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Alterations in the muscarinic M1 and M3 receptor gene expression in the brain stem during pancreatic regeneration and insulin secretion in weanling rats

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Abstract

Muscarinic M1 and M3 receptor changes in the brain stem during pancreatic regeneration were investigated. Brain stem acetylcholine esterase activity decreased at the time of regeneration. Sympathetic activity also decreased as indicated by the norepinephrine (NE) and epinephrine (EPI) content of adrenals and also in the plasma. Muscarinic M1 and M3 receptors showed reciprocal changes in the brain stem during regeneration. Muscairnic M1 receptor number decreased at time of regeneration without any change in the affinity. High affinity M3 receptors showed an increase in the number. The affinity did not show any change. The number of low affinity receptors decreased with decreased K_d at 72 hours after partial pancreatectomy. The K_d reversed to control value with a reversal of the number of receptors to near control value. Gene expression studies also showed a similar change in the mRNA level of M1 and M3 receptors. These alterations in the muscarinic receptors regulate sympathetic activity and maintain glucose level during pancreatic regeneration. Central muscarinic M1 and M3 receptor subtypes functional balance is suggested to regulate sympathetic and parasympathetic activity, which in turn control the islet cell proliferation and glucose homeostasis.

Keywords: Muscarinic receptors; Pancreatectomy; Insulin; Brain stem; Regeneration; RT-PCR

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Introduction

Central nervous system through parasympathetic and sympathetic pathways regulates insulin secretion from pancreatic islets and maintains glucose homeostasis (Ahren, 2000). Brain stem along with hypothalamus serves as the key centres of the central nervous system regulating the body homeostasis. Stimulation of the peripheral vagus nerve leads to an increase in circulating insulin levels. Anatomical studies suggest that the origin of these vagal efferent fibres are nucleus ambiguus and dorsal motor nucleus directly innervating pancreas (Bereiter et al., 1981). Studies from our laboratory reported that NE levels as well as the adrenergic receptors function increased in STZ-diabetic rats (Abraham and Paulose, 1999). The increased sympathetic stimulation leads to hypertension in pyridoxine deficient rats (Paulose et al., 1988) which in turn lead to diabetes (Paulose et al., 1999). It is also reported that the high affinity serotonergic receptor's affinity decreased in the brain stem of diabetic rats (Padayatti and Paulose, 1999). Stimulation of the central nervous system with cholinergic agonist caused a marked increase in the hepatic venous plasma glucose concentration (Iguchi et al., 1986). The muscarinic antagonist atropine suppressed the hyperglycaemia induced by hippocampus administration of neostigmine in a dose-dependent manner (Iguchi et al., 1991). Neostigmine-induced epinephrine and glucagon secretion and increased hepatic glucose output stimulated by direct neural innervation to liver is mediated by central muscarinic receptors (Iguchi et al., 1990).

Stimulation of the cholinergic neurons in the central nervous system resulted in an increase in the hepatic venous glucose, glucagon and epinephrine content (Iguchi et al., 1988). Epinephrine secreted after central nervous system stimulation increased glucagon secretion from the pancreas (Gerich et al., 1976) and inhibited insulin secretion (Porte et al., 1966). Muscarinic receptors is reported to induce cell proliferation in different cell types. In colonic epithelial cells, carbachol through muscarinic M3 receptors bring about transactivation of EGFR with subsequent activation of the ERK isoforms of mitogen activated protein kinase (Ukegawa et al., 2003). The importance of muscarinic receptor involvement in the cell proliferation led to the present study of muscarinic M1 and M3 receptor gene expression in the brain stem during pancreatic regeneration in pancratectomised rats. M1 and M3 receptor gene expression analysed by RT-PCR showed that the functional balance of these receptors during pancreatic regeneration. Acetylcholine esterase kinetic parametes showed a decrease in the cholinergic activity. Norepinephrine and epinephrine decreased in the plasma and adrenals indicating a decrease in the sympathetic activity during pancreatic regeneration.

Materials and methods

Chemicals

All reagents were of analytical grade. HPLC solvents were of HPLC grade obtained from SRL and MERCK, India. Quinuclidinyl benzilate , L-[Benzilic-4,4′-^{3}H]] (Sp. Activity 42 Ci/mmol) and 4-DAMP, [N-methyl-^{3}H] (Sp. Activity 83 Ci/mmol) were purchased from NEN life sciences products Inc., Boston, U.S.A. Radioimmunoassay kits for insulin were purchased from Bhabha Atomic Research Centre (BARC), Mumbai, India.

Molecular biology chemicals were purchased from Bangalore Genei, India. Reverse transcriptase enzyme MuMLV was obtained from Amersham Life Science, UK. Tri-reagent kit was purchased from MRC, USA. PCR primers used in this study was synthesised by Sigma Chemical Co., USA.

Animals

Wistar weanling rats of 80–100g body weight were purchased from Central Institute of Fisheries Technology, Cochin and used for all experiments. They were housed in separate cages under 12 hours light and 12 hours dark periods and were maintained on standard food pellets and water ad libitum.

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 (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 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. Bodyweight and blood glucose levels were checked routinely. The rats were maintained for different time intervals-12, 24, 48 and 72 hours; 7 and 14 days. They were sacrificed by decapitation and brain stem was dissected out quickly over ice according to the procedure of Glowinski and Iversen (1996). The tissues were stored at −70°C until assay.

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

[^{3}H]thymidine, 5μCi, was injected intra-peritoneally into partially pancreatectomised rats to study DNA synthesis at 24, 36, 48, 72 hours; 7 and 14 days of pancreatic regeneration. [^{3}H]thymidine was injected 2 hrs before sacrifice. Pancreas was dissected out and DNA was extracted from pancreatic islets according to Schneider (1957). [^{3}H]thymidine incorporation was quantified in a liquid scintillation counter (WALLAC 1409) after adding cocktail-T containing Triton-X 100.

The adrenal monoamines were assayed according to Paulose et al. (1988) and plasma monoamines were assayed according to Jackson et al. (1997). using high performance liquid chromatography (HPLC) integrated with an electrochemical detector (HPLC-ECD) (Shimadzu, Japan) and fitted with CLC-ODS reverse phase column of 5 μm particle size.

Acetylcholine esterase assay was done using the spectrophotometric method of Ellman et al. (1961). The homogenate (10%) was prepared in sodium phosphate buffer 30mM, pH-8.0. One ml of 1% Triton × 100 was added to the homogenate to release the membrane bound enzyme and it centrifuged at 12,500 × g for 30 minutes at 4°C. Different concentrations, 0.025mM–0.5mM, of acetylthiocholine iodide used as substrate. The mercaptan formed as a result of the hydrolysis of the ester reacts with an oxidising agent 5,5′-dithiobis (2-Nitrobenzoate) read at 412 nm.

[^{3}H] QNB and [^{3}H]DAMP binding assay in the brain stem was done according to the modified procedure of Pinter et al. (1998). Brain tissues were homogenised in a polytron homogeniser with 20 volumes of cold 50mM Tris-HCl buffer, containing 1mM EDTA (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, 50mM Tris-EDTA buffer. Binding assays were done using different concentrations i.e., 0.1–2.5nM of [^{3}H]QNB with 200–250μg protein. Non-specific binding was determined using 100μM pirenzepine.

[^{3}H] DAMP Binding assays were done using different concentrations i.e., 0.01–5nM of ligand with 200–250μg protein. Non-specific binding was determined using 100μM 4-DAMP mustard. Tubes were incubated at 22°C for 60 min. and filtered rapidly through GF/C filters (Whatman). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris-HCl buffer, (pH 7.4). Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter. The non-specific binding determined showed 10% in all our experiments. Protein was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

The data were analyzed according to Scatchard (1949). The specific binding was determined by subtracting non-specific binding from the total. The binding parameters, maximal binding (B_{max}) and equilibrium dissociation constant (K_d), were derived by linear regression analysis by plotting the specific binding of the radioligand on X-axis and bound/free on Y-axis. The maximal binding is a measure of the total number of receptors present in the tissue and the equilibrium dissociation constant is the measure of the affinity of the receptors for the radioligand. The K_d is inversely related to receptor affinity.

RNA was isolated from the brain stem of sham and partially pancreatectomised rats using the Tri reagent (MRC, USA). RT-PCR was carried out in a total reaction volume of 20μl reaction mixture in 0.2ml tubes. RT-PCR was performed in an Eppendorf Personal thermocycler. cDNA synthesis of 2μg RNA was performed in a reaction mixture containing 40units of MuMLV reverse transcriptase, 2mM dithiothreitol, 4 units of human placental RNAse inhibitor, 0.5μg of random hexamer and 0.25mM dNTPS (dATP, dCTP, dGTP and dTTP). The tubes were then incubated at 37°C for one hour. After incubation the reverse transcriptase, MuMLV, was inactivated by heating at a temperature of 95°C. Polymerase Chain Reaction (PCR) was carried out in a 20μl volume reaction mixture containing 4μl of cDNA, 0.25mM dNTPS-dATP, dCTP, dGTP and dTTP-, 0.5units of Taq DNA polymerase and 10 picomoles of specific primers.

PCR primers

The following primers were used for muscarinic M1, M3 receptors and β-actin RT-PCR studies. (M1 f) 5′-GCA.CAG.GCA.CCC.ACC.AAG.CAG -3′, (m1 r) 5′-AGA GCA GCA GCA GGC GGA ACG -3′;

Table 1
Acetylcholine esterase activity in the brain stem of sham and pancreatectomised young rats

Animal status	V_{max} (μmoles/min/mg protein)	K_m (μM)
Sham	3.66 ± 0.13	0.05 ± 0.008
72 hours pancreatectomy	2.52 ± 0.02**,ψψ	0.06 ± 0.003
7 days pancreatectomy	3.66 ± 0.05	0.04 ± 0.007

Values are mean ± S.E.M of 4–6 separate experiments.
Acetylcholine esterase assay was done using the spectrophotometric method using different concentrations of acetylthiocholine iodide, 0.025 mM–0.5 mM as substrate.
** $p < 0.01$ when compared to sham.
ψψ $p < 0.01$ when compared to 7 days.

Table 2
Norepinephrine and epinephrine level (nmoles/ml) in the plasma of sham and pancreatectomised young rats

Animal status	NE	EPI
Sham	2.21 ± 0.44	3.26 ± 0.26
72 hours pancreatectomy	0.88 ± 0.09***	0.83 ± 0.12***,ψψψ
7 days pancreatectomy	1.01 ± 0.06***	2.75 ± 0.28

Values are mean ± S.E.M of 4–6 separate experiments.
NE-Norepinephrine. EPI-Epinephrine.
The contents were determined using HPLC integrated with EC detector.
*** $p < 0.001$ when compared to sham.
ψψψ $p < 0.001$ when compared to 7 days.

(M3 f) 5′-ATT TCT CCT CAA ACG ACA CCT CC -3′ (M3 r) 5′-ATG ACC CAA GCC AGA CCA ATC -3′; (β-actin f) 5′-CAA CTT TAC CTT GGC CAC TAC C-3′, (β-actin r) 5′- TAC GAC TGC AAA CAC TCT ACA CC -3′. The following thermocycling profile is used for the initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 61°C for 30 sec (M3) or 58°C for 30 sec(β-actin) and 72°C for 30 sec, then incubation at 72°C for 7 min at the end of the final cycle . The annealing temperature for the M1 is 62°C for 1 min followed by incubation at 72°C for 1 min. The β-actin was used as an internal standard control gene.

The Polymerase Chain Reaction product was loaded on a 2.0% agarose gel with ethidium bromide. Bromophenol blue was used as the indicator dye. 48V current was used for all the run. The image was captured using an Imagemaster gel documentation system (Pharmacia Biotech) and the bands were densitometrically analysed using Total Lab software.

Results

There was no significant change in the body weights and blood glucose levels of sham operated and partially pancreatectomised rats. [^{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 hours after partial pancreatectomy. The DNA synthesis was peaked at 72 hours after partial pancreatectomy ($p<0.001$). The elevated levels of DNA synthesis reversed back to

Table 3
Norepinephrine and epinephrine content (nmoles/g wet wt) in the adrenals of sham and pancreatectomised young rats

Animal status	NE	EPI
Sham	518 ± 38	3454 ± 312
72 hrs pancreatectomy	105 ± 23***,ψψψ	1245 ± 60***,ψψψ
7 days pancreatectomy	498 ± 33	2546 ± 101

Values are mean ± S.E.M of 4–6 separate experiments.
NE-Norepinephrine. EPI-Epinephrine.
The contents were determined using HPLC connected with EC detector.
*** $p < 0.001$ when compared to sham.
ψψψ $p < 0.001$ when compared to 7days.

Table 4
Scatchard analysis of [^{3}H] QNB binding against pirenzepine in the brain stem of sham and pancreatectomised rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Sham	1432 ± 21	1.21 ± 0.10
72 hours pancreatectomy	1198 ± 17***	1.16 ± 0.02
7 days pancreatectomy	1135 ± 44***	1.29 ± 0.15

Values are mean ± SEM of 4–6 individual experiments.
B_{max}-Maximal binding. K_d-Dissociation constant.
[^{3}H] QNB of different concentrations, 0.01nM-5nM were incubated with and without excess of pirenzepine (100μM) using protein concentration of 200–250μg for the kinetic studies of muscarinic M1 receptor. Specific binding was determined by subtracting non-specific binding from total binding.
*** $p < 0.001$ when compared to sham.

near basal level after 7 and 14 days after partial pancreatectomy. The insulin levels in the plasma of partially pancreatectomised rats showed a significant increase ($p<0.01$) at 72 hours after partial pancreatectomy. The increased insulin levels decreased to near control by 7 and 14 days.

Brain stem AChE activity significantly decreased ($p<0.01$) at 72 hours after partial pancreatectomy with no change in the K_m. The V_{max} increased significantly ($p<0.01$) 7 days after partial pancreatectomy (Table 1).

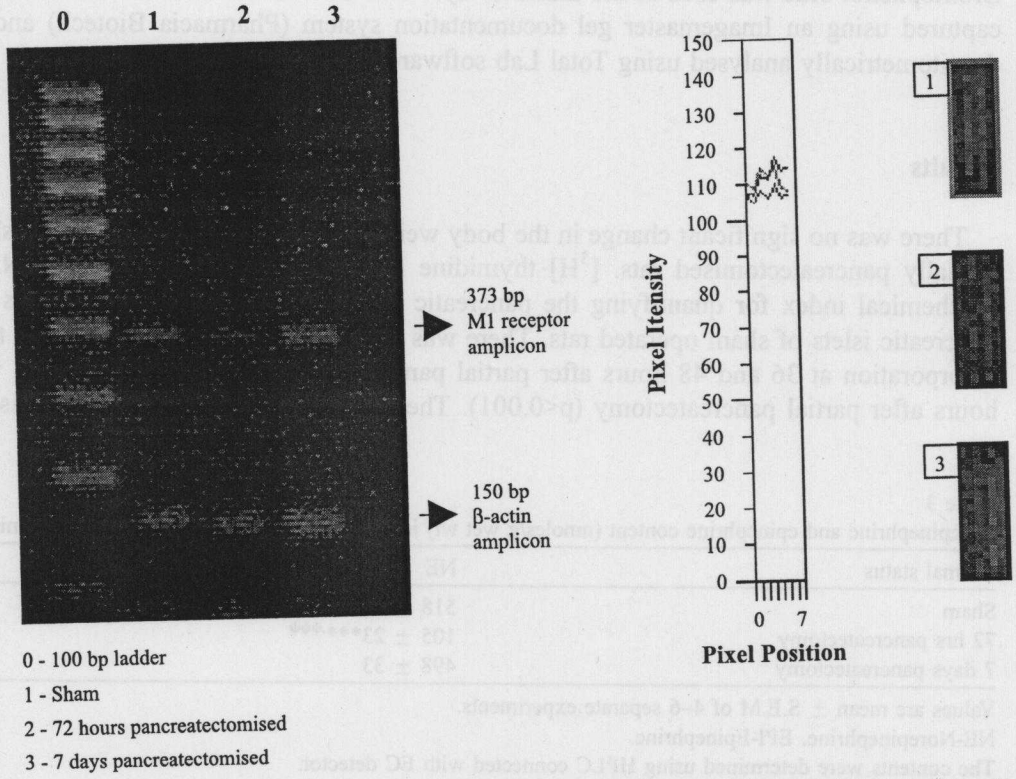

Fig. 1. RT-PCR amplification product of muscarinic M1 receptor mRNA from the brain stem of sham and pancreatectomised rats.

Table 5
Scatchard analysis of high affinity [^{3}H]4-DAMP receptor binding against 4-DAMP mustard in the brain stem of sham and pancreatectomised rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Sham	15.16 ± 1.16	0.12 ± 0.02
72 hours pancreatectomy	23.16 ± 0.66**,ΨΨΨ	0.23 ± 0.05
7 days pancreatectomy	13.00 ± 0.05	0.09 ± 0.03

Values are mean ± SEM of 4–6 individual experiments.
B_{max}-Maximal binding. K_d-Dissociation constant.
[^{3}H] 4-DAMP of different concentrations, 0.1nM–2.5nM were incubated with and without excess of 4-DAMP mustard (100μM) using protein concentration of 200–250μg for the kinetic studies of muscarinic M3 receptor. Specific binding was determined by subtracting non-specific binding from total binding.
** $p < 0.001$ when compared to sham.
ΨΨΨ $p < 0.001$ when compared to 7 days pancreatectomy.

Circulating NE and EPI level significantly decreased ($p<0.001$) in the serum during pancreatic regeneration. The NE level remained decreased ($p<0.001$) at 7 days time period while the EPI reversed to the control level (Table 2). EPI and NE content decreased significantly ($p<0.001$) in the adrenals during pancreatic regeneration. The decreased EPI and NE reversed to control values at 7 days after partial pancreatectomy (Table 3).

Scatchard analysis for brain stem muscarinic M1 receptors showed a significant decrease ($p<0.001$) in the B_{max} at 72 hours after partial pancreatectomy, while the K_d did not show any change. The B_{max} remained decreased at 7 days after partial pancreatectomy (Table 4). RT-PCR analysis showed that the muscarinic M1 receptor mRNA decreased at 72 hours after partial pancreatectomy which was reversed to control level after 7 days (Fig. 1).

The B_{max} of high affinity muscarinic M3 receptors significantly increased ($p<0.01$) indicating an increase in the receptor number during regeneration with no change in K_d. The increased B_{max} was reversed to control valuse after 7 days (Table 5). The B_{max} of the low affinity receptors significantly decreased ($p<0.001$) at 72 hours after partial pancreatectomy which was reversed to near control value

Table 6
Scatchard analysis of low affinity [^{3}H]4-DAMP binding receptors against 4-DAMP mustard in the brain stem of sham and pancreatectomised rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Sham	40.00 ± 2.88	1.08 ± 0.18
72 hour pancreatectomy	18.67 ± 0.06***	0.27 ± 0.02**,ΨΨΨ
7 days pancreatectomy	25.67 ± 2.33*	0.99 ± 0.09

Values are mean ± SEM of 4–6 individual experiments.
B_{max}-Maximal binding. K_d-Dissociation constant.
[^{3}H] 4-DAMP of different concentrations, 0.01nM–5nM were incubated with and without excess of 4-DAMP mustard (100μM) using protein concentration of 200-250μg for the kinetic studies of muscarinic M3 receptor. Specific binding was determined by subtracting non-specific binding from total binding.
* $p < 0.05$ when compared to sham.
** $p < 0.01$ when compared to sham.
*** $p < 0.001$ when compared to sham.
ΨΨΨ $p < 0.01$ when compared to 7 days.

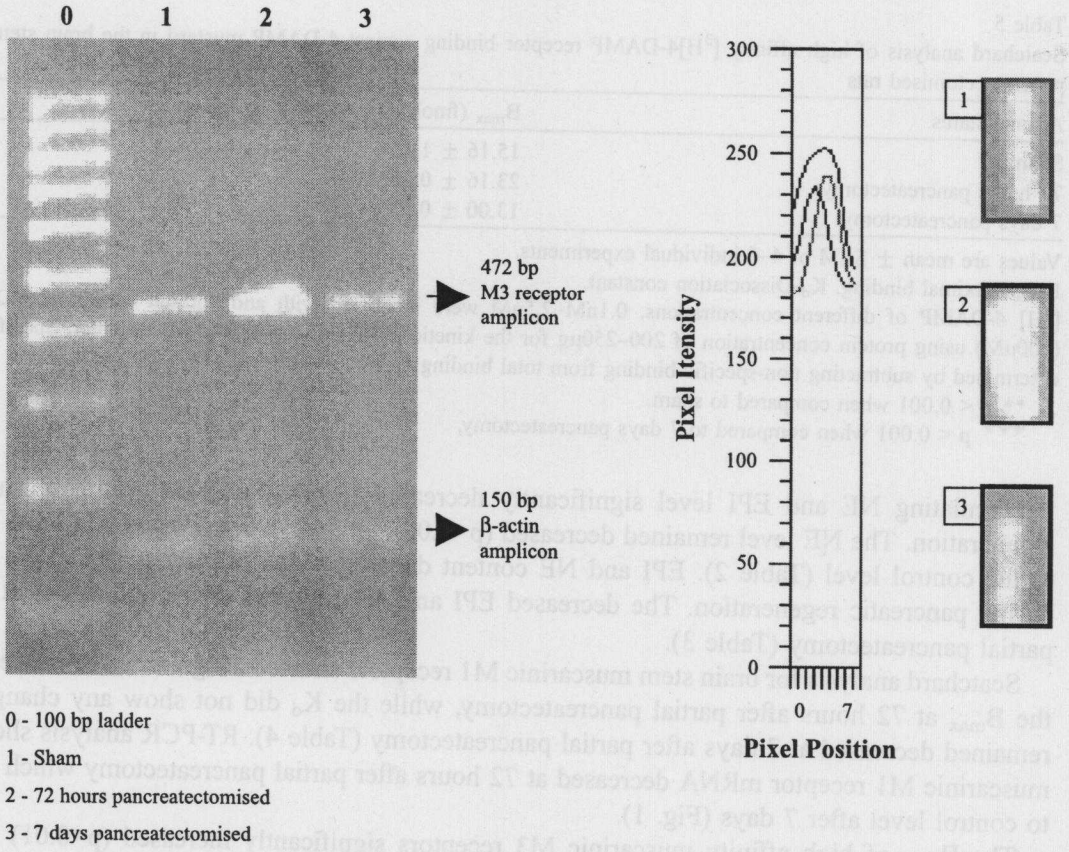

Fig. 2. RT-PCR amplification product of muscarinic M3 receptor mRNA from the brain stem of sham and pancreatectomised rats.

after 7 days. The K_d value decreased significantly ($p<0.01$) during regeneration indicating an increase in the affinity of low affinity receptors which was reversed to control value at 7 days (Table 6). RT-PCR analysis showed that the muscarinic M3 receptor mRNA increased at 72 hours after partial pancreatectomy while it reversed to control level after 7 days (Fig. 2).

Discussion

Partial pancreatectomy is a useful method to study pancreatic regeneration. Removal of 60–70% of the pancreas did not affect the body weight and blood glucose level of pancreatectomised rats. Tritiated thymidine incorporation method was used to study the DNA synthesis in pancreatic islets. Our studies showed that the DNA synthesis was maximum at 72 hours after partial pancreatectomy. The result is in concordant with the previous reports (Brockenbrough et al., 1988).

Circulating insulin levels increased during DNA synthesis keeping the blood glucose level normal. The administration of insulin to diabetic rat implanted with fetal pancreas showed increase in β-cell mass (McEvoy and Hegre, 1978). The increase in the insulin secretion after the pancreatectomy,

besides maintaining the normoglycaemic level, also helps to regain its original mass and volume by inducing cell division. Insulin can stimulate β-cell replication directly possibly through a receptor for multiplication stimulating activity or another insulin like growth factor (Rabinovitch et al., 1982).

Acetylcholine esterase is the enzyme catalysing the degradation of acetylcholine into choline and acetyl CoA. It has been well established that there is a marked change in the acetylcholine esterase in diabetic condition. Akmayev et al. (1978) showed that there is difference in distribution of the enzyme in the neurons of the central vagal nuclei and medulla oblongata in normal and diabetic adult male rats. It is suggested that the changes in the plasma glucose or insulin levels may be the stimulus that influence the activity of cholinergic neurons. This central cholinergic activity will have a role in the insulin secretion. The activation of central cholinergic system by administration of cholinergic agonist into the third cerebral ventricle reported to produce hyperglycaemia in rats (Iguchi et al., 1985). Injection of neostigmine, an inhibitor of cholinesterase, into this ventricle resulted in the increase of not only glucose, but also glucagon, epinephrine, and norepinephrine in the hepatic venous plasma. Neostigmine-induced increments in glucose did not occur in adrenalectomized rats. This suggests that the secreted epinephrine acts directly on the liver to increase hepatic glucose output (Iguchi et al., 1986). Central cholinergic-muscarinic activation with neostigmine stimulates sympathetic nervous activity in the liver, heart, pancreas and interscapular brown adipose tissue (Gotoh and Smythe, 1992). The decreased AChE activity found in the brain regions is a compensatory mechanism to maintain the normoglycaemic level. It is also found that the circulating insulin level increased at the time of regeneration with out any change in the glucose.

The plasma EPI and NE levels and also their content in adrenals decreased at the time of pancreatic regeneration. Sympathetic system is inhibitory to insulin secretion. Epinephrine when used in high doses in vivo or in vitro, reduces the insulin response to stimulators (Malaisse, 1972). EPI and NE have an antagonistic effect on insulin secretion and glucose uptake (Porte et al., 1966). The decrease in the NE and EPI stimulate the β adrenergic receptors which are stimulatory to insulin secretion. Activation of the splanchnic nerves innervating the adrenals results in the catecholamine release from chromaffin cells into the circulation (Bornstein et al., 1990). Injection of neostigmine into the cerebral entricles increased the plasma levels of epinephrine in rats (Nonogaki et al., 1994). Studies of Oda et al. (1988) confirmed the importance of sympathoadrenomedullary system in controlling the activity of pancreatic islets. Intravenous infusion of epinephrine caused increase in plasma glucagon and glucose concentration with decrease in plasma insulin concentration. Studies on insulin secretion in sheep fetus confirmed the inter-relationship of sympathoadrenal activity and fetal insulin secretion. Hypoxia increased the NE and EPI secretion from the adrenals with a marked increase in plasma NE and resulted in the inhibition of insulin secretion (Jackson et al., 1993, 2000). The decrease in the EPI and NE levels suggested to stimulate insulin secretion from pancreatic islets at the time of regeneration.

Scatchard analysis using [^{3}H] QNB showed that M1 receptor decreased at 72 hours after partial pancreatectomy. Muscarinic M1 receptor mRNA also decreased at the time of pancreatic regeneration. Muscarinic receptors are located presynaptically on sympathetic and central noradrenergic neurons where they can modulate release of NE (Stark et al., 1989). Carbachol, muscarine, or neostigmine injection into the third cerebral ventricle caused a dose-dependent increase in the hepatic venous plasma glucose concentration. The increase in glucose concentration caused by neostigmine administration dose-dependently suppressed by the coadministration of muscarinic receptor antagonist, atropine (Iguchi et al., 1986). Among all muscarinic receptors studied M1 receptor gene expression increased in the

hypothalamus of spontaneously hypertensive rats. The increased function reported to increase the sympathetic tone resulting in hypertension (Wei et al., 1995). The decrease in the muscarinic M1 receptors in the brain stem at the time of pancreatic regeneration suggested to stimulate pancreatic insulin secretion and thus regeneration.

[^{3}H] DAMP binding studies revealed that M3 receptors increased at the time of pancreatic regeneration. The M3 subtype receptors are reported to increase glucose induced insulin secretion from the pancreas. (Gilon and Henquin, 2001). Muscarinic M3 receptors activate MAPK in the oligodendrocyte progenitors (Ragheb et al., 2001). The dorsal motor nucleus of the vagus nerve is located in the brain stem. It is connected to the endocrine pancreas exclusively via vagal fibres and has a role in neurally mediated insulin release. Nucleus ambiguus stimulation reported to increase plasma insulin levels in rats (Bereiter et al., 1981). Brain stem adrenergic and serotonergic receptors were altered in diabetic rats (Abraham and Paulose, 1999; Padayatti and Paulose, 1999). Also differential regulation of hypothalamic GABA receptors were reported during the liver regeneration in rats (Biju et al., 2001). The increase in the brain stem muscarinic M3 receptors is suggested to activate pancreatic M3 receptors and stimulate insulin secretion and pancreatric regeneration. Thus the central muscarinic M1 and M3 receptor subtypes functional balance regulate sympathetic and parasympathetic systems which in turn control the islet cell proliferation and glucose homeostasis.

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