Pancreatectomy is a very useful approach to demonstrate the regenerating potential of the β-cells. Removal of 60% of the total pancreas did not cause any reduction in the body weight and elevation in the blood glucose levels of the pancreatectomised rats. This maintenance of glucose homeostasis is due to a regeneration among the remaining pancreatic β-cells and their excess production of insulin (Leahy et al., 1988, Lohr et al., 1989).

[³H]Thymidine incorporation into the islet DNA was used to monitor the entry of quiescent islet cells into the DNA synthetic phase. Sham operated rats showed negligible change in the DNA synthesis. But at 36 hrs and 48 hrs after partial pancreatectomy, DNA synthesis showed a significant increase which was peaked at 72 hrs. This elevated level of DNA synthesis was reversed to near normal after 7 days. This pattern of DNA synthesis is concordant with previous reports (Brockenbrough et al., 1988; Pearson et al., 1977).

During active DNA synthetic period, i.e.; at 72 hrs after 60% pancreatectomy, we found that the insulin secretion is increased. Previous studies suggest that the increase in the β cell proliferation is related to the degree to which insulin biosynthesis and/or release is increased (King & Chick, 1976; Chick et al., 1975). Recent observations indicate that insulin can stimulate pancreatic β-cell growth in vivo. McEvoy and Hegre, in 1978, reported that administration of insulin to diabetic rats implanted with foetal pancreases showed a three-fold increase in β-cell mass. Insulin caused a significant increase in the [³H]Thymidine incorporation as well as the mitotic activity in β-cells of neonatal rats maintained in monolayer cultures. It is suggested that insulin can enhance islet β-cell replication directly, possibly through a receptor for multiplication stimulating activity (MSA) or another insulin-like growth factor (Rabinovitch et al., 1982). Buchner et al reported that pancreatic resection resulted in suppression of liver DNA synthesis at 24 hrs after partial hepatectomy in untreated rats but not in animals that received peripheral injections of insulin and glucagon. (Rabinovitch et al., 1982).

T₃ levels were increased during pancreatic regeneration. Studies on the role of thyroid hormones in influencing DNA synthesis have shown that T₃ can induce
proliferative responses after subcutaneous administration in the intact liver (Francavilla et al., 1984). It is reported that thyrotropin through the cyclic AMP cascade, and in the presence of insulin or IGF-1, activates the proliferation of dog thyroid cells (Taton & Dumont, 1995). TSH induces DNA synthesis, in the presence of insulin. It is proposed that insulin induces the increase of cell mass that is a prerequisite for the mitogenic effect of TSH. Results from our laboratory indicate that thyroid hormones can influence DNA synthesis during liver regeneration by regulating the activity of thymidine kinase, which is a key enzyme for DNA synthesis (Tessy et al., 1997). Insulin can stimulate TSH and TSH stimulation causes increased production of T3. It is reported that in FRTL-5 cells TSH has a mitogenic effect (Takahashi et al., 1990).

5.1 Epinephrine and Norepinephrine content is decreased in the brain regions, pancreatic islets and plasma during pancreatic regeneration.

EPI and NE contents decreased in the brain regions as well as in the pancreatic islets of 72 hrs pancreatectomised rats. They have an antagonistic effect on insulin secretion and glucose uptake (Porte et al., 1966). It is reported that EPI and NE contents in the brain regions were increased significantly in the STZ-diabetic rats (Lackovic et al., 1990; Chen & Yang, 1991; Tasaka et al., 1992). This shows that during pancreatic degeneration the sympathetic stimulation is increasing. The increased sympathetic activity can inhibit the insulin secretion from the pancreatic islets (Efendic et al., 1978; Renstrom et al., 1996; Porte, 1967). Since NE and EPI inhibit insulin secretion they can suppress DNA synthesis as insulin is required for the proliferation of pancreatic islets.

The pancreatic islets form a highly innervated organ, receiving sympathetic neural inflow via the splanchnic nerves (Miller, 1981). A significant increase in the EPI and NE contents in the pancreas was reported during STZ-diabetes. The inhibitory effect of EPI on insulin secretion induced by glucose was reported by Coore and Randle (Coore & Randle, 1964). A limited β-cell regeneration has been observed in STZ-treated neonatal rats (Weir et al., 1981). NE assists the mito-inhibitory effects of TGF-β1 on other cell types like hepatocytes (Michalopoulou & DeFrancis, 1997). Our results showed a significant decrease in the EPI and NE contents in the 72 hrs pancreatectomised rats.
which increased the insulin secretion, thereby stimulating the regenerative capacity of the pancreatic islets.

EPI and NE levels in the plasma showed a significant decrease during active regeneration of pancreatic islets. Decreased levels of EPI and NE facilitate DNA synthesis in the pancreatic islets via increasing the insulin secretion. The insulin secretion is controlled by the sympathetic stimulation. Higher EPI and NE stimulate $\alpha_2$-adrenergic receptors and inhibit the insulin secretion, but at low concentrations, they activate $\beta$-adrenergic receptors thus stimulating insulin secretion from the pancreatic islets (Coore & Randle, 1964). The effect of EPI on islet hormone secretion is dependent on its plasma level. At low levels of EPI, both insulin and somatostatin secretions are stimulated and at extreme stress levels, it produced inhibition (Ahren et al., 1988).

5.2 Brain adrenergic receptors are altered during pancreatic regeneration in rats.

Scatchard analysis of $[^{1}H]$EPI binding showed an increase in the number of high affinity adrenergic receptors in cerebral cortex and the low affinity receptors showed a decrease. The hypothalamic adrenergic receptors are decreased with an increased affinity. Brain stem did not show any change in the receptor number, but the affinity was decreased significantly. All these data showed that the total adrenergic receptors are down regulated during active cell proliferation of pancreatic islets. Decreased adrenergic activity facilitates the insulin secretion and cell proliferation. It is already reported that the insulin secretion is inhibited by the increased sympathetic activity, which is observed in the diabetic state. Chronic adrenergic hyperinnervation acts directly or indirectly on ion flux to partially inhibit insulin release (Grodsky et al., 1997).

5.3 $\alpha_1$-adrenergic receptors are down regulated during active islet cell proliferation

$[^{1}H]$Prazosin binding studies in the cerebral cortex, brain stem and hypothalamus showed that there is a significant decrease in the $B_{\text{max}}$ with an increase in the affinity during active regenerative phase. Thus, from our results it is clear that the $\alpha_1$-adrenoreceptors are down regulated at the time of regeneration of pancreatic islets. Sjoholm et al. (1991) reported that the $\alpha_1$-agonist phenylephrine is a potent inhibitor of
islet DNA synthesis and it inhibited insulin secretion also. Down regulation of α1-adrenergic receptors was reported in liver regeneration after partial hepatectomy (Cruise et al., 1989). α1-adrenergic agonists are known to inhibit insulin secretion (Nilsson et al., 1989). It is suggested that the α1-adrenergic stimulation may cause a reduction in the cAMP synthesis, inhibits insulin secretion and islet DNA synthesis (Sjoholm, 1991).

5.4 α2-adrenergic receptor activity is decreased in the brain regions during islet regeneration

[3H]Yohimbine binding to α2-adrenergic receptors in the cerebral cortex and brain stem of 72 hrs pancreatectomised rats showed a significant reduction in the receptor number with an increased affinity. This decrease in the number of α2-adrenergic receptors indicates that these receptors are down regulated during pancreatic islet cell proliferation. α2-adrenergic receptors are one of the potent inhibitors of insulin secretion from the islets (Moratinos et al., 1988; John et al., 1990). α2-adrenoceptor stimulation by the endogenous catecholamines could lead to inhibition of insulin release, masking any potentiated response that otherwise should have appeared from α1- and β-adrenoceptor stimulation (Garcia-Barrado et al., 1998). In diabetic condition, α2,β-adrenoreceptors are more activated which brought out the insulin inhibition and in turn hyperglycaemia (Lacey et al., 1993). The displacement analysis data showed that in the cerebral cortex, at 72 hrs after partial pancreatectomy, Ki(H) value was found to be decreased but the Ki(L) was increased. The function of low affinity receptors is increased at the time of active regeneration. In the hypothalamus, there was no change in the Bmax, but the affinity of the receptors was significantly increased suggesting that hypothalamic α2-adrenergic receptor activity is increased during pancreatic regeneration. Although there are reports, which say the role of brain α2-adrenergic receptors in the insulin secretion, there are not much studies about the inhibitory action of this receptor subtype on the islet DNA synthesis. Sjoholm in 1991, has suggested that the rate of DNA synthesis, insulin secretion and cAMP content in the isolated pancreatic islets were markedly inhibited by long term exposure to the α2-adrenergic agonist, clonidine (Sjoholm, 1991). Our results showed that in the brain regions the α2-adrenergic function is decreasing during pancreatic
regeneration which then stimulated the insulin secretion and in turn the DNA synthesis in
the islets.

RT-PCR analysis of $\alpha_{2A}$-adrenergic receptor mRNA showed that its expression is
decreased in the cerebral cortex and brain stem of experimental rats during active islet cell
proliferation. This result is in concordant with the receptor data where we observed a
significant decrease in the number of receptors at 72 hrs after partial pancreatectomy.
Cerebral cortex co-ordinates the overall function of the brain and the brain stem has direct
nerves originating from it that extends to the pancreas. the receptor changes observed in
these regions can have a direct effect on the pancreatic function (Coldman & Dampney,
1998). Decreased levels of cAMP led to an increase in $\alpha_{2A}$-adrenergic receptor mRNA
abundance (Sakaue & Hoffman, 1991). It is reported that the cAMP content is decreased
during islet DNA synthesis (Sjoholm, 1991). In the hypothalamus, expression of $\alpha_2$-
adrenergic receptor mRNA remained unchanged confirming the receptor data obtained
from the binding analysis in which there was no change in the receptor number. There
was only the affinity change in the hypothalamus during islet regeneration. Thus, our
results show that the brain $\alpha_2$-adrenergic receptors are down regulated during active islet
cell proliferation.

5.5 Brain $\beta$-adrenergic receptors are up regulated at the time of active DNA
synthesis in the pancreatic islets

In cerebral cortex and hypothalamus, both the low and high affinity $\beta$-adrenergic
receptors increased in numbers in the 72 hrs pancreatectomised rats and the affinity of the
receptors decreased. The displacement analysis of these receptors showed that at 72 hrs
after partial pancreatectomy there was a decrease in the affinity confirming the data
obtained from the Scatchard analysis. In the brain stem, low affinity receptors were found
to increase without change in the affinity. Thus, from our data it is clear that the total $\beta$-
adrenergic receptors are up regulated during islet regeneration. $\beta$-adrenergic receptors
have been reported to couple directly to calcium channels through a stimulatory G-protein
(Brown & Birnbaumer, 1988) thereby, stimulating insulin secretion. It is already reported
that $\beta$-adrenergic receptor populations were decreased in diabetes (Garris, 1990). There
are also reports saying that thyroid hormones can regulate $\beta$-adrenergic receptor number.
In a hyperthyroid state the cardiac β-adrenergic activity is enhanced (Williams et al., 1977). We also observed an increase in the T3 level during active regeneration in pancreatectomised rats. In T3-treated rats myocardial β-adrenergic receptors, as measured by [3H]dihydroalprenolol binding, were significantly increased [Scarpace, 1981 #335].

RT-PCR analysis of β-adrenergic receptor mRNA in the brain regions showed that the mRNA expression is increasing confirming the receptor data. β-adrenergic receptor expression and receptor mRNA levels are down regulated by β-adrenergic agonists and up regulated by glucocorticoids (Haddock et al., 1989) (Haddock & Malbon, 1988). Here we observed an up regulation, because in the brain region agonists EPI and NE, are found to be decreasing. Decrease in cAMP levels results in down regulation of the β2-AR (Hosoda et al., 1995) and it is already known that the cAMP content is increased during islet DNA synthesis (Sjoholm, 1991; Swenne, 1982). These data imply that variations in cellular content of β2-adrenergic receptor mRNA, account for differences in receptor number. Up regulation of the receptor can result from increased production of mRNA due to an increase in the transcription of the gene for the receptor which is stimulated by thyroid hormone or by glucocorticoids (Idem, 1988).

5.6 Pancreatic adrenergic receptors are decreased during active islet cell proliferation

[3H]EPI binding studies in the pancreatic islet membrane showed that the total adrenergic receptors are decreasing. The affinity of the remaining receptors was increased. The EPI and NE contents in the pancreatic islets were found to be decreasing at 72 hrs after partial pancreatectomy. This result was in concordant with the results obtained in the brain regions. Alterations of central neurotransmission and environmental factors can change the relative contribution of sympathetic outflow to the pancreas, liver, adrenal medulla and adipose tissues, leading to the modulation of glucose and fat metabolism (Nonogaki, 2000). The pancreatic islets are richly innervated by parasympathetic, sympathetic and sensory nerves. Several different neurotransmitters are stored within the terminals of these nerves, both the classical neurotransmitters, acetylcholine and noradrenaline, and several neuropeptides. Stimulation of the autonomic nerves and treatment with neurotransmitters affect islet hormone secretion. Thus, insulin
secretion is stimulated by parasympathetic nerves and inhibited by sympathetic nerves (Ahren, 2000). Previous studies have shown that the level to which β-cell proliferation increased is related to the degree to which insulin biosynthesis and/or release is enhanced (Chick et al., 1973). Since the adrenergic activity is decreasing it will eventually help in the insulin secretion thereby enhancing the DNA synthesis in the islets.

5.7 α1-adrenergic receptors in the islets are down regulated during regeneration

α1-adrenergic receptor binding studies in the pancreatic islets showed that the receptor number is decreased without any significant change in the affinity. It is reported that the α1-agonist phenylephrine inhibited the islet DNA synthesis (Sjoholm, 1991). α1-adrenergic receptors are reported to inhibit insulin secretion and they are found to be increased in the streptozotocin diabetic state (Pius, 1996). It was reported in hepatic regeneration that the α1-adrenergic receptors are decreased without any change in their affinity (Sandnes et al., 1986). Reciprocal changes in the expression of α1 and β-adrenergic receptors have been demonstrated to occur in the primary cultures of rat hepatocytes (Kunos et al., 1995).

5.8 Islet α2-adrenergic receptors are down regulated during pancreatic regeneration

Scatchard analysis of [3H]Yohimbine binding in the pancreatic islets showed a significant decrease in the α2-adrenergic receptor number during active islet DNA synthesis. Affinity of the remaining receptors is found to decrease. We also observed a similar change in the brain regions. α2-adrenergic receptors are known to have a critical role in regulating neurotransmitter release from the sympathetic nerves and from the adrenergic neurons in the central nervous system (Miller, 1998). Decreased α2-adrenergic activity can evoke β-adrenergic receptor mediated stimulation of insulin release from the pancreatic islets, which will then enhance the DNA synthesis. It was shown that α2-adrenergic receptor agonist clonidine, suppresses insulin secretion from the islets in vitro (Nilsson et al., 1989). The function of α2-adrenergic receptors is mediated through an inhibitory G-protein which decrease the cAMP content in the islet cells (Gillman, 1987). Decreased cAMP content will inhibit the insulin secretion as well as the DNA synthesis in the pancreatic islets. It is suggested that the long term stimulation of α2-adrenergic
receptor interferes with signalling through pertussis toxin-sensitive G-protein which by suppressing cAMP production inhibits β-cell DNA synthesis and insulin secretion.

5.9 β-adrenergic receptors in the islets are up regulated during regeneration

β-adrenergic receptors are found to be increased without any change in the affinity during active regeneration in the pancreatic islets. Thus from our data it is clear that the total β-adrenergic receptors are up regulated at 72 hrs after partial pancreatectomy. The brain β-adrenergic receptors are also increased at the time of active DNA synthesis. The β-receptor is linked to a stimulatory G-protein leading to the activation of the adenylate cyclase which in turn catalyses the conversion of ATP to cAMP. cAMP is responsible for the physiological response, the nature of which differs with the type of cell (Fraser, 1993).

Increased intracellular levels of cAMP have been proposed to mediate inhibitory as well as stimulatory effects in DNA synthesis and mitosis in fibroblast cell lines (Pessin et al., 1983). It is reported that during primary culture of rat hepatocytes β-adrenergic response is increased (Sandnes et al., 1986). β-adrenergic receptor stimulation evokes an increase in cAMP level which then activates cAMP dependent protein kinase and insulin release.

5.10 Adrenergic receptor antagonists regulate insulin secretion in 1hr incubation and long term culture of islet cells

Insulin secretion is increased with the increase in the glucose concentration. Similar changes were observed by Bombara et al (Bombara et al., 1995) and Castro et al (Castro et al., 1992). cAMP system is positively responsive to increasing glucose concentration. cAMP and the insulinotropic peptides, glucose-dependently increase the cytosolic free Ca²⁺ concentration ([Ca²⁺]ᵢ) in pancreatic β-cells, which is tightly linked to the potentiation of glucose-induced insulin release (Yaekura et al., 1996).

Epinephrine is known to regulate the insulin secretion from the pancreatic islets. It exerts opposite effects on peripheral glucose disposal and glucose stimulated insulin secretion (Avogaro et al., 1996). Our data showed that the EPI regulate the insulin secretion from pancreatic islets in two opposite ways. It is already known that, when used in high doses in vivo or in vitro, epinephrine reduces the insulin response to stimulators
EPI and NE have an antagonistic effect on insulin secretion and glucose uptake (Porte et al., 1966). As judged by Malaisse et al., the inhibitory effect of EPI upon insulin secretion induced by glucose is related to its ability to activate α-adrenoreceptors which then inhibit insulin secretion (Malaisse et al., 1967).

There are different subtypes for adrenergic receptors, of which we studied the involvement of three subtypes in regulation of insulin secretion from the islets. α₁-adrenergic receptors are found to regulate the insulin secretion in two ways, depending upon the concentration of EPI used in the culture. At lower concentrations of EPI, α₁-adrenergic receptors act as stimulatory to insulin secretion. We conclude that α₁-adrenoceptor subtypes are differentially regulated by agonist treatment even if they are expressed in the same cell type. Yang et al., reported that the down regulation of α₁-adrenoceptor subtypes by 100μM phenylephrine was time-dependent, and significant reductions were observed already after 2-4 hrs incubations. In contrast, incubation of α₁D-adrenoceptor-expressing cells with phenylephrine increased receptor number in a time and concentration-dependent manner (Yang et al., 1999). We found that the insulin secretion is regulated by the α₁-adrenoceptors possibly mediated through subtypes differentially expressed according to the EPI concentration in the pancreatic islets.

α₂-adrenergic receptors are inhibitory to the insulin secretion. α₂-adrenergic receptors are coupled by Gi proteins to various effectors, including adenylate cyclase and ion channels. The α₂,₃ adrenergic receptors respond to endogenous NE and EPI to elicit a variety of physiological responses, including inhibition of neurotransmitter release and suppression of insulin release from pancreatic β-cells (MacMillan et al., 1998). Our studies with yohimbine showed that the insulin secretion from the pancreatic islets is significantly increased confirming that the α₂-adrenergic receptors inhibited the insulin secretion from the pancreatic islets. Thus, EPI, a potent activator of this subclass of adrenergic receptors, is a potent inhibitor of insulin secretion from the islets. α₂-adrenergic receptor activation leads to inhibition of insulin release by a mechanism distal to those regulating β-cell cyclic AMP production and [Ca²⁺]i (Ullrich & Wollheim, 1985). The mechanism of action of α₂-adrenergic receptor agonists in mediation of the hyperglycaemic response is of peripheral origin and involves pancreatic β-cell post
synaptic α2-adrenergic receptors, possibly through the inhibition of insulin release (Angel & Langer, 1988).

Our experiments with in vitro cultures of islets showed that propranolol blocking resulted in a marked decrease in the insulin secretion. This explains the stimulatory role of β-adrenergic receptors in the insulin secretion. Stimulation of β-adrenergic receptor normally results in signalling by the stimulatory G protein, leading to the activation of adenylate cyclase, production of cAMP, and activation of cAMP-dependent protein kinase (PKA) in turn stimulating the insulin secretion. EPI and NE, when present at low concentrations, stimulate the β-adrenergic receptors thereby increasing the insulin release from the pancreatic islets.

The long-term insulin secretion studies showed the same pattern of changes as in the 1 hr incubations. The presence of insulin activators or stimulators in the islet cell cultures for 24 hrs showed that they capacitate or loss the ability of the viable cells to synthesise and secrete the insulin. We have done the 24 hrs islet cell culture to study the long-term effect of different adrenergic antagonists to stimulate or block the insulin synthesis and release from the isolated islets.

Thus our results show that the EPI acts through different subclasses of adrenergic receptors bringing about the changes in the insulin secretion. α1-adrenergic receptors can act both as stimulatory and inhibitory, while α2-adrenergic receptors are strictly inhibitory and β-adrenergic receptors are stimulatory to the insulin release from the islets.

5.11 EPI is taken up by the islet cells and binds to different subcellular fractions of islets

[^1H]EPI uptake in the islet cells at different time intervals showed that EPI can go inside the islet cell and bind to the nuclear proteins in a time-dependent manner regardless of the glucose concentration. This explains that the EPI uptake by the islets is not glucose-induced. With the increase in the time of incubation, the concentration of EPI uptake is increased. If the sympathetic stimulation from CNS is increased it can cause a parallel increase in the islet EPI content. It is reported that the EPI and NE content is increased in
the pancreatic islets of young and old diabetic rats (Abraham, 1998). Most released EPI and NE are efficiently removed by neuronal and extraneuronal uptake (Eisenhofer et al., 1992). A number of laboratories have reported the presence of monoamines like 5-HT within the islets (Bird et al., 1980). NE and EPI transporters are present in the plasma membrane of the islets, which can help the passage of EPI into the interior of the islets.

We propose that the presence of increased EPI activate the transporter proteins to transport the EPI which then binds to some protein present in the nuclear fraction. Catecholamines are inactivated mainly by two mechanisms, through the enzyme catechol-o-methyltransferase (COMT) and monoamine oxidase (MAO). A number of reports have appeared with regard to the role of MAO in islets, since the process of deamination of biogenic and exogenic amines lead to concomitant production of hydrogen peroxide (H₂O₂). The generation of H₂O₂ may affect the redox state of the β-cell glutathione system, the balance of which is known to influence nutrient-induced insulin release (Miller, 1981). According to our data it is clear that the EPI uptake by the islets can be irrespective of the glucose concentration and bind to the membrane as well as nuclear fractions of pancreatic islets. The insulin secretion is decreased when we incubated the islets with high concentrations of EPI. This led us to a conclusion that there are some proteins in the nuclear fraction of the islets to which EPI can bind and regulate the insulin secretion.

5.12 The EPI-binding nuclear protein in the islets is identified as a 70-kDa Histidine-rich protein

The EPI-binding nuclear protein was identified by ligand blotting method. The protein was identified as a 70-kDa protein. The amino acid analysis of the protein revealed that the protein is rich in Histidine (69.92%). 70-kDa EPI-binding nuclear protein showed a similarity to a mouse zinc finger protein, which may function as transcription activator. This protein has also a regulatory role during cell division (Cunliffe et al., 1990; Cunliffe et al., 1990).
5.13 EPI-binding nuclear proteins are increased in the islets of pancreatetomised rats

Radioreceptor analysis in the nuclear fraction of the islets using \[^3H\]EPI showed an increase in the number of EPI-binding specific nuclear protein in the pancreatic islets during active regeneration. The affinity of the proteins is also increased. The EPI content in the islets was decreased. The decreased EPI can cause increase in the EPI-binding proteins, which may increase the insulin secretion, observed during regeneration. Central sympathetic system has a parallel control over the peripheral system. Decrease in the brain EPI content causes a significant decrease in the islet EPI. The low EPI content in the islets regulate the membrane and nuclear receptors in two ways. The decrease in EPI results in the reduction of membrane receptors and elevation in the nuclear receptors.

5.14 EPI binding nuclear proteins are down regulated in the pancreatic islets of STZ-diabetic rats

This experiment was done to see the changes in the EPI-binding nuclear proteins in the islets in an insulin-deficient state in rats. We observed that in the STZ-diabetic stage, the nuclear proteins are decreased with an increased affinity. This result showed that the EPI-binding nuclear proteins are down regulated during diabetes. It is reported that the EPI and NE content in the islets are increased in the diabetic rats when compared to control. Thus, from this data it is clear that increase in EPI/NE can decrease the EPI-binding nuclear protein, which eventually decreased the insulin secretion.

5.15 Possible regulation of insulin transcription by the 70-kDa protein

There is no evidence so far explaining the involvement of a 70-kDa zinc finger protein in regulation of insulin gene. The insulin gene has some regulatory sequences which contained many binding sites for highly cell-specific and ubiquitous protein factors (Boam \textit{et al.}, 1990). These proteins when bound to their specific DNA motifs, determine the temporal and spacial expression of the gene and its inducibility by various external stimuli, such as second messenger molecules (Imagawa \textit{et al.}, 1987). It is reported that in the insulinoma cells a 70-kDa DNA-binding protein was identified and reported to bind to the -CACC- promoter element of Gastrin gene. This protein is known to interact with
NEPHRINE → EPI UPTAKE

MEMBRANE

TRANSPORTERS

EPI CONTENT

TRANSPORTERS

EMPHASIS ON:

- CREBIP
- CREB/P BINDING ELEMENT
- ENHANCER BINDING ZINC FINGURE PROTEIN
- β₂AR
- α₂AR
- cAMP
- INS

NUCLEUS

EPI DOWN REGULATES ENHANCER BINDING ZINC FINGURE PROTEIN

NO TRANSCRIPTION
other islet nuclear proteins to synergically activate transcription in a cell-specific manner (Tilloson et al., 1994). We assume that the 70-kDa zinc-finger protein bind directly to the insulin enhancer factor (IEF) binding region in the insulin gene or interact with other DNA-binding proteins to enhance the gene transcription. EPI at high concentrations down regulate these nuclear binding proteins and at lower concentrations it causes up regulation. Thus, it is evident from our results that EPI has a direct role not only in the insulin secretion but also in the insulin gene transcription. As it can involve in the insulin gene transcription, it might have involved in the β-cell regeneration also. To confirm the regulatory role of EPI in the insulin gene transcription further study is required.

5.16 Glucose and growth factors regulate the islet cell proliferation in vitro

Glucose is widely accepted as an important stimulus to the development to β-cell growth and function. Glucose infusion in vivo increased the insulin level in rats (Kervan & Gitard, 1974). In monolayer culture also D-glucose is the principal stimulator that acted as a β-cell mitogen (DeGasparo et al., 1978). We also found in the suspension cultures glucose could increase the DNA synthesis as monitored by the [3H]Thymidine incorporation. Glucose also enhanced the EGF-induced DNA synthesis in the in vitro cultures. One of the mechanisms by which the glucose enhances the DNA synthesis is by increasing the insulin secretion. Some reports say that glucose controls β-cell proliferation by regulating the number of cells which enter the cell cycle (Swenne, 1982). After entering into the cell cycle, then it proceeds irrespective of the glucose concentration. Increase in the glucose will increase the cAMP level which then trigger the mitogenesis in the β-cells (Sjoholm, 1997). Swenne et al., also reported that nutrients like glucose and amino acids have a big role in the regulation of insulin biosynthesis and β-cell growth in vitro (Swenne et al., 1980).

EGF is a prototype stimulator of most epithelial cells. It has mitogenic effect on many of the tissues like hepatocytes. EGF is reported to have a mitogenic role in the pancreatic islets also. Barton et al (Barton et al., 1991) have shown that EGF stimulates the proliferation of pancreas. We also found that the EGF stimulated the DNA synthesis significantly. Chatterjee et al. (1986) have also shown that EGF stimulates proinsulin biosynthesis as well as [3H]Thymidine incorporation into the pancreatic islet DNA. They
suggested that EGF behaves like glucose in stimulating both insulin biosynthesis and β-cell replication. TGF-β1 is known to have inhibitory regulation in the proliferation of various cell types. It is an inhibitor of hepatocyte proliferation in cultures (Michalopoulose & DeFrancis, 1997). TGF-β1 has been implicated as an inhibitor of cell proliferation and a potent inducer of apoptosis in vitro and in vivo after its administration in high doses (Fan et al., 1998). Konturek et al. (1997) have reported that in acute pancreatitis the TGF-β1 showed a marked increase. Our data showed that TGF inhibited the DNA synthesis in the islet cell cultures. But when used in combination with EGF, it suppressed the EGF-induced DNA synthesis.

5.17 EPI controls islet cell proliferation in a concentration dependent manner

EPI at lower concentrations enhanced the EGF-induced [3H]Thymidine incorporation in the islet cell culture whereas higher concentrations inhibited the DNA synthesis. As suggested earlier, the high concentrations of EPI inhibit and low concentrations stimulate the insulin secretion as well as DNA synthesis in the islets. NE offsets the mito-inhibitory effects of TGF-β1 on other cell types like hepatocytes (Michalopoulose & DeFrancis, 1997). The dose-dependent studies showed that the inhibitory effect is increasing with the increase in the EPI concentration. Our results confirm that EPI has both the stimulatory as well as inhibitory effect on the DNA synthesis which is concentration dependent.

5.18 α-adrenergic receptors inhibit and β- adrenergic receptors stimulate the DNA synthesis in islet cells

The pancreatic islets form a highly innervated organ, receiving sympathetic neuron inflow via the splanchnic nerves (Miller, 1981). Ever since the original discovery by Coore and Randle (Coore & Randle, 1964) that the sympathetic neurotransmitter epinephrine inhibits insulin secretion from rabbit pancreas, there has been a great number of studies investigating the effects of adrenergic drugs on insulin secretion. Sjoholm in 1991, has studied the effect of adrenergic agonists and antagonists on the rate of DNA synthesis, insulin secretion and cAMP content in isolated islets. He found that α1-agonist, phenylephrine, the α2-agonist clonidine and β-adrenoceptor antagonist propranolol were
all potent inhibitors of islet cell DNA synthesis and insulin secretion. Our results are in concordant with these results. We observed that $\alpha_1$-adrenergic receptors have two different roles depending upon the concentration of EPI present in the medium. This subclass has the same effect on insulin secretion, which we observed from our insulin secretion studies. The $\alpha_2$-adrenergic receptors are found to be strictly an inhibitor of islet DNA synthesis. $\beta$-adrenergic receptors studied using propranolol, stimulated the EGF-induced DNA synthesis in the islet cells. The possible role of adrenergic receptors in regulating the islet cell proliferation is mediated by cAMP. $\alpha$-adrenergic receptor activation decreases the cAMP content in the islets whereas $\beta$-adrenoreceptor activation causes an increase in the cAMP levels (Sjoholm, 1991). It is suggested from our results that EPI regulates the pancreatic islet DNA synthesis through its different adrenergic receptors. According to the concentration of EPI different subclasses of adrenergic receptors are activated and its function, either stimulatory or inhibitory, is mediated through these receptors. Also, our results suggest that the central and pancreatic adrenergic receptor function have an important role to play in the regulation of islet cell proliferation and insulin homeostasis.