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Enhanced β-adrenergic receptors in the brain and pancreas during pancreatic regeneration in weanling rats

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Abstract

Adrenergic stimulation has an important role in the pancreatic β-cell proliferation and insulin secretion. In the present study, we have investigated how sympathetic system regulates the pancreatic regeneration by analyzing Epinephrine (EPI), Norepinephrine (NE) and β-adrenergic receptor changes in the brain as well as in the pancreas. EPI and NE showed a significant decrease in the brain regions, pancreas and plasma at 72 hrs after partial pancreatectomy. We observed an increase in the circulating insulin levels at 72 hrs. Scatchard analysis using [^{3}H] propranolol showed a significant increase in the number of both the low affinity and high affinity β-adrenergic receptors in cerebral cortex and hypothalamus of partially pancreatectomised rats during peak DNA synthesis. The affinity of the receptors decreased significantly in the low and high affinity receptors of cerebral cortex and the high affinity hypothalamic receptors. In the brain stem, low affinity receptors were increased significantly during regeneration whereas there was no change in the high affinity receptors. The pancreatic β-adrenergic receptors were also up regulated at 72 hrs after partial pancreatectomy. *In vitro* studies showed that β-adrenergic receptors are positive regulators of islet cell proliferation and insulin secretion. Thus our results suggest that the β-adrenergic receptors are functionally enhanced during pancreatic regeneration, which in turn increases pancreatic β-cell proliferation and insulin secretion in weanling rats.

Key words: pancreatic regeneration, epinephrine, norepinephrine, β-adrenergic receptors, propanolol

Introduction

The pancreatic islets form a highly innerved organ, receiving sympathetic neuron inflow via the splanchnic nerves. The insulin secretion from pancreatic islets is controlled by central nervous system through sympathetic and parasympathetic nerves [1-3]. Brain stem along with hypothalamus serves as 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 vagal efferent fibres originating from nucleus ambiguous and dorsal motor nucleus of brain stem directly innervate pancreas [4] and have a role in neurally mediated insulin release [5]. Hypothalamus also has an important regulatory role in pancreatic secretion [6]. It has been reported that the autonomic nervous system is one of the important factors that regulate pancreatic regeneration and stimulate the carcinogenesis [7]. Recent studies from our laboratory reported the regulatory role of sympathetic system and parasympathetic system in pancreatic regeneration [8–11].

Stimulation of insulin release by adrenergic substances is mediated through β-receptors [12]. The potential of the pancreatic islets to regenerate and the effects of sympathetic

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stimulation on regeneration after partial pancreatectomy are not completely understood. Our previous studies reported that DNA synthesis was maximum at 72 hours after partial pancreatectomy [8]. Since insulin favored regeneration of β-cell by activating the neogenesis of the β-cells from precursor cells [13], factors affecting insulin secretion can affect the pancreatic regeneration. Epinephrine (EPI) and norepinephrine (NE), the major sympathetic neurotransmitters control the insulin secretion in a concentration dependant manner, mediated through different adrenergic receptors. Low concentrations of EPI and NE can bind and activate β-adrenergic receptors, which stimulate the insulin secretion from the islets [14]. Since the β-adrenergic receptors are stimulatory to insulin secretion, it can stimulate the pancreatic islet regeneration. The reports so far did not attempt a study of the β-adrenergic receptor alterations in the brain regions and pancreas during pancreatic regeneration, which has a role in the regulation of insulin secretion from the pancreatic islets. In the present study we investigated the involvement of β-adrenergic receptors in pancreatic regeneration. The study showed an enhanced functional β-adrenergic receptor activity during pancreatic regeneration in weanling rats.

Materials and methods

Chemicals

All biochemicals used were of analytical grade. HPLC standards, propranolol and Fetal Calf Serum (FCS) were obtained from Sigma Chemical Co., U.S.A. L-[4-^{3}H] propranolol (21 Ci/mmol) was purchased from NEN life sciences products Inc., Boston, U.S.A. [^{3}H] Thymidine (18 Ci/mmol) and Radioimmunoassay kits were purchased from BARC, Mumbai, India.

Animals

Weanling Wistar rats (3–4 weeks old) 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 under 12 hrs light and 12 hrs dark periods and controlled temperature and were maintained on standard food pellets and water *ad libitum*. Animal care and procedures were done according to the institutional and National Institute of Health Guide lines.

Partial pancreatectomy

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 [15]. The removal of most of the pancreas was done by gentle abrasion with cotton applications, leaving the major blood vessels supplying other organs intact [16]. 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 a.m. and 9 a.m. to avoid diurnal variations in responses. Body weight and blood glucose levels were checked routinely. The rats were maintained for different time intervals, 24, 48 and 72 hrs; 7 and 14 days. They were sacrificed by decapitation at the end of the time interval. The brain regions and pancreas were dissected out quickly over ice according to the procedure of Glowinski and Iversen [17] and stored at −70 °C until assay.

Estimation of blood glucose and circulating insulin

Blood glucose was estimated by Glucose estimation kit (Merck) using glucose oxidase-peroxidase method. The insulin assay was done according to the procedure of BARC radioimmunoassay kit. The radioimmunoassay method is based on the competition of unlabelled insulin in the standard or samples and [^{125}I] insulin for the limited binding sites on a specific antibody.

Isolation of pancreatic islets

Pancreatic islets were isolated from male weanling Wistar rats by standard collagenase digestion procedures using aseptic techniques [18]. 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. The pancreas was aseptically transferred to a sterile glass vial containing 2.0 ml collagenase type XI solution (1.5 mg/ml in HBSS), pH 7.4. The collagenase digestion was carried out for 15 minutes at 37 °C in an environmental shaker with vigorous shaking (300 rpm/minute). The tissue digest was filtered through 500 μm nylon screen and the filtrate was washed with three successive centrifugations and resuspensions in cold HBSS. The pancreatic islet preparation having a viability of > 90% as assessed by Trypan Blue exclusion was chosen for cell culture and other experiments.

Quantification of EPI and NE

Circulating EPI and NE were assayed according to Jackson *et al.* [19] using high performance liquid chromatography

(HPLC) integrated with an electrochemical detector (HPLC-ECD) (Shimadzu, Japan) fitted with C18-CLC-ODS reverse phase column. 1.0 ml plasma was diluted in equal volume of distilled water. 50 μl of 5 mM sodium bisulphite was added to it followed by 250 μl of 1 mM Tris buffer of pH 8.6. Acid alumina (20 mg) was then added, mixed for 20 minutes, the supernatant aspirated off and alumina was washed with 2.0 ml of sodium bisulphite. To the final pellet of alumina, 0.2 ml of 0.1N perchloric acid was added. The supernatant was filtered through 0.22 μm HPLC grade filters and used for HPLC determinations. 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/minute. 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.

EPI and NE contents of brain regions and pancreatic islets were quantified according to Paulose *et al.* [20]. A 10% homogenate of the tissue was made in 0.4N perchloric acid. The homogenate was centrifuged at 5000 × *g* for 10 minutes at 4 C (Kubota Refrigerated Centrifuge, Japan) and the clear supernatant was filtered through 0.22 μm HPLC grade filters and used for HPLC analysis as mentioned above. Data from different brain regions, pancreas and plasma of the experimental and control rats were statistically analysed.

β-Adrenergic receptor analysis

[^{3}H] propranolol, a specific antagonist, was used to analyse the β-adrenergic receptors. The assay was done according to the modified procedure of Lefkowtiz *et al.* [21]. Protein was measured by the method of Lowry *et al.* [22]. Membrane binding assays were done in 0.5 ml incubation buffer containing appropriate protein concentrations (150–200 μg) and different concentrations of [^{3}H] propranolol ranging from 0.5 nM – 50 nM. Non-specific binding was determined using 100μM unlabelled propranolol. The tubes were incubated at 37 °C for 30 min and filtered rapidly through GF/C filters (Whatman). The filters were washed quickly by three successive washing with 10 ml of ice cold buffer containing 50 mM Tris and 10 mM $MgCl_2$, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

The data were analyzed according to Scatchard [23]. The specific binding was determined by subtracting non-specific binding from the total. The binding parameters, maximal binding (B_{max}) and equilibrium dissociation constant (K_d), were derived by linear regression analysis by plotting the specific binding of the radioligand on X-axis and bound/free on Y-axis. The maximal binding is a measure of the total number of receptors present in the tissue and the equilibrium dissociation constant is the measure of the affinity of the receptors for the radioligand. The K_d is inversely related to receptor affinity.

In vitro DNA synthesis study in the pancreatic islets

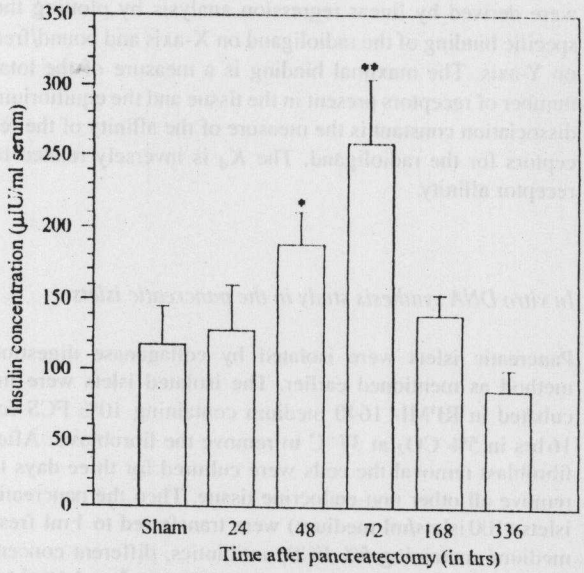
Pancreatic islets were isolated by collagenase digestion method as mentioned earlier. The isolated islets were incubated in RPMI- 1640 medium containing 10% FCS for 16 hrs in 5% CO_2 at 37 °C to remove the fibroblasts. After fibroblast removal the cells were cultured for three days to remove all other non-endocrine tissue. Then the pancreatic islets (100 islets/ml medium) were transferred to 1 ml fresh medium containing 5% FCS, antibiotics, different concentrations of glucose (4 and 20 mM), EPI (10^{-8} and 10^{-4} M) and propanolol (10^{-5} M) and incubated for 24 hrs in the presence of 1 μCi of [^{3}H] thymidine [24]. DNA was extracted with 5% TCA according to Schneider [25] and estimated by diphenylamine procedure [26]. The radioactivity incorporated was determined by counting in a scintillation counter.

In vitro insulin secretion study in the pancreatic islets

Pancreatic islets were isolated by collagenase digestion method and islets were suspended in Krebs Ringer Bicarbonate buffer, pH 7.3 (KRB), of following composition: 115 mM NaCl, 4 mM KCl, 2.56 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$ and 20 mM $NaHCO_3$. The islets were pre-incubated for 1 hr in KRB at 37 °C. The cells were then harvested and resuspended in fresh KRB (100 islets/ml medium) with glucose (4 mM and 20 mM), EPI (10^{-8} and 10^{-4} M) and propranolol (10^{-5} M). After incubation, the supernatant was transferred to fresh tubes for insulin assay. Insulin assay was done according to the procedure of BARC radioimmunoassay kit. Insulin concentration in the samples was determined from the standard curve plotted using MultiCalc^{TM}software (Wallac, Finland).

Statistical analysis

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).



Values are Mean ± S.E.M. of 4-6 separate determinations
*p<0.05 when compared to sham
**p<0.01 when compared to sham

Fig. 1. Circulating insulin levels of the sham and pancreatectomised young rats. Circulating insulin level was measured in sham and experimental rats (24, 48 and 72 hrs; 7 and 14 days after partial pancreatectomy). The insulin assay was done according to the procedure of BARC radioimmunoassay kit. Insulin concentration in the samples was determined from the standard curve plotted using MultiCalc™ software (Wallac, Finland). Values are representation of 4–6 separate experiments.

Results

Body weight and blood glucose level

There was no significant change in the body weights and blood glucose levels of sham operated and pancreatectomised rats.

Circulating insulin level

The insulin levels in the serum of pancreatectomised rats showed a significant increase at 48 hrs ($p < 0.05$) and peaked at 72 hrs after partial pancreatectomy ($p < 0.01$). The increased insulin levels then decreased to near normal by 7 and 14 days (Fig. 1).

EPI and NE content in the brain regions, pancreatic islets and plasma were decreased during pancreatic regeneration

The EPI and NE levels in the plasma were significantly reduced ($p < 0.001$) in the pancreatectomised rats at 72 hrs after pancreatectomy (Table 1). In the cerebral cortex, the EPI and NE contents were significantly decreased at 72 hrs after partial pancreatectomy ($p < 0.001$ and $p < 0.01$ respectively). This decrease was partially reversed to the near control by the 7th day of partial pancreatectomy. In the brain stem EPI was not detected and NE showed a significant reduction ($p < 0.001$) during active DNA synthesis. The EPI and NE contents in hypothalamus showed a significant decrease ($p < 0.001$) at 72 hrs after partial pancreatectomy. The HPLC analysis of isolated pancreatic islets of pancreatectomised rats showed a significant reduction ($p < 0.001$) in the EPI and NE content during active regeneration. The decreased levels of these catecholamines were reversed back to near control at 7days after partial pancreatectomy (Tables 2 and 3).

Table 1. Norepinephrine and Epinephrine content (nmoles/g wet wt. of tissue) in plasma of sham and pancreatectomised young rats

Animal status	NE	EPI
Sham	2.21 ± 0.44	3.26 ± 0.26
72hrs pancreatectomy	0.88 ± 0.09***	0.83 ± 0.12***[III]
7days pancreatectomy	1.01 ± 0.06***	2.75 ± 0.28

Values are Mean ± S.E.M. of 4–6 separate experiments.
*** $p < 0.001$ when compared to sham, [III] $p < 0.001$ when compared to 7days

Brain β-adrenergic receptors were increased at the time of pancreatic regeneration

The Scatchard analysis of β-adrenergic receptors using [^{3}H] propranolol showed two affinity sites. There was a significant increase ($p < 0.05$) in the number of both low and high affinity receptors with an increase in the K_d value in the cerebral cortex at the time of active DNA synthesis. The increased parameters were reversed to near control by 7 day after pancreatectomy (Table 4). In the brain stem, low affinity receptors were increased significantly ($p < 0.05$) without any change in the affinity during regeneration whereas the high affinity receptors did not change. The increased parameters were reversed back to near control in 7 days pancreatectomised rats (Table 5). In the hypothalamus, high affinity receptor number increased significantly ($p < 0.001$) accompanied by a significant reduction ($p < 0.01$) in the receptor affinity. The low affinity receptors were also increased significantly ($p < 0.05$) in the 72 hrs pancreatectomised group with out any change in the affinity of the receptor (Table-6). These results showed that the β-adrenergic receptors were up regulated during pancreatic regeneration.

Table 2. Epinephrine content (nmoles/g wet wt. of tissue) in brain regions and pancreatic islets of sham and pancreatectomised young rats

Animal status	Brain regions			
	Cerebral cortex	Brain stem	Hypothalamus	Pancreas
Sham	0.53 ± 0.11	34.99 ± 11.58	23.03 ± 1.07	210.26 ± 10.21
72 hrs pancreatectomy	0.21 ± 0.09***	ND	1.31 ± 0.18***	104.14 ± 7.35***‡
7 days pancreatectomy	0.73 ± 0.11*‡‡‡	46.67 ± 9.02	44.59 ± 5.65***‡‡‡	144.11 ± 8.98***

Values are Mean ± S.E.M. of 4–6 separate experiments.
*** $p < 0.001$ when compared to sham, * $p < 0.05$ when compared to sham.
‡‡‡ $p < 0.001$ when compared to 72 hrs pancreatectomy, † $p < 0.05$ when compared to 7 days EPI – Epinephrine, ND- Not detected.

Table 3. Norepinephrine content (nmoles/g wet wt. of tissue) in brain regions and pancreatic islets of sham and pancreatectomised young rats

Animal status	Brain regions			
	Cerebral cortex	Brain stem	Hypothalamus	Pancreas
Sham	2.03 ± 0.31	28.86 ± 0.36	30.91 ± 0.69	45.37 ± 3.51
72 hrs pancreatectomy	0.29 ± 0.13**	10.05 ± 0.06***	3.29 ± 0.58***	17.68 ± 3.32***
7 days pancreatectomy	1.74 ± 0.26‡‡‡	29.47 ± 0.49‡‡‡	6.09 ± 0.52***‡	47.95 ± 4.28‡‡‡

Values are Mean ± S.E.M. of 4- 6 separate experiments
*** $p < 0.001$ when compared to sham, * $p < 0.05$ when compared to sham,
‡‡‡ $p < 0.001$ when compared to 72 hrs pancreatectomy, † $p < 0.05$ when compared to 7 days NE – Norepinephrine.

Table 4. Scatchard analysis of [^{3}H] Propranolol binding against propranolol in the cerebral cortex of sham and pancreatectomised young rats

Animal status	High affinity		Low affinity	
	B_{max} (fmoles/mgprotein)	K_d (nM)	B_{max} (fmoles/mg protein)	K_d (nM)
Sham	7.67 ± 1.86	0.81 ± 0.07	147.67 ± 26.28	13.58 ± 0.95
72 hrs pancreatectomy	26.67 ± 6.22	4.29 ± 0.44***	314.33 ± 57.20*	113.44 ± 16.21***
7 days pancreatectomy	10.50 ± 2.01	2.26 ± 0.33‡‡	145.00 ± 25.50	40.26 ± 5.36

Values are Mean ± S.E.M. of 4–6 separate experiments.
*** $p < 0.001$ when compared to sham, * $p < 0.05$ when compared to sham.
‡‡ $p < 0.01$ when compared to 72 hr pancreatectomy.
B_{max} – Maximal binding; K_d – Dissociation constant.

Table 5. Scatchard analysis of high affinity [^{3}H] propranolol binding against propranolol in the brain stem of sham and pancreatectomised young rats

Animal status	High affinity		Low affinity	
	B_{max} (fmoles/mg protein)	K_d (nM)	B_{max} (fmoles/mg protein)	K_d (nM)
Sham	22.50 ± 5.95	3.40 ± 0.86	280.00 ± 60.00	44.70 ± 6.20
72 hrs pancreatectomy	31.00 ± 9.71	2.49 ± 0.60	546.00 ± 53.00*	54.17 ± 12.74
7 days pancreatectomy	17.50 ± 2.50	2.16 ± 0.55	306.67 ± 12.02	43.97 ± 3.90

Values are Mean ± S.E.M. of 4–6 separate experiments.
* $p < 0.05$ when compared to sham.

Table 6. Scatchard analysis of high affinity [^{3}H] propranolol binding against propranolol in the hypothalamus of sham and pancreatectomised young rats

	High affinity		Low affinity	
Animal status	B_{max}(fmoles/mgprotein)	K_d(nM)	B_{max}(fmoles/mgprotein)	K_d (nM)
Sham	9.16 ± 1.67	1.32 ± 0.13	370.00 ± 20.00	44.07 ± 4.22
72 hrs pancreatectomy	48.00 ± 5.69***	4.65 ± 0.64**	551.67 ± 16.42*	36.99 ± 1.96
7 days pancreatectomy	36.66 ± 4.41**	3.02 ± 0.78	456.67 ± 86.67	33.37 ± 7.54

Values are Mean ± S.E.M. of 4–6 separate experiments.
*** $p < 0.001$ when compared to sham, ** $p < 0.01$ when compared to sham.
* $p < 0.05$ when compared to sham.

Table 7. Scatchard analysis of [^{3}H] Propranolol binding against propranolol in the pancreatic islets of sham and pancreatectomised young rats

	High affinity		Low affinity	
Animal status	B_{max} (fmoles/mg protein)	K_d (nM)	B_{max} (fmoles/mg protein)	K_d (nM)
Sham	7.50 ± 1.98	0.21 ± 0.05	90.00 ± 9.00	4.09 ± 0.89
72 hrs pancreatectomy	30.00 ± 9.00***	0.36 ± 0.08	290.00 ± 10.58***	3.81 ± 0.91
7 days pancreatectomy	13.00 ± 1.46*	0.14 ± 0.02	110.00 ± 14.33	2.57 ± 0.58

Values are Mean ± S.E.M. of 4–6 separate experiments.
*** $p < 0.001$ when compared to sham, * $p < 0.05$ when compared to sham.

Pancreatic β-adrenergic receptors are up regulated during active pancreatic regeneration

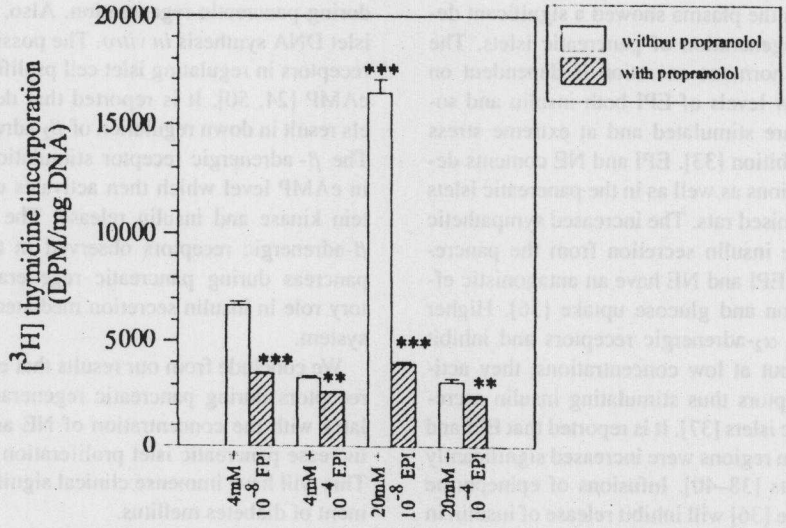
Scatchard analysis of [^{3}H] Propranolol binding against propranolol in the pancreatic islets showed a significant increase ($p < 0.001$) in the B_{max} during active pancreatic DNA synthesis without any change in the K_d (Table-7). This increase was partially reversed back at 7 days after partial pancreatectomy. These results were similar to the receptor changes observed in the brain regions.

Propranolol inhibited DNA synthesis and insulin secretion in vitro

Propranolol, a selective blocker of the β-adrenergic receptors, inhibited the *in vitro* DNA synthesis in the islets significantly ($p < 0.001$). This proves that the β-adrenergic receptors are stimulating the pancreatic growth (Fig. 2). Propranolol could block insulin secretion significantly ($p < 0.01$) (Fig. 3) suggesting that β-adrenergic receptors are positive regulators of pancreatic DNA synthesis and insulin secretion.

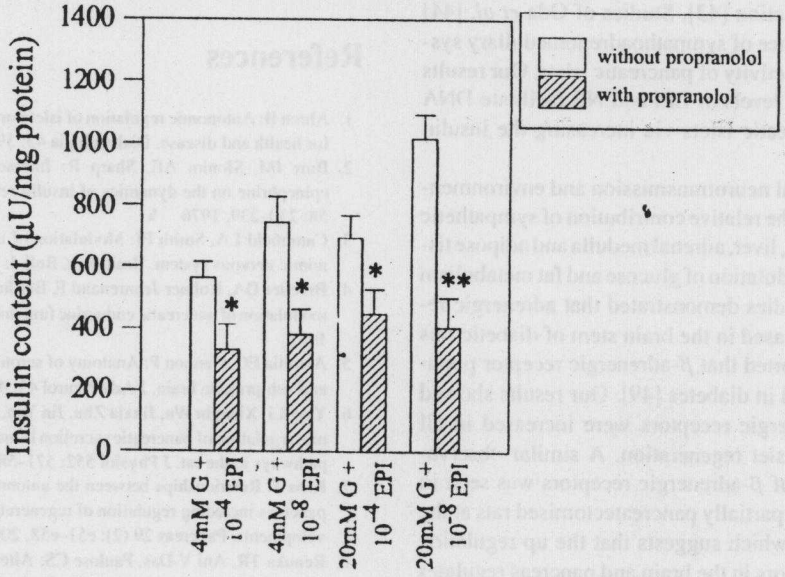
Discussion

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 bodyweight and elevation in the blood glucose levels of the pancreatectomised rats. Pancreatic islets receive innervation from both divisions of the autonomic nervous system and pancreatic endocrine secretion is partly controlled by the autonomic nervous system [27]. Our previous studies reported that the DNA synthesis was maximum at 72 hrs after partial pancreatectomy [8]. The increase in the β-cell proliferation is related to the degree to which insulin biosynthesis and/or release is increased [28, 29]. Also circulating insulin levels increased significantly during pancreatic regeneration keeping the blood glucose level normal [8]. This maintenance of glucose homeostasis is due to regeneration of the remaining pancreatic β-cells and their excess production of insulin [30, 31]. The administration of insulin to diabetic rat implanted with fetal pancreas showed a three-fold increase in β-cell mass. Since insulin favored regeneration of β-cell by activating the neogenesis of the β-cells from precursor cells [13], the factors affecting insulin secretion can affect the pancreatic regeneration. Autonomic nervous system is one of the important factors that regulate pancreatic regeneration and stimulate the carcinogenesis [7]. Recent studies from our laboratory reported the regulatory role of sympathetic system and parasympathetic system in pancreatic regeneration [8–11]. The inhibition of insulin release is an alpha receptor effect while stimulation of insulin release by adrenergic substances is mediated through beta receptors [12, 32].



Values are Mean ± S.E.M. of 4-6 separate determinations
***p<0.001 when compared with respective without antagonists
**p<0.01 when compared with respective without antagonists

Fig. 2. Effect of propanolol on pancreatic islet DNA synthesis *in vitro*. The pancreatic islets were incubated in RPMI-1640 medium with different concentrations of glucose (4 and 20 mM), EPI (10^{-8} and 10^{-4} M), propanolol (10^{-5} M) and 1μCi of [^{3}H] Thymidine. The radioactivity incorporated was determined by counting in a scintillation counter. Values are representation of 4–6 separate experiments.



Values are Mean ± S.E.M. of 4-6 separate determinations
**p<0.01 when compared to without antagonists
*p<0.05 when compared to without antagonists

Fig. 3. Effect of propanolol on insulin secretion from pancreatic islets in 1hr *in vitro* culture. The islets were incubated in KRB with glucose (4 mM and 20 mM), EPI (10^{-8} and 10^{-4} M) and 10^{-5} M propranolol for 1 hr at 37° C. Insulin assay was done according to the procedure of BARC radioimmunoassay kit. Insulin concentration in the samples was determined from the standard curve plotted using MultiCalc™ software (Wallac, Finland). Values are representation of 4–6 separate experiments.

EPI and NE levels in the plasma showed a significant decrease during active regeneration of pancreatic islets. 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 [33]. EPI and NE contents decreased in the brain regions as well as in the pancreatic islets of 72 hrs pancreatectomised rats. The increased sympathetic activity can inhibit the insulin secretion from the pancreatic islets [34, 35, 12]. EPI and NE have an antagonistic effect on insulin secretion and glucose uptake [36]. Higher EPI and NE stimulate α_2-adrenergic receptors and inhibit the insulin secretion, but at low concentrations, they activate β-adrenergic receptors thus stimulating insulin secretion from the pancreatic islets [37]. It is reported that EPI and NE contents in the brain regions were increased significantly in the STZ-diabetic rats [38–40]. Infusions of epinephrine [13] and norepinephrine [36] will inhibit release of insulin in man. This inhibition of insulin release has also been demonstrated in studies on pancreatic slices [37], in isolated islets [41], and in the isolated perfused rat pancreas [42]. Earlier studies from our laboratory reported that EPI and NE content in adrenals decreased at the time of pancreatic regeneration [8]. Activation of splanchnic nerves innervating the adrenals results in the catecholamine release from chromaffin cells into the circulation [43]. Studies of Oda *et al.* [44] confirmed the importance of sympathoadrenomedullary system in controlling the activity of pancreatic islets. Our results suggest that decreased levels of EPI and NE facilitate DNA synthesis in the pancreatic islets via increasing the insulin secretion.

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 [45]. Our previous studies demonstrated that adrenergic receptor's function increased in the brain stem of diabetic rats [46–48]. It is also reported that β-adrenergic receptor populations were decreased in diabetes [49]. Our results showed that the total β-adrenergic receptors were increased in all brain regions during islet regeneration. A similar observation of up regulation of β-adrenergic receptors was seen in the pancreatic islets of partially pancreatectomised rats at the time of regeneration, which suggests that the up regulation of β-adrenergic receptors in the brain and pancreas regulates pancreatic regeneration. The up-regulation of β-adrenergic receptors observed is suggested to be due to the decreased EPI and NE, in the brain regions during active β cell proliferation.

Our experiments with pancreatic islet culture *in vitro* 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 during pancreatic regeneration. Also, propranolol inhibited islet DNA synthesis *in vitro*. The possible role of adrenergic receptors in regulating islet cell proliferation is mediated by cAMP [24, 50]. It is reported that decrease in cAMP levels result in down regulation of β_2 adrenergic receptors [51]. The β- adrenergic receptor stimulation evokes an increase in cAMP level which then activates cAMP dependant protein kinase and insulin release. The increased binding of β-adrenergic receptors observed in the brain regions and pancreas during pancreatic regeneration has its stimulatory role in insulin secretion mediated through sympathetic system.

We conclude from our results that enhanced β-adrenergic receptors during pancreatic regeneration negatively correlates with the concentration of NE and EPI, which in turn increase pancreatic islet proliferation and insulin secretion. This will have immense clinical significance in the management of diabetes mellitus.

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