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# Decreased $\alpha_2$-adrenergic receptor in the brain stem and pancreatic islets during pancreatic regeneration in weanling rats

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**Abstract**

Sympathetic stimulation inhibits insulin secretion. $\alpha_2$-Adrenergic receptor is known to have a regulatory role in the sympathetic function. We investigated the changes in the $\alpha_2$-adrenergic receptors in the brain stem and pancreatic islets using [$^3$H]Yohimbine during pancreatic regeneration in weanling rats. Brain stem and pancreatic islets of experimental rats showed a significant decrease ($p<0.001$) in norepinephrine (NE) content at 72 h after partial pancreatectomy. The epinephrine (EPI) content showed a significant decrease ($p<0.001$) in pancreatic islets while it was not detected in brain stem at 72 h after partial pancreatectomy. Scatchard analysis of [$^3$H]Yohimbine showed a significant decrease ($p<0.05$) in $B_{max}$ and $K_d$ at 72 h after partial pancreatectomy in the brain stem. In the pancreatic islets, Scatchard analysis of [$^3$H]Yohimbine showed a significant decrease ($p<0.001$) in $B_{max}$ and $K_d$ ($p<0.05$) at 72 h after partial pancreatectomy. The binding parameters reversed to near sham by 7 days after pancreatectomy both in brain stem and pancreatic islets. This shows that pancreatic insulin secretion is influenced by central nervous system inputs from the brain stem. In vitro studies with yohimbine showed that the $\alpha_2$-adrenergic receptors are inhibitory to islet DNA synthesis and insulin secretion. Thus our results suggest that decreased $\alpha_2$-adrenergic receptors during pancreatic regeneration functionally regulate insulin secretion and pancreatic β-cell proliferation in weanling rats.


*Keywords:* $\alpha_2$-Adrenergic receptors; Pancreatectomy; Brain stem; Pancreatic islets; Insulin secretion

## Introduction

$\alpha_2$-Adrenoceptors are involved in the inhibition of insulin release induced by sympathetic nerve stimulation (Niddam et al., 1990). The $\alpha_2$-adrenergic receptor appears to be an important modulator of noradrenergic (NE) neurotransmission in the brain (Aantaa et al., 1995). Norepinephrine, a principal neurotransmitter of the sympathetic nervous system inhibits insulin secretion both in vivo and in vitro (Filipponi et al., 1986) via $\alpha_2$-adrenergic receptors on the rat islet cell membrane (Lackovic et al., 1990). The pharmacological characterisation of $\alpha_2$-adrenoceptors has been facilitated by the introduction of [$^3$H]Yohimbine (Cheung et al., 1982). It was reported that the inhibition of insulin release due to epinephrine (EPI) is mediated through $\alpha_2$-adrenergic receptors (Ullrich and Wollheim, 1985).

Central nervous system through parasympathetic and sympathetic pathways regulates insulin secretion from pancreatic islets and maintains glucose homeostasis (Ahren, 2000). Brain stem serves as one of the key centres of the central nervous system regulating the body homeostasis. Stimulation of the peripheral vagus nerve leads to an increase in circulating insulin levels. Anatomical studies suggest that the origin of these vagal efferent fibres is nucleus ambiguus and dorsal motor nucleus directly innervating pancreas (Bereiter et al., 1981). Epinephrine secreted after central nervous system stimulation increased glucagon secretion from the pancreas (Gerich et al., 1976) and inhibited insulin secretion (Porte et al., 1966).

In diabetic condition, $\alpha_2$-adrenergic receptors are more activated which brought out the insulin inhibition and in turn hyperglycaemia (Lacey et al., 1993). The rat islet cell membrane is equipped with $\alpha_2$-adrenoceptors which are linked to

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adenylate cyclase to cause diminution of the cellular content of cyclic AMP; insulin secretion may be inhibited consequently (Yamazaki et al., 1982). Earlier studies from our laboratory have established the central neurotransmitter receptor subtypes functional regulation during pancreatic regeneration, diabetes and cell proliferation (Abraham and Paulose, 1999; Biju et al., 2001; Renuka et al., 2004; Mohanan et al., 2005a,b). There is much evidence to suggest that prolonged stimulation of insulin secretion in vivo leads to a compensatory increase of the total volume of the pancreatic islets in partially pancreatectomised rats (Martin and Lacy, 1963). Studies conducted have demonstrated that insulin secretion in response to glucose from β-cells of the endocrine pancreas can be modified by the activity of both the sympathetic and parasympathetic branches of the autonomic nervous system (Burr et al., 1976; Campfield and Smith, 1980). In the present study, we investigated the $\alpha_2$-adrenergic receptor functional alterations in brain stem and pancreatic islets during pancreatic regeneration in weanling rats.

## Materials and methods

### *Biochemicals*

All the reagents were of analytical grade. Biochemicals used in the present study were purchased from Sigma Chemical Co., USA. [*O*-methyl-$^3$H]Yohimbine (Sp. activity 88 Ci/mmol), [$^3$H]Thymidine (Sp. activity 25 Ci/mmol) were purchased from Amersham Life science, UK.

### *Animals*

Wistar weanling rats of 80–100 g body weight purchased from Central Institute of Fisheries Technology, Cochin were used for all experiments. They were housed in separate cages in 12-h light and 12-h dark periods and were maintained on standard food pellets and water ad libitum. All animal care and procedures were taken in accordance with the institutional and National Institute of Health guidelines.

### *Partial pancreatectomy*

The rats were anaesthetised under aseptic conditions, the body wall was cut opened and 60–70% of the total pancreas, near to the spleen and duodenum, was removed (Pearson et al., 1977). The removal of most of the pancreas was done by gentle abrasion with cotton applications, leaving the major blood vessels supplying other organs intact (Wei et al., 1995). The sham operation was done in an identical procedure except that the pancreatic tissue was only lightly rubbed between fingertips using cotton for a minute instead of being removed. All the surgeries were done between 7 am and 9 am to avoid diurnal variations. The rats were maintained for different time intervals, 72 h and 7 days. The sham, 72-h and 7-day pancreatectomised rats were sacrificed by decapitation and the brain regions were

### *Quantification of EPI and NE*

EPI and NE contents in the brain stem and pancreatic islets were determined using high performance liquid chromatography (HPLC) integrated to electrochemical detector (HPLC-ECD) (Shimadzu, Japan) and fitted with CLC-ODS reverse phase columns of 5 μm particle size. A 10% homogenate of the tissue was made in 0.4 N perchloric acid. The homogenate was centrifuged at 5000×*g* for 10 min at 4 °C (Kubota refrigerated centrifuge) and the clear supernatant was filtered through 0.45 μm HPLC grade filters and used for HPLC analysis (Paulose et al., 1988). Mobile phase was 75 mM sodium dihydrogen orthophosphate buffer containing 1 mM sodium octyl sulphonate, 50 mM EDTA and 7% acetonitrile (pH 3.25), filtered through 0.22 μm filter delivered at a flow rate of 1.0 ml/min. Quantification was by electrochemical detection, using a glass carbon electrode set at +0.80 V. The peaks were identified by relative retention time compared with standards and concentrations were determined using a Shimadzu integrator interfaced with the detector.

### *$\alpha_2$-Adrenergic receptor analysis*

The $\alpha_2$-adrenergic receptor binding status was studied using [$^3$H]Yohimbine. The assay was done according to the modified procedure of Repaske et al. (1987). The brain stem and pancreatic islets were homogenised in 20 volumes of ice cold Tris buffer containing 4 mM $MgCl_2$, 2 mM EGTA, 10 mM benzamidine and 5 mM PMSF (pH 7.4) in a Potter-Elvejhem homogeniser. The homogenate was centrifuged at 900×*g* for 10 min and the supernatant again centrifuged at 30,000×*g* for 60 min. The pellet was resuspended in 50 volumes of 50 mM Tris–HCl, pH 7.5 and re-centrifuged at 17,000×*g* for another 1 h. The final pellet was resuspended in a minimum volume of incubation buffer containing 25 mM glycyl glycine, 10 mM HEPES, 100 mM NaCl, 2 mM EGTA, (pH 7.6).

Membrane binding assays were done using different concentrations [$^3$H]Yohimbine i.e., 1.0 nM–12.5 nM for brain stem and 1.0 nM–50.0 nM for pancreatic islets along with appropriate protein concentrations. Nonspecific binding was determined using 100 μM unlabelled phentolamine. Competition studies were carried out with 0.5 nM and 2.5 nM [$^3$H] Yohimbine in each tube with the unlabelled yohimbine concentrations varying from $10^{-9}$ to $10^{-4}$ M for brain stem and pancreatic islets respectively. The tubes were incubated at 15 °C for 90 min and filtered rapidly through GF/C filters (Whatman). Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter. The nonspecific binding determined showed 30–40% in all our experiments. Protein was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

The receptor binding parameters were determined using Scatchard's (1949) analysis. The maximal binding ($B_{max}$) and equilibrium dissociation constant ($K_d$) were derived by linear

Competitive binding data were analysed using nonlinear regression curve-fitting procedure (GraphPad PRISM™, San Diego, USA). The concentration of competitor that competes for half the specific binding was defined as $EC_{50}$. It is same as $IC_{50}$. The affinity of the receptor for the competing drug is designated as $K_i$ and is defined as the concentration of the competing ligand that will bind to half the binding sites at equilibrium in the absence of radioligand or other competitors (Cheng and Prusoff, 1973).

The data of the competitive binding assays are represented graphically with the negative log of concentration of the competing drug on *X*-axis and percentage of the radioligand bound on the *Y*-axis. The Hill slope was used to indicate a one- or two-site model of curve-fitting.

Statistical evaluations were done by ANOVA using InStat (Ver.2.04a) computer programme. Linear regression Scatchard plots were made using SIGMA PLOT (Ver 2.03).

*Isolation of pancreatic islets*

Pancreatic islets were isolated from male Wistar rats by standard collagenase digestion procedures using aseptic techniques (Howell and Taylor, 1968). The islets were isolated in HEPES-buffered sodium free Hanks Balanced Salt Solution (HBSS) with the following composition: 137 mM choline chloride, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM $KH_2PO_4$, 14.3 mM $KHCO_3$ and 10 mM HEPES (Pipeleers et al., 1985). Pancreas was aseptically dissected and transferred to a sterile glass vial containing 2 ml collagenase type XI solution (1.5 mg/ml in HBSS), pH 7.4. The collagenase digestion was carried out for 20 min at 37 °C in an environmental shaker with vigorous shaking (300 rpm/min). The tissue digest was filtered through 500 μm nylon screen and the filtrate was washed with three successive centrifugations and resuspended in cold HBSS. The islets preparation having a viability of >90% as assessed by Trypan Blue exclusion was chosen for cell culture and other experiments.

*In vitro DNA synthesis studies in the pancreatic islets*

Pancreatic islets were prepared by the collagenase digestion method as mentioned earlier. The isolated islets were then suspended in RPMI-1640 medium containing 10% FCS and incubated for 16 h at 37 °C and 5% $CO_2$ to remove the fibroblasts. After fibroblast removal, the cells were cultured for 3 days to remove all other non-endocrine tissue. Then the pancreatic islets (100 islets/ml medium) were transferred to 1 ml fresh medium containing 5% FCS, antibiotics, 4 mM glucose, $10^{-4}$ M EPI and $10^{-5}$ M yohimbine were incubated for 24 h in the presence of 1 μCi of [$^3$H]Thymidine (Sjoholm, 1991). DNA was extracted with 5% TCA according to Schneider (1957) and estimated by diphenylamine procedure of Burton (1955). The radioactivity incorporated was determined by counting in a scintillation counter.

*In vitro 24-h insulin secretion study in the pancreatic islets*

Pancreatic islets were harvested after removing the fibroblasts and cultured for 24 h in RPMI-1640 medium. The insulin secretion study was carried out by preincubating the cells (100 islets/ml medium) with 4 mM glucose, $10^{-4}$ M EPI and $10^{-5}$ M yohimbine (MacDonald et al., 1990). The cells were then harvested and washed with fresh Krebs Ringer Bicarbonate Buffer, pH 7.3 and then incubated for another 1 h in the presence of same concentration of glucose, EPI and yohimbine. At the end of incubation period, the medium was collected and insulin content was assayed according to the procedure of BARC radioimmunoassay kit. Protein was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard. Insulin concentration in the samples was determined from standard curve plotted using MultiCalc™ software (Wallac, Finland).

## Results

The HPLC analysis of brain stem and pancreatic islets of experimental rats showed a significant decrease ($p<0.001$) in NE content at 72 h after partial pancreatectomy. The EPI content showed a significant decrease ($p<0.001$) in pancreatic islets while it was not detected in brain stem (Table 1). The changed parameters reversed to near sham by 7 days after partial pancreatectomy.

Scatchard analysis of [$^3$H]Yohimbine in the brain stem showed a significant decrease ($p<0.05$) in $B_{max}$ and $K_d$ at 72 h

Table 1
Epinephrine and norepinephrine content (nmol/g wet wt. of tissue) in the brain stem and pancreatic islets of sham and pancreatectomised young rats

| Animal status | Brain stem | | Pancreatic islets | |
|---|---|---|---|---|
| | NE | EPI | NE | EPI |
| Sham | 27.75±0.34 | 32.10±10.24 | 43.53±3.52 | 211.30±9.92 |
| 72-h pancreatectomy | 11.02±0.07[a] | ND | 16.62±3.48[a] | 102.84±6.82 |
| 7-day pancreatectomy | 28.34±0.50[b] | 44.50±8.10 | 46.62±3.68[b] | 142.10±7.74[a,c] |

Values are means±S.E.M. of 4–6 separate experiments.
NE—norepinephrine; EPI—epinephrine.
ND—not detectable.
The contents were determined using HPLC connected with EC detector and the values were integrated Shimadzu chromatopac with standard values.
[a] $p<0.001$ when compared to sham.

Table 2
Scatchard analysis of [$^3$H]Yohimbine binding against phentolamine in the brain stem of sham and pancreatectomised young rats

| Animal status | [$^3$H]Yohimbine binding | |
|---|---|---|
| | $B_{max}$ (fmol/mg protein) | $K_d$ (nM) |
| Sham | 67.00±9.00 | 4.28±0.25 |
| 72-h pancreatectomy | 24.33±28.00* | 2.59±0.81* |
| 7-day pancreatectomy | 71.00±13.53 | 7.63±2.90 |

Values are means±S.E.M. of 4–6 separate experiments.
$B_{max}$—maximal binding; $K_d$—dissociation constant.
Membrane binding assays were done using different concentrations [$^3$H] Yohimbine, i.e., 1.0 nM–12.5 nM for brain stem along with appropriate protein concentrations. Nonspecific binding was determined using 100 μM unlabelled phentolamine
* $p<0.05$ when compared to sham.

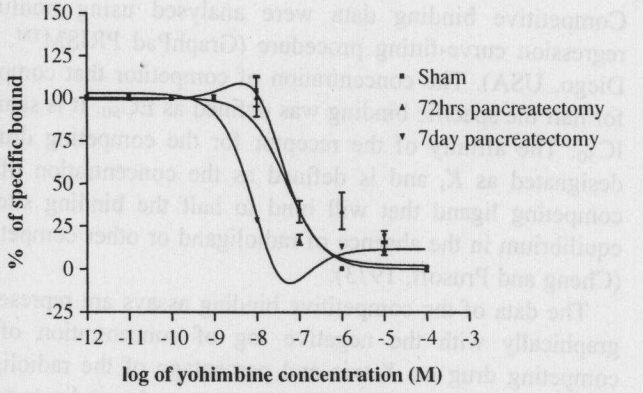


Fig. 1. Displacement of [$^3$H]Yohimbine against yohimbine in the brain stem of sham, 72-h and 7-day pancreatectomised rats. ■: Sham, ▲: 72-h pancretectomy, ▼: 7-day pancreatectomy. Competition studies were carried out with 0.5 nM [$^3$H]Yohimbine in each tube with the unlabelled yohimbine concentrations varying from $10^{-9}$ to $10^{-4}$ M. The tubes were incubated at 15 °C for 90 min and filtered rapidly through GF/C filters (Whatman). The filters were washed quickly by three successive washing with 10.0 ml of ice cold buffer containing 25 mM glycyl glycine, pH 7.6. Values are representation of 4–6 separate experiments.

after partial pancreatectomy compared with sham. The binding parameters reversed to near sham by 7 days after pancreatectomy (Table 2). The competition curve for yohimbine against [$^3$H]Yohimbine fitted a one-site model in all the groups with Unity as Hill slope value. The $K_i$ and log ($EC_{50}$) showed no change in 72-h pancreatectomised rats compared with sham indicating no shift in affinity (Table 3, Fig. 1).

Scatchard analysis of [$^3$H]Yohimbine in the pancreatic islets showed a significant decrease ($p<0.001$) in $B_{max}$ and $K_d$ ($p<0.05$) at 72 h after partial pancreatectomy compared with sham. The binding parameters reversed to near sham by 7 days after pancreatectomy (Table 4). The competition curve for yohimbine against [$^3$H]Yohimbine fitted a one-site model in all the groups with Unity as Hill slope value. The $K_i$ and log ($EC_{50}$) showed no change in 72 h after partial pancreatectomised rats compared with sham indicating no shift in affinity (Table 5, Fig. 2).

Yohimbine showed a significant increase in islets DNA synthesis ($p<0.01$) and insulin secretion ($p<0.001$) in the in vitro culture study (Table 6).

## Discussion

Pancreatic regeneration after pancreatectomy has been well documented in animal models (Pearson et al., 1977). Pancreatectomy is a 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 (Mohanan et al., 2005b). This maintenance of glucose homeostasis is due to regeneration among the remaining pancreatic β-cells and their excess production of insulin (Leahy et al., 1988; Lohr et al., 1989). [$^3$H]Thymidine incorporation into the islet DNA showed that the peak DNA synthesis in pancreatic islets is at 72 h after partial pancreatectomy, i.e., during active pancreatic regeneration and decline at 7 days after pancreatectomy (Mohanan et al., 2005a). The circulating insulin levels showed a significant increase without any change in the glucose at 72 h after partial pancreatectomy, when there is maximum DNA synthesis (Renuka et al., 2004). Increased islet DNA synthesis and glucose-derived lipid and amino acid production in association with β-cell hyperproliferation are reported in normoglycaemic 60% pancreatectomised rats (Liu et al., 2001).

Table 3
Binding parameters of [$^3$H]Yohimbine against yohimbine in the brain stem of sham and pancreatectomised young rats

| Experimental group | Best-fit model | Log ($EC_{50}$) | $K_i$ | Hill slope |
|---|---|---|---|---|
| Sham | One-site | −7.17 | $4.25\times10^{-8}$ | −4.72 |
| 72-h pancreatectomy | One-site | −7.43 | $2.35\times10^{-8}$ | −2.67 |
| 7-day pancreatectomy | One-site | −8.23 | $3.68\times10^{-9}$ | −4.47 |

Values are means of 4–6 separate experiments.
Data were fitted with an iterative nonlinear regression software (Prism, GraphPad, San Diego, CA). $K_i$—the affinity of the receptor for the competing drug.
$EC_{50}$ is the concentration of the competitor that competes for half the specific binding.
Competition studies were carried out with 0.5 nM [$^3$H]Yohimbine in each tube

Table 4
Scatchard analysis of [$^3$H]Yohimbine binding against phentolamine in the pancreatic islets of sham and pancreatectomised young rats

| Animal status | $B_{max}$ (fmol/mg protein) | $K_d$ (nM) |
|---|---|---|
| Sham | 170.00±13.53 | 4.59±0.20 |
| 72-h pancreatectomy | 40.00±6.08[a] | 2.67±0.11[b] |
| 7-day pancreatectomy | 190.00±15.88[c] | 6.67±0.98[c] |

Values are means±S.E.M. of 4–6 separate experiments.
$B_{max}$—maximal binding; $K_d$—dissociation constant.
Membrane binding assays were done using different concentrations [$^3$H] Yohimbine, i.e., 1.0 nM–50.0 nM for pancreatic islets along with appropriate protein concentrations. Nonspecific binding was determined using 100 μM unlabelled phentolamine.
[a] $p<0.001$ when compared to sham.

Table 5
Binding parameters of [$^3$H]Yohimbine against yohimbine in the pancreatic islets of sham and pancreatectomised young rats

| Experimental group | Best-fit model | Log ($EC_{50}$) | $K_i$ | Hill slope |
|---|---|---|---|---|
| Sham | One-site | −5.72 | $1.28\times10^{-6}$ | −2.09 |
| 72-h pancreatectomy | One-site | −7.57 | $1.82\times10^{-6}$ | −1.17 |
| 7-day pancreatectomy | One-site | −8.43 | $2.47\times10^{-6}$ | −7.40 |

Values are means of 4–6 separate experiments.
Data were fitted with an iterative nonlinear regression software (Prism, GraphPad, San Diego, CA). $K_i$—the affinity of the receptor for the competing drug.
$EC_{50}$ is the concentration of the competitor that competes for half the specific binding.
Competition studies were carried out with 2.5 nM [$^3$H]Yohimbine in each tube with the unlabelled yohimbine concentrations varying from $10^{-9}$ to $10^{-4}$ M for pancreatic islets

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 et al., 1978). Recent observations indicate that insulin can stimulate pancreatic β-cell growth in vivo. Also, it is reported that administration of insulin to diabetic rat implanted with fetal pancreas showed a three-fold increase in β-cell mass (McEvoy and Hegre, 1977). Insulin caused a significant increase in the [$^3$H] Thymidine incorporation as well as the mitotic activity in β-cells of neonatal rats maintained in monolayer cultures.

The result of the present study indicates a significant decrease in EPI and NE content in the brain stem and pancreatic islets at 72 h after partial pancreatectomy in weanling rats compared to sham. It is reported that EPI and NE contents in the brain regions were increased significantly in the streptozotocin-diabetic rats (Lackovic et al., 1990; Chen and Yang, 1991; Tasaka et al., 1992). This shows that during pancreatic regeneration, there is sympathetic stimulation. The increased sympathetic activity inhibits the insulin secretion from the pancreatic islets (Efendic et al., 1978; Renstrom et al., 1996) which in turn suppresses β-cell proliferation during pancreatic

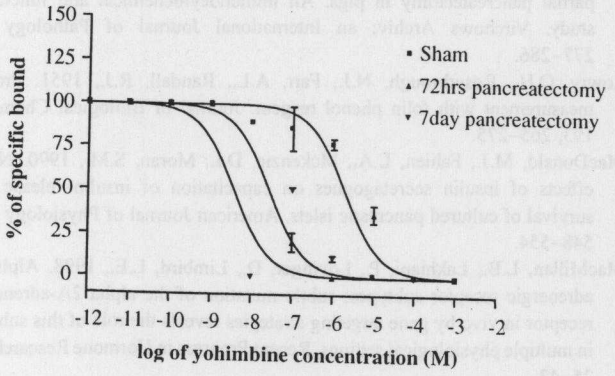


Fig. 2. Displacement of [$^3$H]Yohimbine against yohimbine in the pancreatic islets of sham, 72-h and 7-day pancreatectomised rats. ■: Sham, ▲: 72-h pancretectomy, ▼: 7-day pancreatectomy. Competition studies were carried out with 2.5 nM [$^3$H]Yohimbine in each tube with unlabelled yohimbine concentrations varying from $10^{-9}$ to $10^{-4}$ M. The tubes were incubated at 15 °C for 90 min and filtered rapidly through GF/C filters (Whatman). The filters were washed quickly by three successive washing with 10.0 ml of ice cold buffer containing 25 mM glycyl glycine, pH 7.6. Vol

Table 6
Effect of yohimbine on pancreatic islet DNA synthesis and 24-h insulin secretion in vitro

| Experimental status | Islet DNA synthesis[a] (DPM/mg DNA) | Insulin secretion[b] (μU/mg protein) |
|---|---|---|
| Without yohimbine | 3321.11±99.04 | 55.22±8.42 |
| With yohimbine | 5826.21±126.32* | 149.45±7.26** |

Values are means±S.E.M. of 4–6 separate experiments.
[a] Pancreatic islets were incubated in RPMI-1640 medium with 4 mM glucose, $10^{-4}$ M EPI, $10^{-5}$ M yohimbine and 1 μCi of [$^3$H]Thymidine. The radioactivity incorporated was determined by counting in a scintillation counter.
[b] Pancreatic islets were incubated in RPMI-1640 medium with 4 mM glucose, $10^{-4}$ M EPI and $10^{-5}$ M yohimbine for 24 h. Later 1-h incubation was carried out in KRB. Insulin content was assayed according to the procedure of BARC radioimmunoassay kit. Insulin concentration in the samples was determined from standard curve plotted using MultiCalc™ software (Wallac, Finland).
* $p<0.01$ when compared to without yohimbine.
** $p<0.001$ when compared to without yohimbine.

regeneration. The decrease in the EPI and NE levels stimulates insulin secretion from pancreatic islets at the time of regeneration. Previous studies from our laboratory reported the decrease in norepinephrine and epinephrine content in the adrenals during pancreatic regeneration in rats (Renuka et al., 2004).

Adrenal medullary hormones inhibit insulin release via the mediation of $\alpha_2$-adrenergic receptor (Lacey et al., 1993). The pancreatic islet β-cells have more $\alpha_2$- than $\alpha_1$-adrenergic receptors (Ahren, 2000). [$^3$H]Yohimbine binding to $\alpha_2$-adrenergic receptors of the brain stem and pancreatic islets at 72-h pancreatectomised rats showed a significant reduction in the receptor number with an increased affinity. This decrease in the number of $\alpha_2$-adrenergic receptors indicates that these receptors are down-regulated during pancreatic islet cell proliferation. The affinity increase observed might be a compensatory mechanism. $\alpha_2$-Adrenergic receptors are one of the potent inhibitors of insulin secretion from the islets (Moratinos et al., 1988; John et al., 1990). $\alpha_2$-Adrenoceptor stimulation by the endogenous catecholamines could lead to inhibition of insulin release, masking any potentiated response that otherwise should have appeared from $\alpha_1$- and β-adrenoceptor stimulation (Garcia-Barrado et al., 1998). In diabetic condition, $\alpha_{2A}$ receptors are more activated which brought out the insulin inhibition and in turn hyperglycaemia (Lacey et al., 1993). Although there are reports, which say the role of brain $\alpha_2$-adrenergic receptors in the insulin secretion, there are not much studies about the inhibitory action of this receptor subtype on the islet DNA synthesis. The rate of DNA synthesis, insulin secretion and cAMP content in the isolated pancreatic islets were markedly inhibited by long-term exposure to the $\alpha_2$-adrenergic agonist, clonidine (Sjoholm, 1991).

In vitro studies with yohimbine showed that the insulin secretion from the pancreatic islets increased significantly suggesting that when the $\alpha_2$-adrenergic receptors are blocked, it enhances islet cell proliferation and insulin secretion. $\alpha_2$-

possible role of adrenergic receptors in regulating the islet cell proliferation is mediated by cAMP (Sjoholm, 1991). The $\alpha_{2A}$-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 results show that the $\alpha_2$-adrenergic receptors functional decrease in the brain stem and pancreatic islets stimulate insulin secretion and islets cell proliferation during pancreatic regeneration in weanling rats.

## Conclusion

The pancreatic insulin secretion and islet cell proliferation is regulated by the central nervous system function. The decrease in the EPI and NE levels stimulates insulin secretion from pancreatic islets at the time of regeneration. $\alpha_2$-adrenergic receptors functional decrease in the brain stem and pancreatic islets at 72-h pancreatectomised rats, stimulate insulin secretion and pancreatic β-cell proliferation in weanling rats during pancreatic regeneration. Our in vitro studies on islet DNA synthesis and insulin secretion confirm the functional role of $\alpha_2$-adrenergic receptors. This will have immense clinical significance in therapeutic application of diabetes.

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