotic rate during development of hyperglycaemia followed by a notable decrease in the β-cell renewal. In the diabetic mouse the decreased proliferation of β-cells is paralleled by the appearance of fulminant diabetes. These data suggest a limited capacity for β-cell proliferation, which may differ
between species. According to this hypothesis, there is an increased risk of developing diabetes once the predetermined potential for β-cell division has been exhausted (Hellerstrom et al., 1976).

Hyperglycaemia, even when mild, can attenuate the secretory response of pancreatic β- and α-cells to increments in glucose and can impair insulin-mediated glucose transport, thus impeding its own correction and initiating a cycle of progressive self-exacerbation and metabolic deterioration. Both reduced islet function and insulin action may be the consequence of a generalized down-regulation and/or occupation of glucose transporters by hyperglycaemia so that the islets respond less to further increments in glycaemia. The postulated hyperglycaemic cycle can be initiated by any environmental perturbation that increases insulin demand in previously normoglycaemic patients in whom insulin secretion has already reached a maximum level of compensation for peripheral insulin resistance (Unger & Grundy, 1985). Sreenan et al. (1999) observed in the NOD mouse, β-cell destruction begins soon after the onset of insulitis. Diabetes may be present when residual β-cell mass represents 30% of control levels. Defects in any one of the three pronged regulatory mechanisms, viz. insulin synthesis, insulin secretion and changes in β-cell mass, result in a relative or complete insulin deficiency leading to diabetes (Ashcroft & Ashcroft, 1992; Taylor, 1999; Kahn, 1998).

In Type 1 diabetes, β-cell mass is depleted due to autoimmune destruction and the remaining β-cells are insufficient in mounting a growth response to counter the increasing hyperglycaemia. Although, the pathogenesis of Type II diabetes is multifactorial and less well defined, there is increasing evidence that defective β-cell replication and growth may constitute an additive predisposition to the development of the disease. It is likely that in the face of defective β-cell growth, followed by insulin deficiency may develop diabetes (Kloppel et al., 1985). The low growth rate and the reduced proliferation potential in Type II diabetes could be due to defects in growth regulatory proteins. However, studies aimed at identifying the key growth modulatory genes in impairment of β-cell growth and its low proliferation potential have yielded very few clues regarding mechanisms responsible for the deficient β-cell mass in these patients (Mares & Welsh, 1993; Welsh et al., 1993).
It is generally agreed that the pancreatic β-cell plays a key role in the aetiology of diabetes mellitus. Insufficient production of biologically active insulin is a common denominator in almost all forms of diabetes and the degree of insulin deficiency determines both the severity of disease and the choice of therapy. This has stimulated worldwide research efforts to elucidate the function and the natural history of the β-cell, leading to spectacular advancements in the basic understanding of the cell. Most research has been concerned with the regulation and molecular biology of insulin biosynthesis and release, and that other aspects of islet histophysiology have remained relatively neglected, despite their potential importance for a full understanding of the role of β-cell in diabetes.

The concept of the β-cell was born in 1907 (Lane, 1907; Bensley, 1911). At that time nothing was known to the hormone-producing capability of the β-cell, but there was nevertheless morphological evidence saying that this cell type would somehow be involved in the development of diabetes (Schaefer, 1895; Laguesse, 1893). Today the β-cell is defined not only by its histological or cytochemical staining characteristics, but also by its ability to express a complicated set of genes which provide the cell with a unique mechanism to synthesize and store insulin and to release the hormone in exact concert with the peripheral demand. The β-cell may also express on its plasma membrane certain antigens which are specific for the cell type and which may lead to autoimmune reactions (MacLaren et al., 1975) (Lernmark et al., 1978; Dyrberg et al., 1982; DeWinkel et al., 1982). Each of these properties serves to distinguish the β-cell from other cells of the body and may be specifically involved in the pathogenesis of diabetes.

The number of functionally intact β-cells in the islet organ is of decisive importance for the development, course and outcome of diabetes mellitus. Generally speaking, the total β-cell mass reflects the balance between the renewal and loss of these cells. In early foetal life both the endocrine and exocrine pancreatic cells probably arise from a common "protodifferentiated" cell type. The islets subsequently grow according to a regular pattern characterised by a symmetrical distribution of the islet volume in relation to islet diameter. The growth of the islets reflects the replication of both α- and β-cells in individual islets. Whether new islet cells are derived also from duct epithelium or by
transformation of differentiated acinar cells remains a matter of controversy (Hellerstrom et al., 1976). There is now evidence to suggest that β-cells arises from a pool of undifferentiated precursor cells in the fetal and newborn pancreas. These cells may contribute to islet growth and, if inappropriately stimulated, also to early islet hyperplasia. In the postnatal state, β-cell function is finely tuned by a complex set of incoming signals, one of which is the nutrient supply provided by the blood. Recent studies indicate that a disproportionately high fraction of pancreatic blood is diverted to the islets and that the islet blood flow is increased by glucose.

An acute stimulus to insulin release may be accompanied by a process, which enhances the distribution of the hormone to the target cells. Adaptive growth responses to an increased insulin demand occur in a number of hereditary diabetic syndromes in animals, but in some of these there is an inherited restriction on the capacity for β-cell proliferation leading to further deterioration of the glucose tolerance. Some evidence suggests that a similar mechanism may operate also in human non-insulin-dependent diabetes.

In eukaryotes, progression of the cell cycle is associated with periodic transcription activation/repression of growth-regulatory genes. Although the functional connections between transcription and cell-cycle regulators is far from being understood, recent progress has been made in connecting cell-cycle progression to dedicated components of the RNA polymerase II transcription apparatus complex (Lania et al., 1999). Efrat. (1998) developed a number of highly differentiated β-cell lines in transgenic mice. These cells produce insulin amounts comparable to normal pancreatic islets and release it in response to physiological insulin secretagogues.

2.10 DIFFERENTIATION OF THE PANCREATIC β-CELL

Knowledge of the embryonic origin of the β-cell will provide important information on β-cell function later in life. It is unfortunate that methods are not available to identify directly an apparently non-differentiated precursor cell, committed to become a β-cell. In the rat foetus the growth of the β-cell mass between gestation days 20 and 22 is considerable, with a total increase of more than 100% in 48hrs. However, growth that can be accounted for the formation of new β-cells from pre-existing β-cells is only 20% and
the remaining 80% must be accounted for the mechanisms other than β-cell division (Erikkson & Swenne, 1982; Swenne & Erikkson, 1982). Recent evidence suggests that, in the early postnatal period also, differentiation of precursor cells to β-cells might contribute to islet cell growth. It was reported that when STZ was injected into 1-2 day old rats, the ensuing hyperglycemia was only transient and was completely reversed by the 14th postnatal day (Portha et al., 1974; Weir et al., 1981). These changes were accompanied by marked initial destruction and loss of β-cells followed by active repair (Bonner-Weir et al., 1981, Cantenys et al., 1981, Dutrillaux et al., 1982). The latter process was characterised by the appearance of numerous insulin positive cells throughout the exocrine parenchyma and in the duct epithelium. Budding of the islets from ducts was a prominent feature. These observations suggest a rapid formation of β-cells, primarily through multiplication and differentiation of precursor cells, which may be located both in the acinar part and the ducts. It is still unclear whether precursor cells contribute to islet growth in the adult animals. While in vitro studies with foetal and neonatal pancreas strongly suggest that new islet tissue is derived from ductal epithelium, it is not established whether the primary cell is a committed endocrine cell or duct-like cell capable of transdifferentiation (Rosenberg, 1995).

Sarvetnick & Gu, in 1992 have shown that the pancreatic duct cells of IFN-gamma mouse are actively multiplying and that many duct cells differentiate to become endocrine cells. This islet regenerating process closely parallels the islet development during normal organogenesis in the foetus and offers a model for studying the cell lineage relationships of islet cells. They found that duct cells retain the ability to proliferate and to differentiate into islet cells. Under normal conditions, duct cells do not continue to multiply or to differentiate suggesting that in the transgenic mice, the progenitor cells of embryonic multipotential duct cells transform into adult cells, but in the presence of appropriate signals or stimuli can resume their multipotential property.

The ability of the adult pancreas to generate new insulin β-cells has been controversial because of difficulties in identifying the precursor population. Fernandes et al. (1997) recently determined that β-cells were generated during development from precursors that expressed the homeodomain-containing transcription factor, pancreas
duodenum homeobox gene-1 (PDX-1) and the existence of PDX-1 in the β-cell precursors and indicate that their differentiation is induced by islet injury (Fernandes et al., 1997).

2.11 MECHANISM OF PANCREATIC β-CELL GROWTH

Pancreatic β-cells, like all other cells of our body are under the regulatory checks and balances enforced by changes in cell cycle progression. However, very little is known regarding the key components of the cell cycle machinery regulating cell cycle control of β-cells. Knowledge of key elements involved in cell cycle regulation of β-cells will go a long way in improving our understanding of the replication capacity and developmental biology of β-cells (Rane & Reddy, 2000). Like other somatic cells, β-cell passes through a cell cycle, which can be subdivided into several distinct phases. By knowing the normal β-cell cycle is of considerable importance for a full understanding of the mechanism β-cell proliferation.

THE CELL CYCLE OF THE PANCREATIC β-CELL

By using isolated foetal rat islets (Hellerstrom et al., 1979), in which the progress of the β-cells through the cycle had been synchronised in vitro with the aid of hydroxyurea, Swenne recently made an extensive study of the lengths of the various
phases (Swenne, 1982; Swenne, 1982). The β-cell cycle could be subdivided into a $G_1$ phase of 2.5 hrs, an S phase of 6.4 hrs, a $G_2$ phase of 5.5 hrs and a mitosis time of 0.5 hrs, with a total generation time of 14.9 hrs.

The pancreas is an organ containing two distinct populations of cells, the exocrine cells that secrete enzymes into the digestive tract, and the endocrine cells that secrete hormones into the blood stream. It arises from the endoderm as a dorsal and a ventral bud which fuse together to form the single organ. Mammals, birds, reptiles and amphibians have a pancreas with similar histology and mode of development, while in some fish, the islet cells are segregated as Brockmann bodies. Invertebrates do not have a pancreas, but comparable endocrine cells may be found in the gut or the brain. In the developing pancreatic buds, the endocrine cells start to differentiate before the exocrine cells, and co-expression of different hormones by the same cell is often observed at early stages. Although pancreatic endocrine cells produce many gene products also characteristic of neurons, evidence from in vitro cultures and from quail-chick grafts shows that they are of endogenous and not of neural crest origin. Observational studies suggest strongly that both endocrine and exocrine cells arise from the same endodermal rudiment. Development of the pancreas in embryonic life requires a trophic stimulus from the associated mesenchyme. In postnatal life, all cell types in the pancreas continue to grow. Destruction of acinar tissue by duct ligation or ethionine treatment is followed by rapid regeneration. Surgical removal of parts of the pancreas is followed by moderate but incomplete regeneration of both acini and islets. Poisoning with alloxan or streptozotocin can lead to permanent depletion of β-cells (Slack, 1995).

2.12 FACTORS REGULATING PANCREATIC β-CELL GROWTH

The factors regulating islet cell proliferation and differentiation may permit proto-undifferentiated cells and islets to be grown in culture and to induce endocrine cell differentiation in vitro. Furthermore, islet cell growth factors could be used to provide 'trophic support' to islet transplants maintaining graft viability. There may also be greater scope for gene therapy when the growth factors have been isolated, purified, sequenced and cloned (Vinik et al., 1993). The adult β-cell is normally virtually quiescent, but its replicatory activity can be enhanced in vitro by certain nutrients and growth factors. The long-term alterations in β-cell mass constitute an important means to accommodate an
increased demand for insulin (Sjoholm, 1996). Recent studies have revealed that islet cells differentiate from the epithelial cells of primitive pancreatic ducts during embryogenesis, and can regenerate in response to the loss of islet cells even in adult pancreas. The ability of islet cells to regenerate raises the possibility that impaired and decreased islets of diabetic patients can be restored.

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 & Swenne; 1985; Swenne, 1982; Swenne, 1983). 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 (Nielsen, 1982; Brelje et al., 1989; Brelje & Sorenson, 1991; Swenne et al., 1987; Swenne et al., 1988). 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 (Cantenys et al., 1981; Yamaoka & Itakura, 1999). GH 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 foetal and adult rat islets leading to mitogenesis (Swenne et al., 1987; Swenne et al., 1988; Swenne & Hill, 1989). The presence of high-affinity IGF-1 receptors on β-cells and the finding that exogenous IGF-1 stimulates β-cell replication (Schravendijk et al., 1987), supported a concept that GH mitogenic activities might be mediated by a paracrine regulation involving IGF-1. 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, 1991), nicotinamide (Sandler & Andersson, 1986), amniotic fluid (Dunger et al., 1990) and serum (Hellerstrom & Swenne, 1985).
2.12.1 Glucose

Glucose is one of the stimulators of β-cell replication activity. There is evidence of compensatory growth of β-cells in adult rats after short-term glucose infusion (Bonner-Weir et al., 1989). Factors believed to increase the rate of replication in vivo include a high caloric intake, hypoglycemic sulfonylureas, various hormones and hyperglycemia. Recent studies in vitro have so far confirmed the mitogenic action of a high extracellular glucose concentration (Hellerstrom et al., 1976). Chronic exposure to glucose in excess of basal levels can induce insulin secretion in the neonatal β-cells by increasing the cAMP levels. In late foetal life, glucose is also a strong stimulus to replication, and metabolism of glucose is a prerequisite for this process. Glucose stimulates proliferation by recruiting β-cells from a resting state into an active division state. Severe hyperglycemia, at least when induced in rats, seems to retard rather than stimulate β-cell growth (Hellerstrom & Swenne, 1991).

2.12.2 Insulin

Recent observations indicate that insulin can stimulate pancreatic islet β-cell growth in vivo. McEvoy and Hegre reported that administration of insulin to diabetic rats implanted with foetal pancreas resulted in a three-fold increase in β-cell mass in some of the pancreatic recipients (McEvoy & Hegre, 1978). Rabinovitch et al have demonstrated that insulin can stimulate islet β-cell replication directly, possibly through a receptor for multiplication stimulating activity or another insulin like growth factor (Rabinovitch et al., 1982). Insulin favored regeneration of β-cell, by activating the neogenesis of the β-cells from precursor cells (Movassat et al., 1997). It is reported that mannoheptulose, an agent believed to inhibit insulin release, inhibits β-cell replication in vitro (King et al., 1978). It has been reported that foetal rat pancreas explanted in vitro in the presence of added insulin had greater β-cell volume and a greater insulin content than those grown without insulin (McEvoy, 1981).
2.12.3 Role of growth factors

There are several reports on effects of growth factors in the normal β-cell growth. The growth hormone, prolactin and placental lactogen were found to stimulate proliferation of normal rat β-cells (Nielsen, 1986). Among the large number of protein hormones GH and lactogenic peptides, prolactin (PRL) and placental lactogen (PL) have an important role in β-cell proliferation. GH has been reported to stimulate the in vitro replication of foetal (Dudek et al., 1984), neonatal (Brelje et al., 1989) and adult rat β-cells (Swenne & Hill, 1989). In most of the studies there was also a stimulatory effect of GH on the insulin content or secretion, and the majority of effects were mimicked by PRL and PL. GH appears to elicit its biological activities by inducing local production of insulin-like growth factors (IGF) in target cells (Milner & Hill, 1984). It is reported that in both fetal and adult islets growth factors, but not glucose, stimulated release of IGF-I partially counteracted the mitogenisity of GH (Swenne et al., 1987; Swenne & Hill, 1989). Culture of islets with Platelet Derived Growth Factor (PDGF) and IGF-I caused an increase in the islet content of polyamines resembling the effect of GH. These two growth factors elicited a stimulation of DNA synthesis in islets (Sjoholm et al., 1990). Epidermal growth factor is known to stimulate DNA replication in many systems like hepatocytes, pituitary cells etc. Chatterjee et al., (1986) have shown that EGF stimulates proinsulin biosynthesis as well as [³H]thymidine uptake into pancreatic islets. They suggested that EGF behaves like glucose in stimulating both insulin biosynthesis and β-cell replication.

2.12.4 Amino acids and polyamines

The amino acid enrichment in the organ culture appears to favour the growth of pancreatic rudiments suggesting that metabolites other than glucose might influence the development of pancreatic β-cells. The mechanism by which amino acids provoke an increased response of growth is unknown. DeGaspero et al., (1978) have shown that enrichment of amino acids in the culture medium is a factor which induce the growth of β-cells in organ culture. Amino acids are also able to stimulate β-cell replication, and it appears in the early foetal life as they are more important than glucose in this respect (DeGaspero et al., 1978). Amino acids, and human amniotic fluid, were recently also identified as potent stimulators of cell proliferation in adult mouse islets (Dunger et al.,
It is shown that glucose regulates polyamine content \textit{in vitro}. Polyamines like, putrescine and spermidine are necessary for the maintenance of normal insulin and protein biosynthesis, whereas spermine may exert a role in some other cellular processes such as DNA replication, RNA transcription and glucose stimulated insulin release (Welsh & Sjoholm, 1988).

\subsection*{2.12.5 Regulatory proteins}

A pancreatic gene called Reg., encoding a 165-amino acid protein was isolated from regenerating rat islets after partial pancreatectomy. The \textit{reg} gene is expressed in experimentally induced regenerating or hyperplastic islets. Unno \textit{et al.} reported that ectopic expression of the \textit{reg} gene occurs in some human colonic and rectal tumors, suggesting that enhanced \textit{reg} expression may be related to the proliferative state of tumor cells. At present, any direct relationship between \textit{reg} protein and \( \beta \)-cell replication remains to be established. However, since the \textit{reg} protein is a secretory protein and \textit{reg} can be expressed at an early stage of pancreatic cell differentiation, the \textit{reg} protein may act on the stem cells of \( \beta \)-cells in an autocrine or paracrine manner. In normal mature exocrine cells, the \textit{reg} gene is expressed and the gene product may be necessary to maintain adequate exocrine pancreatic function (Unno \textit{et al.}, 1992). The \textit{reg} protein is synthesised and secreted from regenerating \( \beta \)-cells, and that the expression of \textit{reg} was closely associated with \( \beta \)-cell regeneration. Recently, \textit{reg} protein was shown to stimulate pancreatic \( \beta \)-cell growth, further strengthening the notion that \textit{reg} is involved in pancreatic islet growth and regeneration (Watanabe \textit{et al.}, 1994).

\subsection*{2.12.6 Inhibitors of pancreatic beta cell proliferation}

Inhibitors of pancreatic \( \beta \)-cell proliferation include transforming growth factor \( \beta \) (TGF-\( \beta \)), the cytokine interleukin 1\( \beta \) (IL1\( \beta \)), pancreastatin and the diazepam binding inhibitor, all of which inhibit fetal rodent \( \beta \)-cell proliferation. TGF-\( \beta \) is a multifunctional cytokine which has implicated in various biological processes including an inhibition of the epithelial, endothelial and hemopoietic cell proliferation. Recent studies have demonstrated an involvement of TGF-\( \beta \) in the pathogenesis of acute and chronic pancreatitis (Gress \textit{et al.}, 1994). TGF-\( \beta \) inhibits glucose stimulated \( \beta \)-cell replication.
Poypeptides like cytokines has also role in β-cell proliferation because it helps in the secretion of macrophages (Jiang & Woda, 1991) which is known to precede the onset of clinically manifest diabetes mellitus. Interleukin-1β can exert cytotoxic and cytostatic actions on β-cells in culture. Interleukin-1β can function as an inhibitor of rat insulinoma cell proliferation (Sandler et al., 1989) and of adult islet cell replication in rats and mice (Southern et al., 1990; Sandler et al., 1991). 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., (1991) 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; Tatemoto et al., 1986; Efendic et al., 1987) and inhibit insulin secretion and may function as inhibitors of β-cell replication in vivo.

2.13 MODELS OF PANCREATIC β-CELL PROLIFERATION IN DIABETES

Since the β-cell is the only source of insulin production, mechanisms responsible for regeneration of β-cells lost or severely reduced in diabetes have been a focus of several studies. These studies have led to the generation and characterisation of many animal models, which have yielded important clues regarding the regenerative capacity of β-cells. Pancreatic β-cell toxins, alloxan or streptozotocin, have been used to selectively destroy β-cells and produce an IDDM-like state. Pancreatic β-cells, which survive the massive destruction in response to these reagents are capable of replicating, suggesting a replication capacity in a fraction of β-cells (Korcakova, 1971; Steiner et al., 1970). The degree of β-cell regeneration, however, is insufficient to cure diabetes (McEvoy & Hegre, 1977). Administration of streptozotocin to neonatal rats leads to hyperglycemia due to destruction of β-cells. This is followed by evidence of increased mitotic activity in the surviving β-cells and β-cell neogenesis from undifferentiated precursor cells, resulting in reversion of the hyperglycemia to a normoglycemic state (Portha et al., 1974, Bonner-Weir, 1981; Dutrillaux et al., 1982).

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 a regeneration among the remaining pancreatic β-cells (Leahy et al., 1988, Lohr et al., 1989). However, when 85-90% partial pancreatectomy is performed, mild hyperglycemia ensues which is followed by increased β-cell replication and a 40% increased β-cell mass (Bonner-Weir et al., 1983). Interestingly, 95% pancreatectomy results in severe hyperglycemia 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 relative contribution of replication, neogenesis or increased β-cell size to the increased β-cell mass is not very clear at this time. Also, it is likely that the degree of hyperglycemia may dictate the extent of the β-cell replication capacity, with severe hyperglycemia or diabetes negatively affecting the compensatory replication of β-cells. 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. The endogenous cholecystokinin released by FOY-305 stimulates regeneration after partial pancreatectomy. FOY-305 may be a useful agent in the treatment of pancreatic insufficiency after extensive subtotal pancreatectomy or chronic pancreatitis (Parekh et al., 1991).

2.14 NEUROTRANSMITTERS AS GROWTH SIGNALS

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 J.H, 1993).

2.14.1 Norepinephrine

NE is reported to amplify the mitogenic signals of both EGF and HGF by acting through the α1 adrenergic receptor. It induces the production of EGF and HGF at distal sites and also enhances the response to HGF at target tissues (Broten, et al 1999). Norepinephrine rises rapidly in the plasma within one hour after 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 (Michalopoulou & DeFrancis, 1997). Prazosin, a
specific antagonist of α1 adrenergic receptor, as well as sympathetic denervation greatly decrease DNA synthesis at 24 hrs after PH (Cruise et al., 1989). Addition of NE to hepatocytes stimulates Ca\textsuperscript{2+} mobilisation or PI turnover and either or both of these processes was proposed to be involved in the mitogenicity of NE (Exton et al., 1981; Exton et al. 1988; Nagano et al. 1999). Rat hepatomas lacked the α\textsubscript{1A} and α\textsubscript{1B} mRNA and receptor binding, while in the human hepatocyte cancer cell line, HepG2, their expression is high but they lack receptor binding (Kost et al., 1992). Hepatic neoplasm are characterised by an increase in α\textsubscript{2}-and β-adrenergic receptors and a concomitant decline in α\textsubscript{1} receptors (Sanae et al., 1989).

2.14.2 Adrenergic receptors and regeneration

Recent studies have shown that proliferation and insulin secretion of foetal rat β-cells could be significantly suppressed by α-adrenergic stimulation (Sjoholm, 1991). When α-adrenergic agonists were given together with Sp-cAMP[S] or to pertussis toxin-pretreated islets, the suppressed β-cell proliferation and insulin secretion were partially prevented, suggesting that α-adrenergic stimulation represses β-cell growth and hormone release in part by interfering with GTP binding proteins that connect cell surface receptors to adenylate cyclase (Sjoholm, 1991).

2.15 Effect of aging on the regenerative capacity of the pancreatic β-cells

Since an increased insulin resistance could be expected to lead to a compensatory β-cell hyperplasia, it may be speculated that human type II diabetes becomes manifest only in those individuals who are unable to respond to an increased insulin demand with a higher rate of β-cell proliferation. The proliferating islet cells were synchronized with hydroxyurea and their progression through the cell cycle studied by pulse labeling with \textsuperscript{3}HThymidine and it was possible to calculate the rate of formation of new β-cells from the cell cycle data. The growth rate of islet cells in \textit{in vitro} cultures was increased with increase in the glucose concentrations and decreased with increasing age. The fraction of cells that can enter cell cycle composed about of 10% in the fetal islet cells but was less
than 3% in the adult islets. The small pool of proliferating cells in adult islets could explain why β-cell multiplication, although present in the aging rat, is insufficient to increase the insulin output to levels at which normal glucose tolerance is maintained. This forms an interesting parallel to the development of type II diabetes in man, in which an inherited low capacity for β-cell regeneration may predispose to the disease (Swenne, 1983).

Proliferation of islet cells may compensate for both increased peripheral insulin resistance and islet cell destruction but the capacity for regeneration may be genetically determined. For the latter reason, glucose-stimulated islet cell replication was estimated in both inbred C57BL/6J (BL/6) and C57BL/KsJ (BL/Ks) mice. Islets isolated from both strains were exposed to high concentrations of glucose in vitro or in vivo for a prolonged time period. In both strains high glucose concentration culture was found to increase replicatory activity of the islets but decreased with age (Swenne & Andersson, 1984).

2.16 MOLECULAR BIOLOGY OF PANCREATIC β-CELL

The endocrine pancreas is an organ of enormous importance, since its dysfunction causes diabetes, one of the most common human diseases in the world. Regulation of pancreatic endocrine cell determination and differentiation requires a unique set of transcription factors, including basic helix-loop-helix and homeodomain-containing proteins. The physiological role of individual transcription factor has been characterised by gene disruption in the mouse. The results indicate that these genes are not only involved in tissue-specific activation of downstream target genes for islet-specific hormones, but also critical for the proper islet morphogenesis. Future elucidation of the genetic relationship of these genes will lead to a better understanding of the molecular mechanisms controlling endocrine pancreas formation and will contribute to the development of new therapeutic approaches to diabetes (Huang & Tsai, 2000).

The proliferative response of pancreatic islets to a prolonged glucose stimulation may be genetically determined. This may play a significant role in the development of different diabetic syndromes both in laboratory animals and man (Swenne & Andersson, 1984). At the genetic level, the regulatory regions in islet-specific genes are being
characterised. Transcription factors that interact with these regions have been cloned and these will be instructive in elucidating how islet-specific genes are regulated during development and regeneration (Steiner & James, 1992). Islet duodenal homeobox 1 (IDX-1/PF-1/STF-1/PDX-1), a homeodomain protein that transactivates the insulin promoter, has been shown by targeted gene ablation to be required for pancreatic development. The PDX-1 mRNA levels were not significantly different for common pancreatic ducts of pancreatectomised rats. PDX-1 protein expression was found to increase during active regeneration. Thus, in pancreatic regeneration PDX-1 is upregulated in newly divided ductal cells as well as in islets. The timing of enhanced expression of PDX-1 implies that it is not important in the initiation of regeneration but may be involved in the differentiation of ductal cells to \( \beta \)-cells (Sharma et al., 1999).

2.17 PERSPECTIVE

The discovery of insulin more than 75 years ago fueled enthusiastic optimism regarding insulin therapy of diabetes. However, complications of diabetes still produce devastating consequences and it is believed that better control of glucose levels will reduce the rate and severity of these complications. Although, insulin therapy is now better due to the availability of insulin pumps and automated glucose monitoring, only a small portion of patients with IDDM obtain sufficient glycaemic control. Pancreatic and islet transplantation approaches have been experimented upon with the goal of providing exogenous sources for insulin.

In animal models of IDDM, pancreatic and islet transplantation has been successful in establishing euglycaemia. Two feasible routes for such a therapy are (1) \( \beta \)-cell transplantation and (2) a mechanical \( \beta \)-cell. The first successful islet transplants were performed in rodents in the 1970s, but unfortunately, very few human diabetic patients have received any benefit from \( \beta \)-cells replacement therapy. Islet allografts as a mode of therapy offer a theoretically convenient approach since islets can be delivered to the liver via the portal vein with a relatively simple procedure. However, the initial success rate of this procedure has been very low. The two major problems facing islet transplantation being (1) finding a satisfactory source of insulin producing cells and (2) how can the
transplanted cells be protected from destruction by the immune system through the processes of autoimmunity and transplant rejection (Rane & Reddy, 2000).

The source for insulin producing tissue has been from either pancreatic tissue from cadaver donors or half of the pancreas from living donors. Both of these approaches have potential drawbacks, the most striking of which is the lack of supply of available pancreatic tissue and also the possibility of inducing a diabetic state in living pancreatic tissue donors, due to a reduction in their β-cell mass. Use of fetal or neonatal pancreatic tissue, which provides an attractive source because of its increased growth potential have been explored with limited success. Efforts to expand β-cells and create insulin-producing cells with genetic engineering offer an attractive option for therapy. Although β-cells have capacity for some growth, it has not been possible to efficiently expand β-cells in the laboratory. Efforts are underway to create β-cell lines that might be useful for transplantation. By adding additional genes that influence glucose metabolism, it may be possible to manipulate these cells so they secrete insulin when exposed to glucose. These genes can be transferred into β-cells by genetic approaches. The transplanted islets can be protected from transplant rejection or autoimmune attack by similar genetic modifications, which may help the β-cell to escape recognition by the immune system (Rane & Reddy, 2000).

Knowledge about genes necessary to replicate and increase the division potential of these β-cells will definitely aid the process of genetic manipulation of β-cells and a breakthrough allowing expansion of human β-cells would solve the supply problem for transplantable insulin-producing cells. Certain nutrients, pharmacological agents and growth factors can stimulate pancreatic β-cell proliferation; however, mitogenic signal transduction pathways in β-cells have not been particularly well characterised (Rhodes, 2000).

The expansion of the β-cell mass by recruitment of β-cells to proliferate may constitute a means by which the organism can compensate for the loss or dysfunction of β-cells occurring in diabetes. Thus, if β-cells could be induced to replicate at a higher rate, which may prove beneficial in maintaining normoglycaemia, since the β-cell mass is a
major determinant of the total amount of insulin that can be secreted by the pancreas (Sjoholm, 1996).