Decreased 5-HT₁ₐ Receptor Gene Expression and 5-HT₁ₐ Receptor Protein in the Cerebral Cortex and Brain Stem during Pancreatic Regeneration in Rats

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The present study was to investigate the role of central 5-HT and 5-HT₁ₐ receptor binding and gene expression in a rat model of pancreatic regeneration using 60% pancreatectomy. The pancreatic regeneration was evaluated by 5-HT content and 5-HT₁ₐ receptor gene expression in the cerebral cortex (CC) and brain stem (BS) of sham operated, 72 h and 7 days pancreatectomised rats. 5-HT content significantly increased in the CC ($P < 0.01$) and BS ($P < 0.05$) of 72 h pancreatectomised rats. Sympathetic activity was decreased as indicated by the significantly decreased norepinephrine (NE) and epinephrine (EPI) level ($P < 0.001$ and $P < 0.05$) in the plasma of 72 h pancreatectomised rats. 5-HT₁ₐ receptor density and affinity was decreased in the CC ($P < 0.01$) and BS ($P < 0.01$). These changes correlated with a diminished 5-HT₁ₐ receptor mRNA expression in the brain regions studied. Our results suggest that the brain 5-HT through 5-HT₁ₐ receptor has a functional role in the pancreatic regeneration through the sympathetic regulation.

INTRODUCTION

Serotonin (5-hydroxytryptamine or 5-HT)-containing neurons are concentrated in the raphé nuclei of the brain stem and connect to the cerebral cortex, hypothalamus, and major autonomic nuclei (1). 5-HT receptors can be classified into seven classes from 5-HT₁ to 5-HT₇ (2). 5-HT₁ₐ receptors occur in high density in the brain stem and cerebral cortex (3). Brain serotonergic activity plays an important role in the autonomic regulation of pan-

creatic function (4). Anatomical studies suggest that the vagal efferent fibres originating from nucleus ambiguous and dorsal motor nucleus of brain stem directly innervate the pancreas (5) and have a role in neurally mediated insulin release (6).

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 (7). 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 (8,9). Electrical stimulation of the sympathetic nerves to the pancreas or exposure of the pancreas to exogenous norepinephrine (NE) decreased glucose-induced insulin secretion (9,10). 5-HT₁ₐ receptor-selective agonist 8-Hydroxy-2-(di-*n*-propylamino) tetralin (8-OH DPAT) has been reported

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to trigger adrenal catecholamine release and hyper-glycaemia by the activation of central 5-HT$_{1A}$ receptors (11). Though the effects of 8-OH DPAT on glucose and insulin are centrally mediated, they are dependent upon the adrenal glands being intact (12). The relationship between the serotonergic and the sympathoadrenal system leads in turn to a control of both plasma glucose levels and insulin release (13). Although there are several studies regarding the brain control of pancreatic function how the central 5-HT and 5-HT$_{1A}$ receptors responds to pancreatic regeneration is not well studied. In the present study we investigated the changes in the 5-HT content and 5-HT$_{1A}$ receptor gene expression in the cerebral cortex and brain stem and their relationship between sympathoadrenal secretions and insulin secretion during pancreatic regeneration in rats.

EXPERIMENTAL PROCEDURE

Chemicals. All biochemicals used were of analytical grade. 8-Hydroxy-DPAT [propyl-2,3-ring-1,2,3-^{3}H] (Sp. activity 127.0 Ci/mmol), was purchased from NEN Life Sciences products, Inc., Boston, USA. [^{3}H]Thymidine (Sp. Activity 25 Ci/mmol) was purchased from Amersham Life Science, UK. Random hexamers, Taq DNA polymerase, Human placental RNAse inhibitor and DNA molecular weight markers were purchased from Bangalore Genei Pvt. Ltd., India. MuMLV and dNTPs were obtained from Amersham Life Science, UK. Tri-reagent was purchased from Sigma Chemical Co., USA. RT-PCR primers used in this study were synthesized by Sigma Chemical Co., USA.

Animals. Male Wistar weanling rats of 80–100 g body weight purchased from Kerala Agricultural University, Mannuthy, 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 (14). The removal of most of the pancreas was done by gentle abrasion with cotton applications, leaving the major blood vessels supplying other organs intact (15). 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. The rats were maintained for different time intervals–72 h and 7 days.

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 (16). The tissues were stored at −70°C for various experiments.

In vivo DNA Synthesis Studies in Pancreatic Islets. [^{3}H]Thymidine of 5 µCi was injected intra-peritoneally into sham and pancreatectomised rats to study DNA synthesis at 72 h and 7 days of pancreatic regeneration. [^{3}H]thymidine was injected 2 h before sacrifice. DNA was extracted from the islets according to Schneider (17). 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 (18). DNA extract was counted in a liquid scintillation counter (WALLAC 1409) after adding cocktail-T containing Triton-X 100. The amount of DNA synthesized was measured as DPM/mg DNA.

Estimation of Circulating Insulin. The assay was done according to the procedure of BARC (Bhabha Atomic Research Centre, India) 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.

5-HT Quantification by HPLC. Brain 5-HT was quantified by HPLC determinations using electrochemical detection (19–21). A 10% homogenate of the tissue was made in 0.4 N perchloric acid. The homogenate was centrifuged at $5000 \times g$ for 10 min 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 in Shimadzu HPLC system with electrochemical detector fitted with C18-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/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.

5-HT$_{1A}$ Receptor Binding Studies. 5-HT$_{1A}$ receptor assay was done by using specific agonist [^{3}H]8-OH DPAT binding to the 5-HT$_{1A}$ receptors (22). Brain tissues were homogenised in a polytron homogeniser with 50 volumes of 50 mM Tris-HCl buffer, pH 7.4. The supernatant was then centrifuged at $30,000 \times g$ for 30 min and the pellets were resuspended in appropriate volume of incubation buffer, 50 mM Tris buffer.

Binding assays were done using different concentrations i.e. 1–100 nM of [^{3}H]8-OH DPAT in 50 mM Tris buffer, pH 7.4 in a total incubation volume of 250 µl. Specific binding was determined using 100 µM unlabelled 5-HT. Competition studies were carried out with 1.0 nM [^{3}H]8-OH DPAT in each tube with unlabelled ligand concentrations varying from 10^{-12}–10^{-4} M of 5-HT.

Tubes were incubated at 25°C for 60 min and filtered rapidly through GF/C filters (Whatman). The filters were washed quickly by three successive washing with 3 ml of ice-cold 50 mM Tris buffer, pH 7.4. Bound radioactivity was determined 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.* (23) using bovine serum albumin as standard.

Receptor Data Analysis. The receptor binding parameters were determined using Scatchard analysis (24). The maximal binding (B_{max}) and equilibrium dissociation constant (K_d) were derived by linear regression analysis by plotting the specific bind-

ing 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 Ki 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 (25).

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-sited model of curve-fitting. A one-site competitive binding curve that follows the law of mass action has a slope of -1.0. If the curve is more shallow, the slope factor will be a negative fraction (i.e., -0.85 or -0.60). The slope factor is negative because curve goes downhill. If slope factor differs significantly from 1.0, then the binding does not follow the law of mass action with a single site, suggesting a two-site model of curve fitting.

Analysis of Gene Expression by RT-PCR. Total RNA was isolated from the brain regions of sham and pancreatectomised rats using Tri reagent. RNA was reverse transcribed using MuM-LV Reverse Transcriptase with random hexamers in 20 µl reaction volume, from which 2 µl was used for detection of 5-HT$_{1A}$ receptor gene transcripts using PCR. Beta actin mRNA expression was used as the internal standard to which mRNA levels were normalized. Primer pairs and conditions used were: 5-HT$_{1A}$ (357 bp), 5'- TGG CTT TCT CAT CTC CAT CC -3' and 5'- CTC ACT GCC CCA TTA GTG C -3', 30 cycles, beta actin (150 bp), 5'- CAA CTT TAC CTT GGC CAC TAC C -3' and 5'- TAC GAC TGC AAA CAC TCT ACA CC -3', 30 cycles. PCR reactions for these genes were performed at an annealing temperature of 56°C in an eppendorf personnel thermocycler. 10 µl of reaction mixture was electrophoretically separated on 2% agarose gel containing ethidium bromide in Tris-borate-EDTA buffer. The image of the bands was captured using an Imagemaster VDS gel documentation system (Pharmacia Biotech).

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

[3H] Thymidine incorporation into replicating DNA was used as a biochemical index for quantifying islet DNA synthesis during pancreatic regeneration. DNA synthesis was negligible in the pancreatic islets of sham operated rats. The DNA synthesis was peaked at 72 h after pancreatectomy and it decreased at 7 days after pancreatectomy (Fig. 1). Plasma insulin level was increased significantly at 72 h ($P < 0.01$) and 7 days ($P < 0.05$) after pancreatectomy when compared with the sham (Fig. 2). Plasma EPI and NE levels showed significant decrease ($P < 0.001$ and $P < 0.05$) in 72 h pancreatectomised rats com-
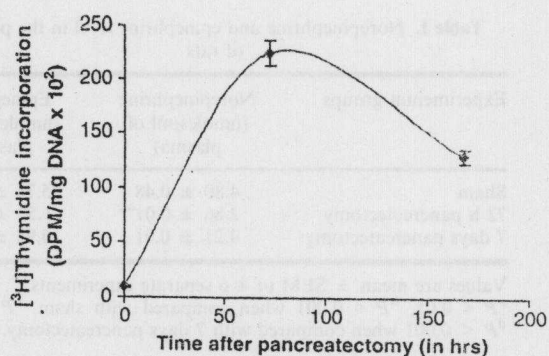

Fig. 1. DNA synthesis in the pancreatic islets of sham (represented as 0 h), 72 h and 7 days (168 h) pancreatectomised rats. 5 µCi of [³H]thymidine was injected intra-peritoneally into rats 2 h before sacrifice. DNA was extracted from islets and the amount of DNA synthesized was measured as DPM/mg DNA. Values are mean of 4–6 separate experiments.
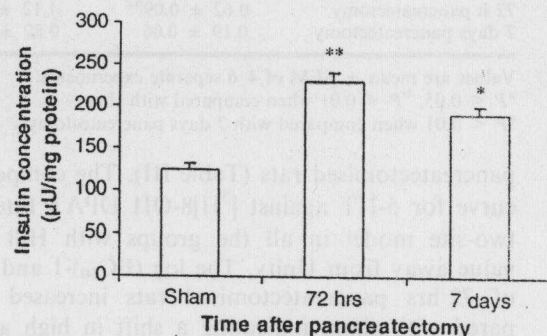

Fig. 2. Insulin concentration in the plasma of sham, 72 h and 7 days pancreatectomised rats. Values are mean of 4–6 separate experiments. **$P < 0.01$, *$P < 0.05$ when compared with sham.

pared with the sham. The decreased level of EPI and NE in the plasma reversed to sham value by 7 days ($P < 0.001$ and $P < 0.05$) after pancreatectomy (Table I). 5-HT content in the cerebral cortex and brain stem showed a significant increase ($P < 0.01$ and $P < 0.05$) at 72 h after pancreatectomy when compared with the sham. The increased content reversed to near sham value by 7 days after pancreatectomy in the cerebral cortex ($P < 0.01$) and it showed a normalizing trend in the brain stem (Table II).

Scatchard analysis of [³H]8-OH DPAT binding to the membrane preparation of cerebral cortex showed a significant decrease ($P < 0.01$) in the B_{max} of 72 h pancreatectomised rats. K_d showed a significant increase ($P < 0.01$) in 72 hrs pancreatectomised rats. The B_{max} and K_d reversed to sham level in 7 days ($P < 0.01$ and $P < 0.01$)

Table I. Norepinephrine and epinephrine level in the plasma of rats

Experimental groups	Norepinephrine (nmoles/ml of plasma)	Epinephrine (nmoles/ml of plasma)
Sham	4.80 ± 0.48	5.75 ± 0.11
72 h pancreatectomy	$2.86 \pm 0.01^{a,c}$	$1.50 \pm 0.32^{b,d}$
7 days pancreatectomy	4.21 ± 0.91	4.95 ± 0.17

Values are mean $\pm$ SEM of 4–6 separate experiments.
[a]$P < 0.05$, [b]$P < 0.001$ when compared with sham. [c]$P < 0.05$, [d]$P < 0.001$ when compared with 7 days pancreatectomy.

Table II. 5-HT content in the cerebral cortex and brain stem of rats

Experimental groups	Cerebral cortex (nmoles/g wet weight of tissue)	Brain stem (nmoles/g wet weight of tissue)
Sham	0.25 ± 0.07	0.28 ± 0.04
72 h pancreatectomy	$0.62 \pm 0.09^{b,c}$	1.12 ± 0.03^{a}
7 days pancreatectomy	0.19 ± 0.06	0.80 ± 0.07

Values are mean $\pm$ SEM of 4–6 separate experiments.
[a]$P < 0.05$, [b]$P < 0.01$ when compared with sham.
[c]$P < 0.01$ when compared with 7 days pancreatectomy.

Table III. [^{3}H]8-OH DPAT receptor binding parameters in the cerebral cortex of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Sham	395 ± 15	43.9 ± 2.6
72 h pancreatectomy	$315 \pm 15^{a,b}$	$63.0 \pm 1.4^{a,b}$
7 days pancreatectomy	405 ± 13	42.7 ± 1.7

Values are mean $\pm$ SEM of 4–6 separate experiments.
[a]$P < 0.01$ when compared with sham.
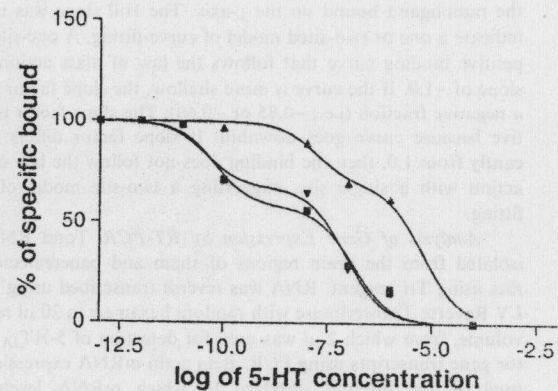[b]$P < 0.01$ when compared with 7 days pancreatectomy.

Fig. 3. Displacement of [^{3}H]8-OH DPAT with 5-HT in the cerebral cortex of sham, 72 h and 7 days pancreatectomised rats. ■ Sham, ▲ 72 h pancretectomy, ▼ 7 days pancreatectomy. Incubation was done at 25°C for 60 min with 1 nM [^{3}H]8-OH DPAT in each tube with cold concentration varying from 10^{-12}–10^{-4} M. Reaction was stopped by rapid filtration through GF/C (Whatman) filters with ice cold Tris buffer pH 7.4. Values are representation of 4–6 separate experiments.

pancreatectomised rats (Table III). The competition curve for 5-HT against [^{3}H]8-OH DPAT fitted for two-site model in all the groups with Hill slope value away from Unity. The log (EC_{50})-1 and $Ki_{(H)}$ of 72 hrs pancreatectomised rats increased compared with sham indicating a shift in high affinity towards low affinity. $Ki_{(L)}$ also showed an increase in 72 h pancreatectomised rats with an increase in log (EC_{50})-2 denoting a shift in the low affinity site towards much lower affinity (Fig. 3 and Table IV). These changes were reversed to sham level in 7 days pancreatectomised rats.

The B_{max} of [^{3}H]8-OH DPAT binding decreased significantly ($P < 0.01$) without any significant change in K_d in the brain stem of 72 h pancreatectomised rats compared with sham. The decreased B_{max} partially reversed to sham level by 7 days ($P < 0.05$) after pancreatectomy (Table V). The competition curve for 5-HT against [^{3}H]8-OH DPAT fitted for two-site model in all the groups with Hill slope value away from Unity. The $Ki_{(H)}$ increased in 72 h with an increased log (EC_{50})-1. This shows a shift from the high-affinity site to low affinity. $Ki_{(L)}$ and the log (EC_{50})-2 value showed no change indicating no shift in affinity (Fig. 4 and Table VI). These changes were reversed to sham level in 7 days pancreatectomised rats.

RT-PCR analysis showed that the 5-HT$_{1A}$ receptor mRNA expression decreased at 72 h after pancreatectomy while it reversed to sham level after 7 days (Fig. 5) in the cerebral cortex. In the brain stem RT-PCR analysis revealed a decreased expression of 5-HT$_{1A}$ receptor mRNA in 72 h pancreatectomised rats and it showed a reversing trend to sham level in 7 days pancreatectomised rats (Fig. 6).

DISCUSSION

Pancreatic regeneration after pancreatectomy has been well documented in animal models (14). Removal of 60–70% of the pancreas did not affect the body weight and blood glucose level of pancreatectomised rats. [3H] Thymidine incorporation studies showed that the peak DNA synthesis in pancreatic islets is at 72 h after pancreatectomy i.e during active pancreatic regeneration (14,26) and declined at 7 days after pancreatectomy. Increased islet DNA synthesis and glucose-derived lipid and

Table IV. Binding parameters of [^{3}H]8-OH DPAT against 5-HT in the cerebral cortex of rats

Experimental groups	Best-fit model	log EC$_{50}$)-1	log (EC$_{50}$)-2	Ki$_{(H)}$	Ki$_{(L)}$	Hill slope
S	Two-site	−10.10	−7.05	3.1×10^{-11}	8.5×10^{-8}	−0.29
P-72 hrs	Two-site	−8.00	−4.60	9.5×10^{-9}	2.2×10^{-5}	−0.38
P-7 days	Two-site	−11.14	−6.70	7.0×10^{-11}	1.7×10^{-7}	−0.26

S-Sham, P-72 hrs- 72 h pancreatectomy, P-7 days- 7 days pancreatectomy. Data were fitted with an iterative nonlinear regression software (Prism, GraphPad. San Diego, CA). Ki - The affinity of the receptor for the competing drug. The affinity for the first and second site of the competing drug are designated as Ki$_{(H)}$ (for high affinity) and Ki$_{(L)}$ (for low affinity). EC$_{50}$ is the concentration of the competitor that competes for half the specific binding. Values are mean of 4-6 separate experiments.

Table V. [^{3}H]8-OH DPAT receptor binding parameters in the brain stem of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Sham	350.0 ± 18.0	35.6 ± 9.2
72 h pancreatectomy	258.5 ± 15.0[a,b]	30.6 ± 5.4
7 days pancreatectomy	295.0 ± 10.0	41.9 ± 6.1

Values are mean ± SEM of 4-6 separate experiments.
[a] $P < 0.01$ when compared with sham.
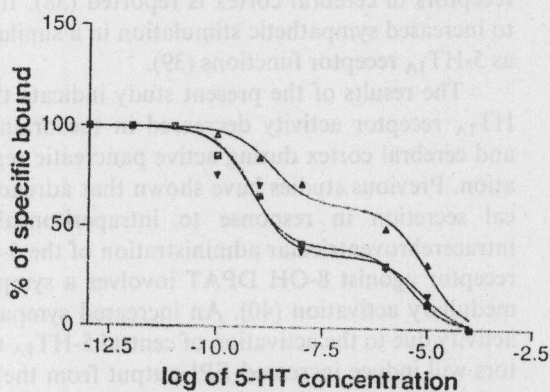[b] $P < 0.05$ when compared with 7 days pancreatectomy.

Fig. 4. Displacement of [^{3}H]8-OH DPAT with 5-HT 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 1 nM [^{3}H]8-OH DPAT in each tube with cold concentration varying from 10^{-12}–10^{-4} M. Reaction was stopped by rapid filtration through GF/C (Whatman) filters with ice cold Tris buffer pH 7.4. Values are representation of 4-6 separate experiments.

amino acid production in association with beta-cell hyperproliferation is reported in normoglycaemic 60% pancreatectomy rats (27).

Pancreatic islets receive innervation from both divisions of the autonomic nervous system, and pancreatic endocrine secretion is partly controlled by the autonomic nervous system (28). Brain serotonergic changes are reported to regulate autonomic nerve function in rats (29). 5-HT content was increased in the cerebral cortex and brain stem during active pancreatic regeneration. Large alterations in brain monoamine contents in diabetic rats (30) and the relationship between enhanced monoamine content in the sympathetic centre of hypothalamus (VMH), a characteristic of hyperinsulinemic and insulin-resistant animals and islet dysfunction is reported (4). Our studies have shown that circulating insulin level is increased during peak DNA synthesis in pancreatectomised rats. Observations have indicated the ability to increase proinsulin mRNA levels as an adaptation to pancreatectomy (31). Insulin tends to release the tryptophan bound to albumin and hence increasing the concentration of free tryptophan in plasma (32). The increase in brain 5-HT content observed in 72 h pancreatectomised rats may be due to the increased uptake of tryptophan into the brain (33).

The 5-HT$_{1A}$ receptor binding parameters as determined by [^{3}H]8-OH DPAT against 5-HT indicate a decrease in number and affinity of the receptor in the cerebral cortex of 72 h pancreatectomised rats.

Table VI. Binding parameters of [^{3}H]8-OH DPAT against 5-HT in the brain stem of rats

Experimental group	Best-fit model	log (EC$_{50}$)-1	log (EC$_{50}$)-2	Ki$_{(H)}$	Ki$_{(L)}$	Hill slope
S	Two-site	−9.2	−5.43	5.5×10^{-10}	3.7×10^{-6}	−0.32
P-72 hrs	Two-site	−8.7	−5.03	1.8×10^{-9}	9.0×10^{-6}	−0.33
P-7 days	Two-site	−9.2	−5.20	6.5×10^{-10}	6.8×10^{-6}	−0.30

S-Sham, P-72 hrs- 72 h pancreatectomy, P-7 days- 7 days pancreatectomy. Data were fitted with an iterative nonlinear regression software (Prism, GraphPad. San Diego, CA). Ki - The affinity of the receptor for the competing drug. The affinity for the first and second site of the competing drug are designated as Ki$_{(H)}$ (for high affinity) and Ki$_{(L)}$ (for low affinity). EC$_{50}$ is the concentration of the competitor that competes for half the specific binding. Values are mean of 4-6 separate experiments.

0 1 2 3

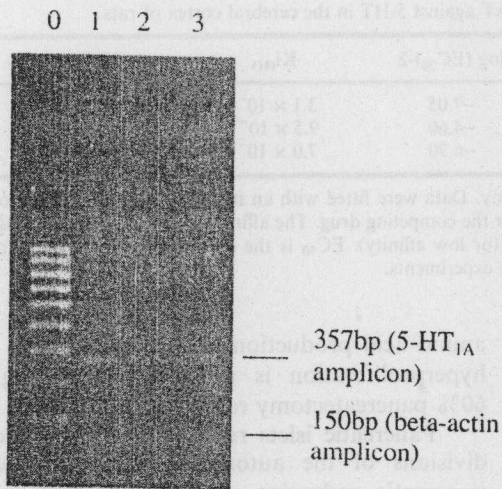357bp (5-HT_1A amplicon)

150bp (beta-actin amplicon)

Fig. 5. RT-PCR analysis of mRNA from the cerebral cortex of sham, 72 h and 7 days pancreatectomised rats (lanes 1, 2 and 3 respectively) with primers specific for 5-HT_1A receptor and beta actin mRNAs. Lane 0 indicates 100 bp ladder. Numbers on the right indicate product size in base pairs.
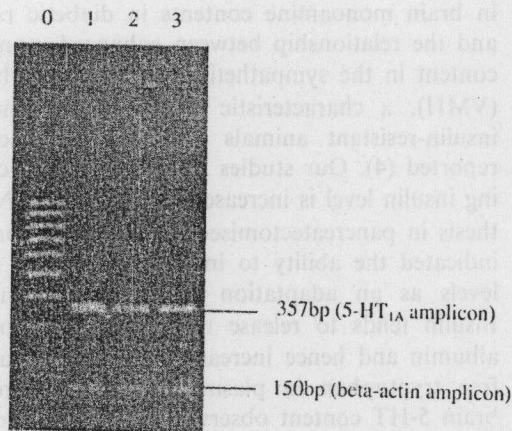
0 1 2 3

357bp (5-HT_1A amplicon)

150bp (beta-actin amplicon)

Fig. 6. RT-PCR analysis of mRNA from the brain stem of sham, 72 h and 7 days pancreatectomised rats (lanes 1, 2 and 3 respectively) with primers specific for 5-HT_1A receptor and beta actin mRNAs. Lane 0 indicates 100 bp ladder. Numbers on the right indicate product size in base pairs.

This shows that 5-HT_1A receptor activity decreased in the cerebral cortex during peak DNA synthesis in pancreas. The decreased activity may be due to the 5-HT induced downregulation of 5-HT_1A receptors (34). Displacement analysis showed that both the affinity sites shifted towards their corresponding lower affinity site as indicated the increase in the $Ki_{(H)}$, $Ki_{(L)}$, log (EC_{50})-1 and log (EC_{50})-2 in 72 h pancreatectomised rats. Agonist induced desensitiza-

tion and loss of high-affinity binding sites of 5-HT_1A receptors is already reported (35,36). The observation that the decrease in [^{3}H]8-OH DPAT binding is due to a reduction in the number of binding sites is evidence that the lowered binding is a reflection of increased serotonin concentration.

In the case of brain stem 5-HT_1A receptors also there was a decrease in number of receptors in 72 h pancreatectomised rats. The decrease in receptor number during pancreatic proliferation may be due to increased 5-HT brain levels in the brain stem of these rats, since the 5-HT_1A receptor expression is sensitive to auto inhibition (37). Displacement analysis showed a shift in the high affinity site to low affinity site and low affinity site to much lower affinity site as indicated by the increase in the $Ki_{(H)}$ and log (EC_{50})-1, $Ki_{(L)}$ and log (EC_{50})-2 in 72 h pancreatectomised rats. This shows decreased functioning of the receptor in 72 h pancreatectomised rats. The decreased brain content leads to an up-regulation of 5-HT_2A in brain stem and increased affinity of these receptors in cerebral cortex is reported (38). It leads to increased sympathetic stimulation in a similar way as 5-HT_1A receptor functions (39).

The results of the present study indicate that 5-HT_1A receptor activity decreased in the brain stem and cerebral cortex during active pancreatic regeneration. Previous studies have shown that adrenocortical secretion in response to intraperitoneal and intracerebroventricular administration of the 5-HT_1A receptor agonist 8-OH DPAT involves a sympathomedullary activation (40). An increased sympathetic activity due to the activation of central 5-HT_1A receptors will induce increased EPI output from the adrenal medulla that will inhibit insulin secretion (12,41). The EPI releasing effect of 8-OH DPAT are blocked by the 5HT_1A antagonist (−)-pindolol (13). The decreased 5-HT_1A receptor number and affinity observed in the cerebral cortex and brain stem can decrease the sympathetic nerve discharge and thereby decreasing the circulating NE and EPI levels. Plasma NE and EPI levels in the present study were in accordance with the functioning of the 5-HT_1A receptors. The effect of EPI on islet hormone secretion is dependent on its plasma level (42). EPI and NE at low concentrations activate β-adrenergic receptors thus stimulating insulin secretion from the pancreatic islets (43). Insulin treatment after major pancreatectomy in dogs enhances the proliferation of the remnant pancreas and maintains endogenous insulin secretion for a long period, prolonging survival and promoting pancreatic regeneration (44).

Down-regulation of the receptor by 5-HT is associated with an equivalent decrease in the level of receptor mRNA (34). Our RT-PCR analysis revealed decreased 5-HT$_{1A}$ receptor mRNA expression in the cerebral cortex and brain stem of 72 h pancreatectomised rats. This is concordant with our receptor data. Serotonin modulates the expression of 5-HT receptor mRNA by transcriptional regulation (45). Decreased expression of 5-HT$_{1A}$ mRNA in long-term adrenalectomised rats in the dentate gyrus and its reversal by dexamethasone has been reported in rats (46). Our result shows that pancreatectomy may affect 5-HT$_{1A}$ receptors in the cerebral cortex and brain stem through 5-HT release which regulates 5-HT$_{1A}$ receptor gene transcription.

Thus, the major conclusion that emerges from this study is that pancreatectomy exerts a modulatory effect on the 5-HT$_{1A}$ receptor subtype in the cerebral cortex and brain stem. The decreased 5-HT$_{1A}$ receptor binding and gene expression in the cerebral cortex and brain stem is stimulatory to insulin release mediated through the sympathetic system in pancreatic regeneration which is suggested to have clinical significance in diabetes management.

ACKNOWLEDGMENT

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