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Hypothalamic 5-HT functional regulation through 5-HT_{1A} and 5-HT_{2C} receptors during pancreatic regeneration

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Abstract

5-HT receptors are predominantly located in the brain and are involved in pancreatic function and cell proliferation through sympathetic nervous system. The objective of this study was to investigate the role of hypothalamic 5-HT, 5-$HT1_A$ and 5-$HT2_C$ receptor binding and gene expression in rat model of pancreatic regeneration using 60% pancreatectomy. The pancreatic regeneration was evaluated by 5-HT content, 5-$HT1_A$ and 5-$HT2_C$ receptor gene expression in the hypothalamus of sham operated, 72 h and 7 days pancreatectomised rats. 5-HT content was quantified by HPLC. 5-$HT1_A$ receptor assay was done by using specific agonist [^{3}H]8-OH DPAT. 5-$HT2_C$ receptor assay was done by using specific antagonist [^{3}H]mesulergine. The expression of 5-$HT1_A$ and 5-$HT2_C$ receptor gene was analyzed by RT-PCR. 5-HT content was higher in the hypothalamus of 72 h pancreatectomised rats. 5-$HT1_A$ and 5-$HT2_C$ receptors were down-regulated in the hypothalamus. RT-PCR analysis revealed decreased 5-$HT1_A$ and 5-$HT2_C$ receptor mRNA expression. The 5-$HT1_A$ and 5-$HT2_C$ receptors gene expression in the 7 days pancreatectomised rats reversed to near sham level. This study is the first to identify 5-$HT1_A$ and 5-$HT2_C$ receptor gene expression in the hypothalamus during pancreatic regeneration in rats. Our results suggest the hypothalamic serotonergic receptor functional regulation during pancreatic regeneration.

Keywords: Hypothalamus; 5-HT_{1A} receptor; 5-HT_{2C} receptor; Pancreas; Regeneration

Introduction

Brain serotonergic changes are reported to regulate autonomic nerve function in rats (Kuhn et al., 1980). The autonomic nervous system influences many of the functions of the body, including the pancreas. It has been reported that the autonomic nervous system is one of the important factors that regulate pancreatic regeneration (Kiba, 2004). The autonomic fibers supplying the pancreas travel via the vagus and splanchnic nerves. These nerves are clearly related to the ventral hypothalamus (Helman et al., 1982). Studies have shown that a lesion in the ventromedial hypothalamus stimulates cell proliferation in pancreas (Kiba et al., 1996).

The hypothalamus plays a central role in the integration of neurohormonal function (Oommura and Yoshimatsu, 1984). Hypothalamic neuronal activity is modulated by serotonin (5-hydroxy tryptamine or 5-HT) and serotonergic receptors (Kang et al., 2004). 5-HT receptors can be classified into seven classes from 5-HT_1 to 5-HT_7 (Bradley et al., 1986). 5-HT_{1A} and 5-HT_{2C} (formerly called 5-HT_{1C}) receptors are present in the hypothalamus (Pazos and Palacios, 1985) and activation of these receptors triggers adrenal catecholamine release (Bagdy et al., 1989). Previous studies from our laboratory have shown that the brain 5-HT through 5-HT_{1A} receptor has a functional role in pancreatic regeneration through the sympathetic regulation (Mohanan et al., 2005). It is also reported that epinephrine (EPI) and norepinephrine (NE) contents in the adrenals are decreased during pancreatic regeneration (Renuka et al., 2004). EPI and NE at low concentrations are stimulatory to insulin secretion from the pancreatic islets (Coore and Randle, 1964). Since hypothalamic neuronal activity is modulated by serotonin and serotonergic receptors, the receptor

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alterations occurring in this region could have a role in sympathoadrenal secretions and insulin secretion during pancreatic regeneration. Therefore, the present study was performed to investigate the functional role of hypothalamic 5-HT_{1A} and 5-HT_{2C} receptor gene expression during pancreatic regeneration in rats.

Materials and methods

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. [N^6-methyl-^{3}H]mesulergine (Sp. activity — 79.0 Ci/mmol) were 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. PCR primers used in this study were synthesized by Sigma Chemical Co., USA.

Animals

Male Wistar weanling rats of 80–100g body weight were purchased from Kerala Agriculture 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 anaesthetized under aseptic conditions, the body wall was cut open 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 (Zangen et al., 1997). 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. Body weight and blood glucose levels were checked routinely. Blood glucose was estimated using Glucose estimation kit (Merck). Pancreatectomy referred in the text is 60–70% partial pancreatectomy.

Sacrifice of rats

The sham, 72 h and 7 days pancreatectomised rats were sacrificed by decapitation. The hypothalamus was dissected out the line between the posterior hypothalamus and the mammillary bodies as the caudal limit (Glowinski and Iverson, 1966). The tissues were stored at −70 °C for various experiments. Total hypothalamus was used for analyses.

5-HT quantification by HPLC

Hypothalamic 5-HT was quantified by HPLC determinations using electrochemical detection (Paulose et al., 1988). 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, 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/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.

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 (Nenonene et al., 1994). The 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×*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 nM–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} to 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.

5-HT_{2C} receptor binding studies

5-HT_{2C} receptor assay was done by binding to specific antagonist [^{3}H]mesulergine binding to the synaptic membrane preparations as previously described (Herrick-Davis et al., 1999). Crude synaptic membrane preparation was suspended in

lergine was incubated with and without excess of unlabelled 5-HT (100 μM) and in competition binding experiments the incubation mixture contained 1 nM of [^{3}H]mesulergine with and without 5-HT at a concentration range of 10^{-12} M to 10^{-4} M. 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. (1951) using bovine serum albumin as standard.

Receptor data analysis

The receptor binding parameters were determined using Scatchard analysis (Scatchard, 1949). 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 analyzed 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 (Chen and Prusoff, 1973).

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.

Analysis of gene expression by RT-PCR

The expression of 5-HT_{1A} and 5-HT_{2C} receptor gene was analyzed by RT-PCR according to the method described by Wong et al. (1994). Total RNA was isolated from the brain regions of sham and pancreatectomised rats using Tri-reagent. RNA was reverse transcribed using muMLV Reverse Transcriptase with random hexamers in 20 μl reaction volume, from which 2 μl was used for detection of 5-HT_{1A} and 5-HT_{2C} receptor gene transcripts using PCR. β-actin mRNA expression was used as the internal standard. 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, 5-HT_{2C} (252 bp), 5′-CCA ACG AAC ACC TTC TTT CC-3′ and 5′-GCA TTG TGC AGT TTC TTC TCC-3′, 30 cycles, β-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) and densitometrically analyzed using Imagemaster ID software to quantitate the 5-HT_{1A} and 5-HT_{2C} receptor mRNA expression in sham, 72 h and 7 days pancreatectomised rats.

Statistics

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

Results

Body weights and blood glucose levels showed no significant change in sham operated and pancreatectomised rats (Table 1). 5-HT content in the hypothalamus showed a significant increase ($p<0.01$) at 72 h after pancreatectomy when compared with the sham. The increased content significantly reversed ($p<0.01$) to near sham value by 7 days after pancreatectomy (Table 2). Scatchard analysis of [^{3}H]8-

Table 1
Body weight (g) and blood glucose level (mg/dL) of sham operated and pancreatectomised rats

Experimental group	Body weight (g)	Glucose level (mg/dL)
Sham	90±4	99.6±6.6
72 h pancreatectomy	87±2	85.3±5.1

Table 2
5-HT content in the hypothalamus of rats

Experimental group	Hypothalamus
Sham	0.31±0.04
72 h pancreatectomy	1.08±0.14**††
7 days pancreatectomy	0.47±0.05

Values are mean±S.E.M. of 4–6 separate experiments.
**$p<0.01$ when compared with sham, ††$p<0.01$ when compared with 7 days pancreatectomy.

Table 3
[^{3}H]8-OH DPAT receptor binding parameters in the hypothalamus of rats

Experimental Group	B_{max} (fmol/mg protein)	K_d (nM)
Sham	152.0±7.0	30.1±2.1
72 h pancreatectomy	110.5±4.5**†	71.3±5.8*†
7 days pancreatectomy	138.5±5.5	41.9±6.4

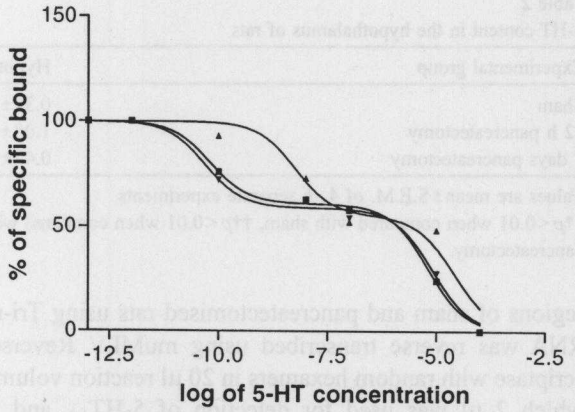

Fig. 1. Displacement of [^{3}H]8-OH DPAT with 5-HT in the hypothalamus of sham, 72 h and 7 days pancreatectomised rats. ■ Sham, ▲ 72 h pancreatectomy, ▼ 7 days pancreatectomy. Incubation was done at 25 °C for 60 min with 1 nM [^{3}H]8-OH DPAT in each tube with cold concentration varying from 10^{-12}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

OH DPAT binding to the membrane preparation of hypothalamus showed a significant decrease ($p<0.01$) in the B_{max} of 72 h pancreatectomised rats. K_d showed a significant increase ($p<0.05$) in 72 h pancreatectomised rats. The decreased B_{max} and increased K_d indicate a reduction in the density as well as the affinity of the receptor in 72 h pancreatectomised rats. The B_{max} and K_d were coming back ($p<0.05$) to sham value in 7 days pancreatectomised rats (Table 3). 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 $K_{i(H)}$ of 72 h pancreatectomised rats increased compared with sham indicating a shift in high affinity towards low affinity. $K_{i(L)}$ 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. 1 and Table 4). These changes were reversed to sham level in 7 days pancreatectomised rats.

There was a significant decrease ($p<0.01$) in the B_{max} of the [^{3}H]mesulergine binding to the membrane preparation of hypothalamus denoting a decreased 5-HT_{2C} receptor number in 72 h pancreatectomised rats. The K_d of the receptor binding showed a significant increase ($p<0.01$) in 72 h pancreatectomised rats compared with sham. This suggests a decreased receptor number as well as the affinity of the receptor in 72 h pancreatectomised rats. The altered parameters, B_{max} and K_d, significantly reversed ($p<0.01$ and $p<0.05$) to sham level by 7 days after pancreatectomy (Table 5).

Table 5
[^{3}H]Mesulergine binding parameters in the hypothalamus of rats

Experimental group	B_{max} (fmol/mg protein)	K_d (nM)
Sham	17.8±1.55	2.29±0.07
72 h pancreatectomy	10.8±1.75**†	3.10±0.10**†
72 days pancreatectomy	16.5±1.050	2.80±0.09

Values are mean±S.E.M. of 4–6 separate experiments.
**$p<0.01$ when compared with sham.
†$p<0.05$ when compared with 7 days pancreatectomy.

The competition curve for 5-HT against [^{3}H]mesulergine fitted for one-sited model in all the groups with Unity as the Hill slope value. There was an increase in the K_i and log (EC_{50}) in 72 h pancreatectomised rats compared with sham indicating a shift in affinity of the receptor towards low affinity (Fig. 2 and Table 6).

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. 3). 5-HT_{2C} receptor mRNA decreased in 72 h pancreatectomised rats and it showed a reversing trend to sham level by 7 days after pancreatectomy (Fig. 4).

Discussion

Partial pancreatectomy is a well established model to study pancreatic regeneration in animal models (Bonner-Weir et al., 1983; Sharma et al., 1999). Removal of 60–70% of the pancreas did not affect the body weight and blood glucose level of pancreatectomised rats. The maintenance of glucose homeostasis is due to regeneration among the remaining pancreatic β-cells (Leahy et al., 1988; Lohr et al., 1989). Tritiated thymidine incorporation studies from our laboratory (Mohanan et al., 2005) and previous reports (Brockenbrough et al., 1988) showed that the DNA synthesis in pancreatic islet was maximum at 72 h after pancreatectomy i.e., during active pancreatic regeneration. Increased islet DNA synthesis and glucose-derived lipid and amino acid production in association with beta-cell hyperproliferation is reported in normoglycaemic 60% pancreatectomy rats (Liu et al., 2001).

Pancreatic islets receive innervation from both divisions of the autonomic nervous system, and pancreatic endocrine secretion is partly controlled by the autonomic nervous system (Holst et al., 1986). Brain serotonergic changes are reported to regulate autonomic nerve function in rats (Kuhn et al., 1980). 5-HT content was increased in the hypothalamus during active pancreatic regeneration. Large alterations in brain monoamine

Table 4
Binding parameters of [^{3}H]8-OH DPAT against 5-HT in the hypothalamus of rats

Experimental group	Best-fit model	Log (EC_{50}) − 1	Log (EC_{50}) − 2	$K_{i(H)}$	$K_{i(L)}$	Hill slope
S	Two-site	−10.21	−5.21	6.2×10^{-11}	6.01×10^{-6}	−0.28
P-72 h	Two-site	−8.25	−4.6	5.4×10^{-9}	2.5×10^{-5}	−0.28
P-7 days	Two-site	−10.32	−5.1	4.6×10^{-11}	7.8×10^{-6}	−0.26

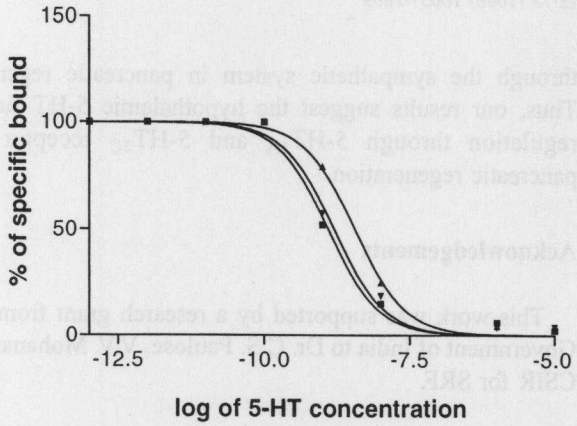

Fig. 2. Displacement of [^{3}H]mesulergine with 5-HT in the hypothalamus of sham, 72 h and 7 days pancreatectomised rats. ■ Sham, ▲ 72 h pancreatectomy, ▼ 7 days pancreatectomy. Incubation was done at 25 °C for 60 min with 1 nM mesulergine in each tube with cold concentration varying from 10^{-12} to 10^{-4} M. Reaction was stopped by rapid filtration through GF/C (Whatman) filters with ice-cold Tris buffer pH 7.4.

contents in diabetic rats (Bitar et al., 1987) 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 (Liang et al., 1999). It is generally accepted that the biosynthesis and release of 5-HT in the CNS of rodents are at least partially controlled through feedback mechanisms involving specific receptors (Moret and Briley, 1997). The increase in 5-HT content may be a homeostatic feedback mechanism by the hypothalamus to trigger the sympathetic innervation and thereby DNA synthesis in pancreas.

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 72 h pancreatectomised rats. This shows that 5-HT$_{1A}$ receptor activity decreased in the hypothalamus during peak DNA synthesis in pancreas. The decreased activity may be due to the 5-HT induced down-regulation (Ivins and Molinoff, 1991) of 5-HT$_{1A}$ receptors. Displacement analysis showed a shift in the high affinity site to low affinity site and the low affinity site towards much lower affinity in 72 h pancreatectomised rats indicating a decreased functioning of the receptor. Agonist induced desensitization and loss of high-affinity binding sites of 5-HT$_{1A}$ receptors are already reported (Harrington et al., 1994). The observation that the decrease in [^{3}H]8-OH DPAT binding is due to a reduction

Table 6
Binding parameters of [^{3}H]mesulergine in the hypothalamus of rats

Experimental group	Best-fit model	Log (EC_{50})	K_i	Hill slope
Sham	One-site	−8.9	1.2×10^{-9}	−1.02
P-72 h	One-site	−7.4	3.5×10^{-8}	−1.07
P-7 days	One-site	−8.7	1.6×10^{-9}	−0.93

S — sham, P-72 h — 72 h pancreatectomy, P-7 days — 7 days pancreatectomy. 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

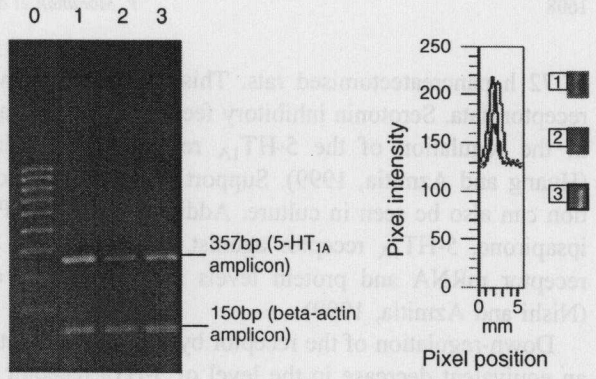

Fig. 3. RT-PCR analysis of 5-HT$_{1A}$ mRNA from the hypothalamus of sham, 72 h and 7 days pancreatectomised rats (lanes 1, 2 and 3 respectively) with primers specific for 5-HT$_{1A}$ receptor and β-actin mRNAs. Lane 0 indicates 100 bp ladder. Figure on the right indicates pixel intensity of bands analyzed densitometrically.

in the number of binding sites is evidence that the lowered binding is a reflection of increased serotonin concentration.

5-HT$_{2C}$ receptor number and affinity towards its ligand decreased in the hypothalamus of 72 h pancreatectomised rats. Displacement analysis showed a shift in affinity of the receptor towards lower affinity indicating a decreased functioning during active pancreatic regeneration. An increase in local release of 5-HT may be responsible for the decrease in [^{3}H]mesulergine binding during peak DNA synthesis in the pancreas.

The decreased expression of 5-HT$_{1A}$ and 5-HT$_{2C}$ receptor mRNA observed in the hypothalamus of 72 h pancreatectomised rats and its reversal by 7 days may be due to the alterations in serotonin concentrations. Serotonin modulates the expression of 5-HT receptor mRNA by transcriptional regulation (Rydelek-Fitzgerald et al., 1993). The distribution and abundance of 5-HT$_{1A}$ receptor mRNA in different rat brain areas generally correlate with those of the binding sites (Pompeiano et al., 1992). Our RT-PCR analysis revealed decreased 5-HT$_{1A}$ and mRNA expression in the hypothalamus

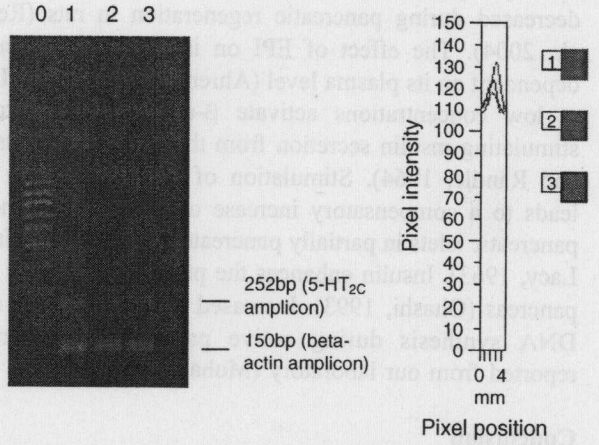

Fig. 4. RT-PCR analysis of 5-HT$_{2C}$ mRNA from the brain stem of sham, 72 h and 7 days pancreatectomised rats (lanes 1, 2 and 3 respectively) with primers specific for 5-HT$_{2C}$ receptor and β-actin mRNAs. Lane 0 indicates 100 bp ladder. Figure on the right indicates pixel intensity of bands analyzed

of 72 h pancreatectomised rats. This is concordant with our receptor data. Serotonin inhibitory feedback plays a major role in the regulation of the 5-HT_{1A} receptor mRNA in brain (Huang and Azmitia, 1999). Support for receptor autoregulation can also be seen in culture. Addition of 8-OH-DPAT and ipsapirone, 5-HT_{1A} receptor agonist, decreases both 5-HT_{1A} receptor mRNA and protein levels in hippocampal cultures (Nishi and Azmitia, 1999).

Down-regulation of the receptor by 5-HT is associated with an equivalent decrease in the level of 5-HT_2 receptor mRNA (Ivins and Molinoff, 1991). We observed a decreased 5-HT_{2C} receptor mRNA expression in the hypothalamus of 72 h pancreatectomised rats. Distribution of 5-HT_2 receptor binding sites also correlates with 5-HT_2 receptor mRNA (Mengod et al., 1990a). Thus, from our study the distribution of the 5-HT_{2C} receptor mRNA corresponded well to that of the 5-HT_{2C} receptors (Mengod et al., 1990b).

Central serotonergic neurons participate in the regulation of sympathetic nerve discharge. It is reported that 5-HT_{1A} (Bagdy et al., 1989) and 5-HT_{2C} (Baudrie and Chouloff, 1992) receptor activation in the hypothalamus leads to adreno-medullary catecholamine release. This was proved by intrahypothalamic injection of 5-HT_{1A} agonist, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), the 5-HT_{1C} agonist, *m*-chlorophenylpiperazine (*m*-CPP), and the 5-HT_2/5-HT_{2C} agonist, 1-(2,5-dimethoxy-4-iodophenyl)2-amino-propane (DOI) (Bagdy et al., 1989). Our results showed decreased number and affinity of 5-HT_{1A} and 5-HT_{2C} receptors during pancreatic regeneration, which reduces the sympathetic nerve discharge and thereby decreasing the circulating norepinephrine and epinephrine levels. Previous studies have shown that sympathoadrenal secretions are inhibited by pre-treatment with 5HT_{1A} receptor antagonist (−)-pindolol (Chauloff et al., 1990) and the 5-HT_{2C} receptor antagonist ritanserine (Bagdy et al., 1989) suggesting reduced 5-HT_{1A} and 5-HT_{2C} receptor mediated mechanism.

Studies from our laboratory reported that norepinephrine and epinephrine content in the adrenals and plasma are decreased during pancreatic regeneration in rats (Renuka et al., 2004). The effect of EPI on islet hormone secretion is dependent on its plasma level (Ahren et al., 1986). EPI and NE at low concentrations activate β-adrenergic receptors thus stimulating insulin secretion from the pancreatic islets (Coore and Randle, 1964). 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). Insulin enhances the proliferation of the remnant pancreas (Ohashi, 1993). Increased insulin secretion and islet DNA synthesis during active pancreatic regeneration is reported from our laboratory (Mohanan et al., 2005).

Conclusion

We conclude from our study that pancreatectomy trigger a serotonergic regulatory effect on the 5-HT_{1A} and 5-HT_{2C} receptors in the hypothalamus. The decreased 5-HT content through the sympathetic system in pancreatic regeneration. Thus, our results suggest the hypothalamic 5-HT functional regulation through 5-HT_{1A} and 5-HT_{2C} receptor during pancreatic regeneration.

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