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ORIGINAL PAPER

Decreased α_1-Adrenergic Receptor Binding in the Cerebral Cortex and Brain Stem during Pancreatic Regeneration in Rats

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Abstract The purpose of this study was to investigate the role of brain α_1-adrenergic receptor binding in the rat model of pancreatic regeneration using 60–70% pancreatectomy. The α_1-adrenergic receptors kinetics was studied in the cerebral cortex and brain stem of sham operated, 72 h pancreatectomised and 7 days pancreatectomised rats. Scatchard analysis with [^{3}H]prazosin in cerebral cortex and brain stem showed a significant decrease ($P < 0.01$), ($P < 0.05$) in maximal binding (B_{max}) with a significant decrease ($P < 0.001$), ($P < 0.01$) in the K_d in 72 h pancreatectomised rats compared with sham, respectively. Competition analysis in cerebral cortex and brain stem showed a shift in affinity during pancreatic regeneration. The sympathetic activity was decreased as indicated by the significantly decreased norepinephrine level in the plasma ($P < 0.001$), cerebral cortex ($P < 0.01$) and brain stem ($P < 0.001$) of 72 h pancreatectomised rats compared to sham. Thus, from our results it is suggested that the central α_1-adrenergic receptors have a functional role in the pancreatic regeneration mediated through the sympathetic pathway.

Keywords α_1-adrenergic receptor binding · Rat model · Pancreatic regeneration · 60–70% pancreatectomy · Receptors kinetics · Brain regions · Cerebral cortex · Brain stem · Sham operated · 72 h pancreatectomised · 7 days pancreatectomised rats · Scatchard analysis · Prazosin · Maximal binding · B_{max} · K_d · Competition analysis · Binding affinity · Sympathetic activity · Norepinephrine · Plasma · Down regulation · Central α_1-adrenergic receptors · Sympathetic pathway

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Introduction

Central nervous system through parasympathetic and sympathetic pathways regulates insulin secretion from pancreatic islets. The pancreatic islets are innervated by the post ganglionic cholinergic nerves emanating from the nerve cell bodies in the pancreatic ganglia [1, 2]. Anatomical studies suggest that the origin of these vagal efferent fibers is nucleus ambiguus and dorsal motor nucleus directly innervating the pancreas [3] and have a role in neurally mediated insulin release. The α-adrenergic receptors are an important modulator of noradrenergic neurotransmission in the brain and in the regulation of blood glucose homeostasis in vivo [4, 5]. Studies with specific α_1, α_2 and β-adrenergic receptor (AR) agonists and antagonists revealed that insulin secretion could be influenced by activation of all three groups of ARs [6]. Previous studies have reported that β-adrenergic receptor agonists were potent inhibitors of insulin release in isolated islet preparation from rats [7], as well as in mice in vivo [8] and in man [9]. The α-adrenergic receptor activation leads to inhibition of insulin release by a mechanism distal to those regulating β-cell cyclic AMP production and [Ca^{2+}] [6, 10]. α_1-antagonist prazosin stimulates insulin secretion from pancreatic islets of fa/fa Zucker rats [11] and these α-adrenergic receptors are found to be increased in the streptozotocin diabetic state [12].

There is much evidence to suggest that prolonged stimulation of insulin secretion in vivo leads to a

Journal : 11064 Dispatch 19-4-2006 Pages : 8
Article No. : 9073 LE TYPESET
MS Code : 9073 CP DISK

compensatory increase of the total volume of the pancreatic islets in partially pancreatectomised rats [13]. 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 [14, 15]. Previous reports from our laboratory have shown that sympathetic activity is decreased during pancreatic regeneration [16]. The role of hypothalamic GABAergic neurotransmission in regulating sympathetic system during liver regeneration has also been reported from our laboratory [17]. Also, the decreased 5-HT_{1A} and 5-HT_{2C} receptor binding in the cerebral cortex and brain stem was reported which is stimulatory to insulin release mediated through the sympathetic system in pancreatic regeneration [18, 19]. Though many reports are there implicating the brain control of pancreatic function how the central α_1-adrenergic receptors respond to pancreatic regeneration is not well studied. In the present study we investigated the role of α_1-adrenergic receptor binding parameters in the cerebral cortex and brain stem and their relationship between sympathoadrenal secretions during pancreatic regeneration in rats.

Experimental procedure

Chemicals

All biochemicals used were of analytical grade, prazosin, sodium octyl sulphonate purchased from Sigma Chemical Co., St. Louis, USA. HPLC solvents were of the grade obtained from SRL and MERCK, India. [furanyl-5-^{3}H] prazosin (27 Ci/mmol) was purchased from NEN life sciences products Inc., Boston, USA.

Animals

Weanling Wistar rats (3–4-week-old) of 80–100 g body weight were purchased from Central Institute of Fisheries Technology, Cochin and used for all experiments. They were housed in separate cages under 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 in accordance with institutional and National Institute of Health guidelines.

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 [20]. The removal of most of the pancreas was done by gentle abrasion with cotton applications, leaving the major blood vessels supplying other organs intact [21]. The sham operation was done in an identical procedure except that the pancreatic tissue was only lightly rubbed between fingertips using cotton instead of being removed. All the surgeries were done between 7 a.m. and 9 a.m. to avoid diurnal variations in responses. The rats were maintained for different time intervals.

Sacrifice of rats

The sham, 72 h and 7 days pancreatectomised rats were sacrificed by decapitation and the brain regions were dissected out quickly over ice according to the procedure of Glowinski and Iversen [22]. The tissues were stored at −70°C for various experiments.

In vivo DNA synthesis studies in pancreatic islets

About 5 μCi of [^{3}H]thymidine was injected intra-peritoneally into partially pancreatectomised rats to study DNA synthesis at 24, 36, 48, 72 h, 7 and 14 days of pancreatic regeneration. [^{3}H]thymidine was injected 2 h before sacrifice. DNA was extracted from pancreatic islets according to Schneider [23]. A 10% trichloroacetic acid (TCA) homogenate was made and DNA was extracted from the lipid free residue by heating with 5% TCA at 90°C for 15 min. DNA was estimated by diphenylamine method [24]. DNA extract was counted in a liquid scintillation counter (WALLAC 1409) after adding cocktail-T containing Triton-X 100. The amount of DNA synthesised was measured as DPM/mg DNA.

Estimation of circulating insulin

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. At the end of incubation, the antibody bound and free insulin are separated by the second antibody-polyethylene glycol (PEG) aided separation method. Measuring the radioactivity associated with bound fraction of sample and standards quantitates insulin concentration of samples.

Norepinephrine quantification by HPLC

The NE was quantified by HPLC determinations using electrochemical detection. [25]. 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

Journal : 11064 Dispatch : 19-4-2006 Pages : 8
Article No. : 9073 LE TYPESET
MS Code : 9073 CP DISK

filtered through 0.45 µm filters and used for HPLC analysis with electrochemical detector (HPLC-ECD) (Shimadzu, Japan) fitted with CLC-ODS reverse phase column. 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.

[^{3}H] Prazosin binding studies

The assay was done according to a modified procedure of Geynet et al. [26]. The brain regions 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 recentrifuged at 17,000 × *g* for another 1 h. The final pellet was resuspended in a minimum volume of incubation buffer—50 mM Tris–HCl, 10 mM $MgCl_2$, 1 mM EGTA, 0.8 mM ascorbic acid and 3 mM catechol, pH 7.7.

Membrane binding assays were performed in 0.5 ml incubations containing protein concentrations ranging from 150 to 200 µg and different concentrations of [^{3}H]prazosin i.e., 0.05–5.0 nM in the incubation buffer. Non-specific binding was determined using 100 µM unlabelled phentolamine. Competition studies were carried out with 0.5 nM [^{3}H]prazosin in each tube with unlabelled ligand concentrations varying from 10^{-12} to 10^{-4} M of prazosin. The tubes were incubated at 25°C for 30 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 50 mM Tris–HCl and 10 mM $MgCl_2$, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter. Specific binding was determined by subtracting non-specific binding from total binding.

Protein determination

Protein was measured by the method of Lowry et al. [27] using bovine serum as standard.

Analysis of the receptor binding data

The receptor binding parameters were determined using Scatchard analysis [28]. The 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 using Sigma plot software (version 2.0, jandel GmbH, Erkrath, Germany). Competitive binding data were analysed using non-linear 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 [29].

Displacement curve analysis

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.

Statistics

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).

Results

[^{3}H]thymidine incorporation into replicating DNA was used as a biochemical index for quantifying the pancreatic regeneration. DNA synthesis was negligible in the pancreatic islets of sham-operated rats. There was a significant increase ($P < 0.01$) in the [^{3}H]thymidine incorporation at 36 and 48 h and was peaked at 72 h after partial pancreatectomy ($P < 0.001$). The elevated levels of DNA synthesis reversed back to near basal level by 7 days. The insulin levels in the serum of pancreatectomised rats showed a significant increase ($P < 0.05$) at 48 h and peaked at 72 h ($P < 0.01$). The increased insulin levels then decreased to near sham by 7 days (Fig. 1).

The plasma NE level showed a significant decrease ($P < 0.001$) in the pancreatectomised rats at 72 h after pancreatectomy (Table 1). In the cerebral cortex and brain stem the NE content were significantly decreased ($P < 0.01$ and $P < 0.001$, respectively) at 72 h after partial pancreatectomy when compared with sham. The decreased contents were reversed to near sham value by 7 days after partial pancreatectomy in the cerebral cortex and brain stem (Table 2).

Journal : 11064 Dispatch : 19-4-2006 Pages : 8
Article No. : 9073 LE TYPESET
MS Code : 9073 CP DISK

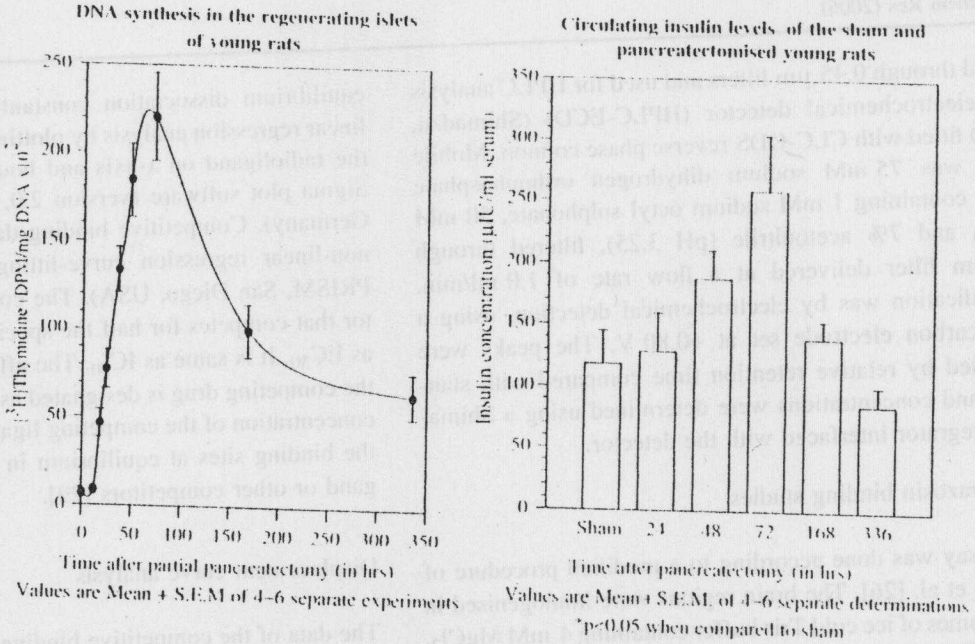

Fig. 1 DNA synthesis in the regenerating islets of young rats. Circulating insulin levels of the sham and pancreatectomised young rats

Values are Mean + S.E.M of 4–6 separate experiments

Values are Mean + S.E.M. of 4–6 separate determinations

*p<0.05 when compared to sham

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

Animal status	NE
Sham	2.21 ± 0.44
72 h pancreatectomy	0.88 ± 0.09*
7 days pancreatectomy	1.01 ± 0.06*

Values are Mean ± S.E.M. of 4–6 separate experiments

*$P < 0.001$ when compared to sham

Table 2 Norepinephrine (NE) content (nmoles/g wet wt. of tissue) in the cerebral cortex, brain stem of experimental rats

Animal status	Cerebral cortex	Brain stem
Sham	2.03 ± 0.31	28.86 ± 0.36
72 h pancreatectomy	0.29 ± 0.13*	10.05 ± 0.06**
7 days pancreatectomy	1.74 ± 0.26***	29.47 ± 0.49***

Values are Mean ± S.E.M. of 4–6 separate experiments

*$P < 0.01$ when compared to sham

**$P < 0.001$ when compared to sham

***$P < 0.001$ when compared to 72 h pancreatectomy

Scatchard analysis of [^{3}H]prazosin binding in the cerebral cortex showed a significant decrease ($P < 0.01$) in the B_{max} of α_1-adrenergic receptors in 72 h pancreatectomised rats with a significant ($P < 0.001$) decrease in the K_d (Table 3). The competitive curve for [^{3}H]prazosin against prazosin was fitted to one-site model in the case of 72 h pancreatectomy and the curve was fitted to a two-sited model in the case of sham and 7 days pancreatectomy with a Hill slope value away from unity. The log (EC_{50})-1 and $K_{i(H)}$ of 7 days pancreatectomised rats increased compared with sham indicating a shift in high affinity towards low affinity. Also, $K_{i(L)}$ showed an increase in 7 days pancreatectomised rats with an increase in log (EC_{50})-2 denoting a shift in the low affinity site towards much lower affinity (Fig. 2 and Table 4).

Scatchard analysis of [^{3}H]prazosin binding in the brain stem showed a significant decrease in the B_{max} ($P < 0.05$) of α_1-adrenergic receptors in 72 h pancreatectomised rats with a significant increase ($P < 0.01$) in the affinity (Table 3). The competitive curve of [^{3}H]prazosin against prazosin was fitted for a one-sited model in sham-operated and 7 days pancreatectomised rats, where as it fitted for two-sited model in the case of 72 h pancreatectomy. $K_{i(H)}$ was decreased and an additional low affinity site was appeared in the 72 h pancreatectomised rats when compared to the sham (Fig. 3 and Table 5). The K_i and log (EC_{50}) value showed no change in 7 days pancreatectomised rats compared with sham indicating no shift in affinity.

Discussion

Pancreatic regeneration after pancreatectomy has been well documented in animal models [20]. Removal of 60–70% of the pancreas did not cause any change in the body weight and the blood glucose levels of the pancreatectomised rats. This maintenance of glucose homeostasis is due to regeneration among the remaining pancreatic β-cells and their excess production of insulin [30, 31]. The increase in insulin secretion after the pancreatectomy besides maintaining the normoglycemic level, also helps to regain its original mass and volume by inducing cell division. Insulin

Journal : 11064 Dispatch : 19-4-2006 Pages : 8
Article No. : 9073 LE TYPESET
MS Code : 9073 CP DISK

Table 3 Scatchard analysis of [^{3}H]prazosin binding against phentolamine in the brain regions of sham and pancreatectomised young rats

Animal status	[^{3}H]prazosin binding parameters			
	Cerebral cortex		Brain stem	
	B_{max} (fmoles/mg protein)	K_d (nM)	B_{max} (fmoles/mg protein)	K_d (nM)
Sham	18.50 ± 5.50	5.36 ± 1.30	23.33 ± 4.67	6.84 ± 0.62
72 h pancreatectomy	9.00 ± 3.22**	2.10 ± 0.64**	7.08 ± 4.24*	1.58 ± 0.82**
7 days pancreatectomy	9.33 ± 3.30*	1.56 ± 0.88***	3.45 ± 0.26*	0.82 ± 0.08***

Values are Mean ± S.E.M. of 4–6 separate experiments

*P < 0.05 when compared to sham

**P < 0.01 when compared to sham

***P < 0.001 when compared to sham

B_{max}, maximal binding; K_d, dissociation constant

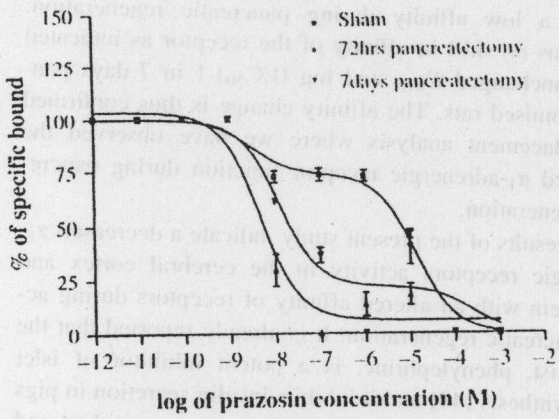

Fig. 2 Displacement of [^{3}H prazosin with prazosin in the cerebral cortex of sham, 72 h and 7 days pancreatectomised rats. ■: Sham, ▲: 72 h pancreatectomy, ▼: 7 days pancreatectomy. Incubation was done at 25°C for 30 min with 0.5 nM [^{3}H]prazosin in each tube with cold concentration varying from 10^{-12} to 10^{-4} M. Reaction was stopped by rapid filtration through GF/C filters (Whatman) filters with ice cold Tris buffer pH 7.4. Values are representation of 4–6 experiments

can stimulate β-cell replication directly possibly through a receptor for multiplication stimulating activity or another insulin like growth factor [32].

Tritiated thymidine incorporation studies from our laboratory [18] and previous reports showed that the DNA synthesis in pancreatic islets was maximum at 72 h after pancreatectomy i.e. during active pancreatic regeneration [20, 33]. Increased islet DNA synthesis and glucose derived lipid and amino acid production in association with β-cell hyperproliferation are reported in normoglycemic 60% pancreatectomy rats [34].

Pancreatic islets receive innervation from both divisions of the autonomic nervous system and pancreatic endocrine secretion is partly controlled by the autonomic nervous system [35]. The epinephrine (EPI) and its receptor regulation in adrenergic nerve ending were controlled by alpha adrenergic receptors [36, 37]. The Norepinephrine (NE) content was decreased in cerebral cortex and brain stem during active pancreatic regeneration. NE has an antagonistic effect on insulin secretion and glucose uptake [9]. At higher concentrations, NE and EPI stimulate α-adrenergic receptors and inhibit the insulin secretion, but at low concentrations, they activate β-adrenergic receptors thus stimulating insulin secretion from the pancreatic islets [38]. This inhibition of insulin release has also been demonstrated in studies on pancreatic slices [38], in isolated islets [39] and in the isolated perfused rat pancreas [40]. Alterations in brain monoamine contents in diabetic rats [41] and the relationship between enhanced monoamine content in the brain, a characteristic of hyperinsulinemic and insulin-resistant animals and islet dysfunction is reported [42].

Table 4 Binding parameters of [^{3}H]prazosin against prazosin in the cerebral cortex of sham and pancreatectomised young rats

Animal status	Best fit model	Log (EC_{50})-1	Log (EC_{50})-2	$K_{i(H)}$	$K_{i(L)}$	Hill slope
Sham	Two-site	−8.52	−4.86	2.95×10^{-9}	1.36×10^{-5}	−0.30
72 h pancreatectomy	One-site	−8.46		3.43×10^{-9}		−5.40
7 days pancreatectomy	Two-site	−7.92	−3.62	1.17×10^{-8}	2.33×10^{-4}	−0.58

Values are mean 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. The affinity for the first and second site of the competing drug is designated as $K_{i(H)}$ (for high affinity) and $K_{i(L)}$ (for low affinity). EC_{50} is the concentration of the competitor that competes for half the specific binding

Journal : 11064 Dispatch : 19-4-2006 Pages : 8
Article No : 9073 LE TYPESET DISK

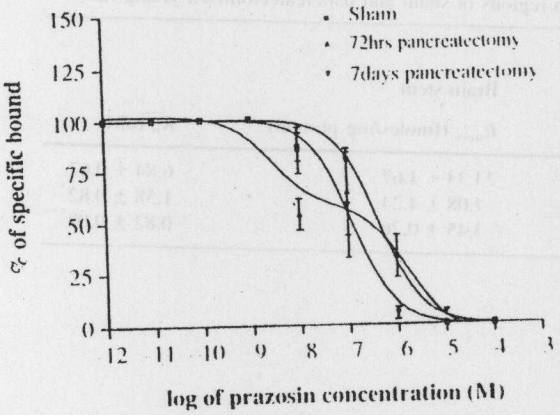

Fig. 3 Displacement of [^{3}H prazosin with prazosin in the brain stem of sham, 72 h and 7 days pancreatectomised rats. ■: Sham, ▲: 72 h pancreatectomy, ▼: 7 days pancreatectomy. Incubation was done at 25°C for 30 min with 0.5 nM [^{3}H]prazosin in each tube with cold concentration varying from 10^{-12} to 10^{-4} M. Reaction was stopped by rapid filtration through GF/C filters (Whatman) filters with ice cold Tris buffer pH 7.4. Values are representation of 4–6 experiments

α-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 [43]. The increased sympathetic activity and the released NE inhibit glucose-stimulated insulin secretion and cyclic adenosine monophosphate (cAMP) contents in rat islets [44–46]. When we analysed the α_1-adrenergic receptor status using [^{3}H]prazosin with phentolamine in the cerebral cortex, we found that α_1-adrenergic receptor number decreased significantly in 72 h after pancreatectomy as indicated by decreased B_{max}. The two affinity sites for phentolamine binding are already reported [47]. Our analysis on the affinity states of these receptors by displacement studies of [^{3}H]prazosin against prazosin showed that in sham, 72 h and 7 days pancreatectomised condition, α_1-adrenergic receptors exist in two populations; one with high affinity $K_{i(H)}$ and another with low affinity $K_{i(L)}$. In the cerebral cortex, the competitive curve was fitted for a model for two-site binding in the case of sham and 7 days pancreatectomy. Both the affinity sites shifted towards their corresponding lower affinity site as indicated the increase in the $K_{i(H)}$, $K_{i(L)}$, log (EC_{50})-1 and log (EC_{50})-2 of 7 days pancreatectomised rats. This shows a decreased functioning of the receptor in 7 days pancreatectomised rats compared to sham. There was no shift in affinity of the receptor in 72 h pancreatectomised rats as indicated by no change in K_i and log (EC_{50}) values and the competitive curve was fitted to one-site model. In the brain stem, Scatchard analysis revealed a decreased B_{max} and decreased K_d of the α_1-adrenergic receptor indicating a reduction in the receptor density with increased affinity of the receptor in 72 h pancreatectomised rats. The competitive curve was fitted for a one-sited model in sham-operated and 7 days pancreatectomised rats, where as it fitted for two-sited model in the case of 72 h pancreatectomy. Displacement analysis showed a shift in affinity with decreased $K_{i(H)}$ towards a low affinity during pancreatic regeneration. There was no shift in affinity of the receptor as indicated by the unchanged $K_{i(H)}$ and log (EC_{50})-1 in 7 days pancreatectomised rats. The affinity change is thus confirmed by displacement analysis where we have observed the decreased α_1-adrenergic receptor function during pancreatic regeneration.

The results of the present study indicate a decreased α_1-adrenergic receptors activity in the cerebral cortex and brain stem with an altered affinity of receptors during active pancreatic regeneration. It is already reported that the α_1-agonist, phenylephrine, is a potent inhibitor of islet DNA synthesis [48] and it inhibits insulin secretion in pigs [49]. The decreased α_1-adrenergic receptor number and affinity observed in the cerebral cortex and brain stem can decrease the sympathetic nerve discharge and thereby decreasing the circulating NE levels during active pancreatic regeneration. Studies from our laboratory reported the decrease in NE and EPI content in the adrenals during pancreatic regeneration [16]. Plasma NE levels in the present study were in accordance with the functioning of the α_1-adrenergic receptors in pancreatic regeneration. Previous studies showed that the administration of α_1-adrenergic agonist, phenylephrine with Sp-cAMP[S] to pertussis toxin-pretreated islets partially prevented the suppressed β-cell proliferation and insulin secretion, sug-

Table 5 Binding parameters of [^{3}H]prazosin against prazosin in the brain stem of sham and pancreatectomised young rats

Animal status	Best fit model	Log (EC_{50})-1	Log (EC_{50})-2	$K_{i(H)}$	$K_{i(L)}$	Hill slope
Sham	One-site	−6.38		4.11×10^{-7}		−0.85
72 h pancreatectomy	Two-site	−8.65	−5.80	2.22×10^{-9}	1.57×10^{-6}	−0.45
7 days pancreatectomy	One-site	−6.93		1.15×10^{-7}		−0.99

Values are mean 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. The affinity for the first and second site of the competing drug is designated as $K_{i(H)}$ (for high affinity) and $K_{i(L)}$ (for low affinity). EC_{50} is the concentration of the competitor that competes for half the specific binding

Journal 11064 Dispatch 19-4-2006 Pages 8
Article No 9073 LE TYPESET
MS Code 9073 CP DISK

gesting that α-adrenergic stimulation represses β-cell growth and hormone release in part by interfering with GTP binding proteins that connect cell surface receptors to adenylate cyclase [48]. Studies from our laboratory have shown that muscarinic M1 and M3 receptor subtypes functional balance can regulate the sympathetic activity, which in turn control islet cell proliferation and glucose homeostasis during pancreatic regeneration [16]. Also theregulatory role of sympathetic system in 5-HT_{1A} and 5-HT_{2C} receptor binding on insulin secretion was reported [18, 19]. This shows that sympathetic tone plays a major regulatory role in the insulin secretion during pancreatic regeneration by acting through different neurotransmitter receptors subtypes.

Thus, we conclude from our studies that pancreatectomy trigger a regulatory effect on sympathetic nerve discharge and the central α_1-adrenergic receptors. The decreased binding of the α_1-adrenergic receptors observed in the cerebral cortex and brain stem during pancreatic regeneration have its stimulatory role on insulin secretion mediated through sympathetic system which is suggested to have clinical significance in diabetes management.

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Journal : 11064 | Dispatch : 19-4-2006 | Pages : 8
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Article No. : 9073 LE TYPESET
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