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Decreased 5-HT_{2C} 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 central 5-HT_{2C} receptor binding in rat model of pancreatic regeneration using 60–70% pancreatectomy. The 5-HT and 5-HT_{2C} receptor kinetics were studied in cerebral cortex and brain stem of sham operated, 72 h pancreatectomised and 7 days pancreatectomised rats. Scatchard analysis with [^{3}H] mesulergine in cerebral cortex showed a significant decrease ($p < 0.05$) in maximal binding (B_{max}) without any change in K_d in 72 h pancreatectomised rats compared with sham. The decreased B_{max} reversed to sham level by 7 days after pancreatectomy. In brain stem, Scatchard analysis showed a significant decrease ($p < 0.01$) in B_{max} with a significant increase ($p < 0.01$) in K_d. Competition analysis in brain stem showed a shift in affinity towards a low affinity. These parameters were reversed to sham level by 7 days after pancreatectomy. Thus the results suggest that 5-HT through the 5-HT_{2C} receptor in the brain has a functional regulatory role in the pancreatic regeneration. (Mol Cell Biochem **272**: 165–170, 2005)

Key words: cerebral cortex, brain stem, 5-HT_{2C} receptor, pancreas, regeneration

Introduction

Brain serotonergic activity plays an important role in the autonomic regulation of pancreatic function [1]. Anatomical studies suggest that the vagal efferent fibres originating from nucleus ambiguus and dorsal motor nucleus of brain stem directly innervate the pancreas [2] and have a role in neurally mediated insulin release [3]. The 5-Hydroxytryptamine or serotonin$_{2C}$ (5-HT_{2C}) receptors (formerly termed 5-HT_{1C}, [4]) are widely expressed in the brain [5] and appear to mediate many important effects of 5-HT. Administration of 5-HT_{2C} receptor agonist, 1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) triggers adrenal catecholamine release and hyperglycaemia. The hyperglycaemic effect of DOI administration is mediated by centrally located 5-HT_{2C} receptors and in turn adrenal epinephrine release due to increase in sympathetic nerve discharge [6]. The hyperglycaemia was caused by an increase in EPI release which mediates its effect through α_2 adrenergic receptors [7].

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 [8]. 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

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autonomic nervous system [9, 10]. Previous reports from our laboratory have shown that sympathetic activity is decreased during pancreatic regeneration [11]. The role of hypothalamic GABAergic neurotransmission in regulating sympathetic system during liver regeneration has also been reported from our laboratory [12]. Though many reports are there implicating the brain control of pancreatic function how the central 5-HT and its receptors respond to pancreatic regeneration is not well studied. In the present study we investigated role of brain 5-HT and 5-HT_{2C} receptor binding parameters during pancreatic regeneration in rats. The study showed that there is a functional regulatory role of brain 5-HT and 5-HT_{2C} receptors in the pancreatic regeneration.

Materials and methods

Chemicals

All biochemicals used were of analytical grade, 5-Hydroxytryptamine, sodium octyl sulphonate purchased from Sigma Chemical Co., St. Louis, U.S.A. HPLC solvents were of the grade obtained from SRL and MERCK, India. [N^6-methyl-^{3}H]Mesulergine (specific activity 79.0 Ci/mmol) were purchased from Amersham, Life Science, U.K.

Animals

Weanling rats of Wistar strain 80–100 g body weight purchased from Kerala Agriculture University, Mannuthy were used for all experiments. They were housed in separate cages in 12 h light and 12 h dark periods, and were maintained on standard food pellets and water *ad libitum*. All animal care and procedures were in accordance with the 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 [13]. The removal of most of the pancreas was done by gentle abrasion with cotton applications, leaving the major blood vessels supplying other organs intact [14]. 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 [15]. The tissues were stored at −70 °C for various experiments.

5-HT quantification by HPLC

5-HT was quantified by HPLC determinations using electrochemical detection [16]. A 10% homogenate of the tissue was made in 0.4N 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_{2C} receptor binding studies

Tritiated mesulergine binding to 5-HT_{2C} receptor in the synaptic membrane preparations were assayed as previously described [17]. Crude synaptic membrane preparation was suspended in 50 mM Tris-HCl buffer (pH 7.4) and used for assay. In saturation binding experiments, 0.1–6 nM of [^{3}H]Mesulergine was incubated with and without excess of unlabelled 100 μM 5-HT 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}–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.* [18] using bovine serum albumin as standard.

Receptor data analysis

The receptor binding parameters were determined using Scatchard analysis [19]. 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, U.S.A.). 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 [20].

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 (version 2.04a) computer programme. Linear regression Scatchard plots were made using SIGMA PLOT (version 2.03).

Results

In the cerebral cortex and brain stem the 5-HT content was increased significantly ($p < 0.01$ and $p < 0.05$, respectively) at 72 h after partial pancreatectomy when compared with sham. The increased contents were reversed to near control value by 7 days after partial pancreatectomy in the cerebral cortex and brain stem (Table 1).

Scatchard analysis of [^{3}H]Mesulergine binding to synaptic membrane preparations of cerebral cortex showed a significant decrease ($p < 0.05$) in B_{max} in 72 h pancreatectomised rats while the K_d remained unaltered compared with Sham. The decreased B_{max} reversed to control level by 7 days after partial pancreatectomy (Table 2).

The competition curve for 5-HT against [^{3}H]Mesulergine fitted a one-site model in all the groups with unity as Hill slope value. The Ki and log (EC_{50}) value showed no change in 72 h pancreatectomised rats compared with sham indicating no shift in affinity (Fig. 1 and Table 3).

Table 1. 5-HT content (nmoles/g wet weight of tissue) in the cerebral cortex, brain stem of experimental rats

Animal status	Cerebral cortex	Brain stem
Sham	0.25 ± 0.07	0.28 ± 0.04
P-72 h pancreatectomy	0.62 ± 0.09**††	1.12 ± 0.03*†
P-7 days pancreatectomy	0.19 ± 0.06	0.80 ± 0.07

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

Table 2. [^{3}H]mesulergine binding parameters in the cerebral cortex of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Sham	21.6 ± 1.9	2.8 ± 0.4
72 h pancreatectomy	10.9 ± 2.5*†	2.9 ± 0.5
7 days pancreatectomy	20.2 ± 1.9	2.2 ± 0.3

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

In the case of brain stem the B_{max} of [^{3}H]Mesulergine binding decreased significantly ($p < 0.01$), whereas K_d increased significantly ($p < 0.01$) in 72 h pancreatectomised rats compared with sham. The altered parameters reversed to near normal in 7 days pancreatectomised rats (Table 4).

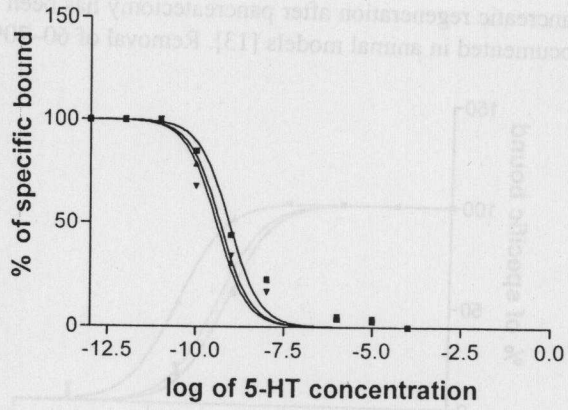

Fig. 1. Displacement of [^{3}H]mesulergine with 5-HT in the cerebral cortex of sham, 72 h and 7 days pancreatectomised rats. ■: Sham, ▲: 72 h pancretectomy, ▼: 7 days pancreatectomy. Incubation was done at 25 °C for 60 min with 1nM [^{3}H]mesulergine in each tube with cold concentration varying from 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.

Table 3. Binding parameters of [^{3}H]mesulergine against 5-HT in the cerebral cortex of experimental rats

Experimental group	Best-fit model	Log (EC_{50})	Ki	Hill slope
Sham	One-site	−9.06	5.9×10^{-10}	−0.96
72 h pancreatectomy	One-site	−9.30	3.2×10^{-10}	−0.97
7 days pancreatectomy	One-site	−9.41	2.5×10^{-10}	−0.92

Data were fitted with an iterative nonlinear regression software (Prism, GraphPad, San Diego, CA). Ki: the affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

Table 4. [^{3}H]Mesulergine binding parameters in the brain stem of rats

Experimental group	B_{max} (fmoles/mg protein)	K_d (nM)
Sham	10.2 ± 1.2	0.65 ± 0.04
72 h pancreatectomy	6.5 ± 1.4*	1.03 ± 0.07**
7 days pancreatectomy	8.1 ± 0.8	0.95 ± 0.04

*$p < 0.01$ when compared with sham.
**$p < 0.01$ when compared with 7 days pancreatectomy.

The competition curve for 5-HT against [^{3}H]Mesulergine fitted for one-site model in all the groups with unity as Hill slope value. The Ki and log (EC_{50}) value increased in 72 h pancreatectomised rats indicating a shift to low affinity (Fig. 2 and Table 5).

Discussion

Pancreatic regeneration after pancreatectomy has been well documented in animal models [13]. Removal of 60–70% of

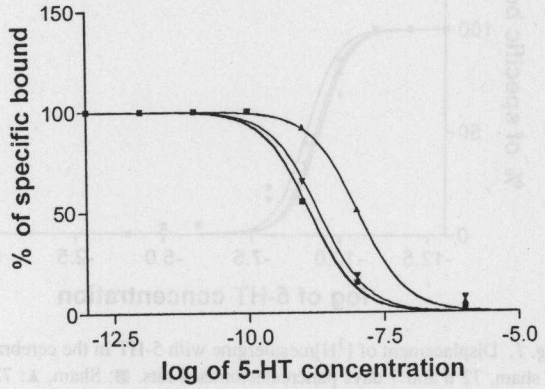

Fig. 2. Displacement of [^{3}H] mesulergine with 5-HT in the brain stem of sham, 72 h and 7 days pancreatectomised rats. ■: Sham, ▲: 72 h pancretectomy, ▼: 7 days pancreatectomy. Incubation was done at 25 °C for 30 min with 1 nM [^{3}H]mesulergine in each tube with cold concentration varying from 10^{-12}–10^{-4} 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.

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

Experimental group	Best-fit model	Log (EC_{50})	Ki	Hill slope
Sham	One-site	−8.86	5.6×10^{-10}	−1.03
72 h pancreatectomy	One-site	−7.97	4.4×10^{-9}	−0.98
7 days pancreatectomy	One-site	−8.70	8.3×10^{-10}	−1.01

Values are mean of 4–6 experiments.
Data were fitted with an iterative nonlinear regression software (Prism, GraphPad, San Diego, CA). Ki: the affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding.

the pancreas did not affect the body weight and blood glucose level of pancreatectomised rats. Tritiated thymidine incorporation studies from our laboratory [21] and previous reports 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 are reported in normoglycaemic 60% pancreatectomy rats [22].

Pancreatic islets receive innervation from both divisions of the autonomic nervous system and pancreatic endocrine secretion is partly controlled by the autonomic nervous system [23]. Brain serotonergic changes are reported to regulate autonomic nerve function in rats [24]. The 5-HT content was increased in the cerebral cortex and brain stem during active pancreatic regeneration. Alterations in brain monoamine contents in diabetic rats [25] and the relationship between enhanced monoamine content in the brain, a characteristic of hyperinsulinemic and insulin-resistant animals and islet dysfunction is reported [1].

Stimulation of centrally located 5-HT_2 receptor leads to adrenal epinephrine release that elevates plasma glucose levels and inhibits insulin release [26]. It is already reported that the 5-HT_2 receptor agonist, 1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) was able to produce tremendous increase in sympathetic nerve discharge, thus increasing EPI concentration. The 5-HT_2 receptor antagonists, Ketanserin and LY53857, were able to reverse the increase in sympathetic nerve discharge produced by DOI [7, 27–29].

When we analysed the 5-HT_{2C} receptor status in the cerebral cortex, we found that 5-HT_{2C} receptor number decreased significantly in 72 h after pancreatectomy as indicated by a decreased B_{max}. There was no shift in affinity of the receptor in 72 h pancreatectomised rats as indicated by the unchanged Ki and log (EC_{50}). In the brain stem, Scatchard analysis revealed a decreased B_{max} and increased K_d of the 5-HT_{2C} receptor indicating a reduction in the receptor density as well as the affinity of the receptor in 72 h pancreatectomised rats.

Previous studies showed that administration of the 5-$HT_{2C/2B}$ receptor agonist 1-(3-chlorophenyl) piperazine (mCPP) induced hyperglycaemia in rats and it is mediated by the activation of 5-$HT_{2C/2B}$ receptors. The effects of mCPP are connected to the activation of the sympathoadrenomedullary system and catecholamine release [30]. Pretreatment with the 5-HT_{1C} receptor antagonist ritanserine dose dependently attenuated norepinephrine and epinephrine responses suggesting 5-HT_{1C} receptor mediated mechanism [31].

Our results showed a decreased binding of 5-HT_{2C} receptors in the cerebral cortex and brain stem with a decreased affinity of receptors during active pancreatic regeneration. The decreased number and affinity of 5-HT_{2C} receptors decrease the sympathetic nerve discharge, the circulating norepinephrine and epinephrine levels, during active pancreatic regeneration. Previous studies from our laboratory reported the decrease in norepinephrine and epinephrine content in the adrenals during pancreatic regeneration in rats [11]. Also, it was reported that the epinephrine and its receptor regulation in adrenergic nerve ending were controlled by alpha2 adrenergic receptors [32, 33]. This shows that the stimulatory role of 5-HT_{2C} receptors in insulin secretion is mediated through sympathetic system during pancreatic regeneration. Studies have also shown that methysergide, which acts as a partial 5-HT_1 receptor agonist and as a 5-HT_{2C} receptor antagonist, potentiates both insulin and glucagon release [34].

Thus, the results of the present study suggest that pancreatectomy trigger a regulatory effect on 5-HT and the central 5-HT_{2C} receptors. The decreased binding of the 5-HT_{2C} receptors observed in the cerebral cortex and brain stem during pancreatic regeneration have its stimulatory role on insulin secretion mediated through sympathetic system.

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