RESULTS

Body Weights and Blood Glucose Levels

The body weights and blood glucose levels of rats showed no significant change in sham operated and pancreatectomised rats (Table - 1).

DNA Synthesis in the Regenerating Pancreas

Tritiated thymidine incorporation into replicating DNA was used as a biochemical index for quantifying DNA synthesis during pancreatic regeneration. DNA synthesis was negligible in the pancreatic islets of sham operated rats. A significant increase ($p<0.01$) in the [^{3}H]thymidine incorporation was observed at 36 hrs and 48 hrs after partial pancreatectomy. The DNA synthesis was peaked at 72 hrs after partial pancreatectomy ($p<0.001$). It reversed to near normal levels by 7 days and reached the basal level by 14 days after partial pancreatectomy (Fig. 1).

Circulating Insulin Level

The insulin levels in the plasma of pancreatectomised rats showed a significant increase at 48 hrs ($p<0.05$) and peaked at 72 hrs ($p<0.01$) after partial pancreatectomy. The elevated insulin levels then reversed to basal levels by 7 and 14 days (Fig. 2).

5-HT Content in the Brain Regions (CC, BS and Hypo) of Experimental Rats

In the cerebral cortex and hypothalamus the 5-HT content was increased significantly ($p<0.01$) at 72 hrs after partial pancreatectomy when compared with control. 5-HT content was also increased significantly ($p<0.05$) in the brain stem during active DNA synthesis. The increased contents were reversed to near normal by 7 days after partial pancreatectomy in the cerebral cortex and hypothalamus while it remained unchanged in the brain stem (Table - 2).

5-HT and 5-HIAA Content in the Pancreas of Experimental Rats

There was a significant ($p<0.05$) decrease in the pancreatic 5-HT conte during active cell proliferation when compared with control. The decreased conte was reversed to near normal at 7 days after partial pancreatectomy. The 5-HIA content and the turnover rate of 5-HIAA/5-HT were significantly increased ($p<0.0$ $P<0.001$) in 72 hrs pancreatectomised rats when compared with control. Th increased 5-HIAA content and the turnover of 5-HIAA/5-HT were reversed by days after partial pancreatectomy (Table - 3).

5-HT, NE and EPI Levels in the plasma of Experimental Rats

There was a significant decrease in the plasma EPI ($p<0.001$) and N ($p<0.05$) levels in 72 hrs pancreatectomised rats compared with control. The plasm 5-HT level increased significantly ($p<0.01$). The decreased NE and EPI levels a the increased 5-HT level reversed to normal levels by 7 days after parti pancreatectomy (Table - 4).

NE and EPI contents in the Adrenals of Experimental Rats

NE and EPI contents decreased significantly ($p<0.001$) in the adrenals duri pancreatic regeneration. The decreased NE and EPI reversed to control levels at days after partial pancreatectomy (Table - 5).

Receptor Alterations in the Brain Regions of Experimental Rats

5-HT_{1A} Receptor Analysis

Cerebral Cortex

[^{3}H]8-OH DPAT Binding Parameters

Scatchard analysis in the cerebral cortex of rats showed that there were two affinity sites for [^{3}H]8-OH DPAT binding. The K_d value of the high affinity receptor significantly increased ($p<0.01$) in 72 hrs pancreatectomised rats. There was no significant change in the B_{max} of [^{3}H]8-OH DPAT high affinity receptor binding to the membrane preparation of 72 hrs pancreatectomised rats (Figure 3 - & Table - 6). The B_{max} of low affinity receptor binding was decreased significantly in 72 hrs pancreatectomised rats ($p<0.01$) compared with control. The K_d of the receptor increased significantly ($p<0.01$) in 72 hrs after partial pancreatectomy. The K_d of high affinity receptor and the B_{max} and K_d of low affinity receptor reversed to near normal in the 7 days pancreatectomised rats (Figure - 4 & Table - 7).

Displacement Analysis of [^{3}H] 8-OH DPAT by 5-HT

The competition curve for 5-HT against [^{3}H]8-OH DPAT fitted for two-sited model in all the groups with Hill slope value away from Unity. The $Ki_{(H)}$ increased in 72 hrs pancreatectomised rats along with an increase in the log (EC_{50})-1 indicating a shift in high affinity towards low affinity. $Ki_{(L)}$ also showed an increase in 72 hrs pancreatectomised rats with an increase in log (EC_{50})-2 denoting a shift in the low affinity site towards much lower affinity (Figure -5 & Table -8).

RT – PCR Analysis of 5 – HT_{1A} Receptor

5- HT_{1A} receptor mRNA expression decreased in 72 hrs pancreatectomised rats and it reversed to control level at 7 days after partial pacreatectomy (Figure – 6 & Table – 9).

Brain Stem

[^{3}H]8-OH DPAT Binding Parameters

The B_{max} of the high affinity receptor binding decreased ($p<0.01$) and th K_d increased significantly ($p<0.01$) in 72 hrs pancreatectomised rats compared wi control. The decreased B_{max} and increased K_d reversed to control level by 7 da after partial pancreatectomy (Figure - 7 & Table - 10). The B_{max} of low affini receptor binding was decreased significantly ($p<0.01$) without any change in K_d in 7 hrs pancreatectomised rats compared with control. The B_{max} partially reversed control level by 7 days after partial pancreatectomy (Figure - 8 & Table - 11).

Displacement Analysis of [^{3}H]8-OH DPAT by 5-HT

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 $Ki_{(H)}$ increas in 72 hrs with an increased log (EC_{50})-1. This indicates a shift of high affini towards low affinity. $Ki_{(L)}$ and the log (EC_{50})-2 value showed no change (Figure - & Table - 12).

RT-PCR Analysis of 5-HT_{1A} Receptor

RT-PCR analysis revealed a decreased expression of 5-HT_{1A} receptor mRN in 72 hrs and it reversed to near normal level in 7 days pancreatectomised ra (Figure - 10 & Table 13).

Hypothalamus

[^{3}H]8-OH DPAT binding parameters

A significant increase ($p<0.01$) in the K_d of the high affinity [^{3}H]8-OH DPAT receptor binding was observed in 72 hrs pancreatectomised rats compared with control. There was no significant change in the B_{max}. The increased K_d value reversed to normal level by 7 days after partial pancreatectomy (Figure - 11 & Table - 14). The B_{max} of the low affinity [^{3}H]8-OH DPAT receptor binding decreased significantly ($p<0.01$) and the K_d value increased significantly ($p< 0.05$) in 72 hrs pancreatectomised rats compared with control. The B_{max} and K_d value reversed to control level by 7 days after partial pancreatectomy (Figure - 12 & Table - 15).

Displacement Analysis of [^{3}H]8-OH DPAT by 5-HT

The competition curve for 5-HT against [^{3}H]8-OH DPAT fitted for two-sited model in all the groups with Hill slope value away from unity. The $Ki_{(H)}$ and log (EC_{50})-1 increased in 72 hrs pancreatectomised rats indicating a shift in affinity of the high affinity receptor binding site towards low affinity. $Ki_{(L)}$ and log (EC_{50})-2 increased in 72 hrs pancreatectomised rats indicating a decrease in the low affinity site towards much lower affinity (Figure - 13 & Table - 16).

RT-PCR Analysis of 5-HT$_{1A}$ Receptor

A decreased 5-HT$_{1A}$ receptor mRNA expression was observed at 72 hrs pancreatectomised rats and it reversed to control level at 7 days (Figure - 14 & Table - 17).

5-HT_{2C} Receptor Analysis

Cerebral cortex

[^{3}H]Mesulergine Binding Parameters

There was a significant decrease ($p<0.05$) in the B_{max} of [^{3}H]mesulergi binding without any change in K_d in 72 hrs pancreatectomised rats compared wi control. The decreased B_{max} reversed to control level by 7 days after parti pancreatectomy (Figure - 15 & Table - 18).

Displacement Analysis of [^{3}H]Mesulergine by 5-HT

The competition curve for 5-HT against [^{3}H]mesulergine fitted for one-sit model in all the groups with Unity as Hill slope value. The Ki and log (EC_{50}) val showed no change in 72 hrs pancreatectomised rats compared with control indicati no shift in affinity (Figure - 16 & Table - 19).

RT-PCR Analysis of 5-HT_{2C} Receptor

5-HT_{2C} receptor mRNA expression decreased in 72 hrs and it reversed control level in 7 days pancreatectomised rats compared with control (Figure - 17 Table - 20).

Brain stem

[^{3}H]Mesulergine Binding Parameters

The B_{max} of [^{3}H]mesulergine binding decreased significantly ($p<0.01$). T K_d of the receptor binding showed a significant increase ($p<0.01$) in 72 h pancreatectomised rats compared with control. The altered parameters reversed near normal in 7 days pancreatectomised rats (Figure - 18 & Table - 21).

Displacement Analysis of [^{3}H]Mesulergine by 5-HT

The competition curve for 5-HT against [^{3}H]mesulergine fitted for one-sited model in all the groups with Unity as Hill slope value. There was an increase in the Ki and log (EC_{50}) in 72 hrs pancreatectomised rats (Figure - 19 & Table - 22).

RT-PCR analysis of 5-HT$_{2C}$ receptor: RT-PCR analysis revealed a decreased mRNA in 72 hrs pancreatectomised rats (Figure - 20 & Table 23).

Hypothalamus

[^{3}H]Mesulergine Binding Parameters

There was a significant decrease ($p<0.01$) in the B_{max} of the [^{3}H]mesulergine binding to the membrane preparation of hypothalamus in 72 hrs pancreatectomised rats. The K_d of the receptor binding showed a significant increase ($p<0.01$) in 72 hrs pancreatectomised rats compared with control. The decreased B_{max} and increased K_d reversed to control level by 7 days after partial pancreatectomy (Figure - 21 & Table - 24).

Displacement Analysis of [^{3}H]Mesulergine by 5-HT

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 Ki and log (EC_{50}) in 72 hrs pancreatectomised rats compared with control indicating a shift in affinity of the receptor towards low affinity (Figure - 22 & Table - 25).

RT-PCR Analysis of 5-HT$_{2C}$ Receptor: 5-HT_{2C} receptor mRNA decreased in 72 hrs pancreatectomised rats and it reversed to near normal level by 7 days after partial pancreatectomy (Figure - 23 & Table - 26).

RECEPTOR ALTERATIONS IN THE PANCREATIC ISLETS DURING PANCREATIC REGENERATION

5-HT_{1A} Receptor Analysis

[^{3}H]8-OH DPAT Binding Parameters

There was a significant increase ($p<0.01$) in the B_{max} of [^{3}H]8-OH DPAT receptor binding to the pancreatic islet membrane preparation of 72 hrs and 7 days pancreatectomised rats compared with control (Figure - 24 & Table - 27). The K_d of the receptor binding was increased significantly in 7 days pancreatectomised rats ($p<0.05$) compared with control.

Displacement Analysis of [^{3}H] 8-OH DPAT by 5-HT

The competition curve for 5-HT against [^{3}H]8-OH DPAT fitted for one-sited model in all the groups with Unity as the Hill slope value. The Ki and log (EC_{50}) value showed no change in 72 hrs pancreatectomised rats compared with control indicating no shift in affinity. While the Ki and log (EC_{50}) value of 7 days pancreatectomised rats increased significanly (Figure - 25 & Table - 28).

RT-PCR analysis of 5-HT_{1A} receptor: 5-HT_{1A} receptor mRNA expression increased in 72 hrs and 7 days pancreatectomised rats (Figure - 26 & Table -29).

5-HT_{2C} receptor analysis in the pancreatic islets

[^{3}H]Mesulergine Binding Parameters

There was a significant increase ($p<0.01$) in the B_{max} of the [^{3}H]mesulergine binding to the membrane preparation of pancreatic islets in 72 hrs and 7 days pancreatectomised rats. The K_d of the receptor binding showed no significant change in 72 hrs and 7 days pancreatectomised rats compared with control (Figure - 27 & Table - 30).

Displacement Analysis of [^{3}H]Mesulergine by 5-HT

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. The Ki and log (EC_{50}) values were unchanged in all the experimental groups (Figure - 28 & Table - 31).

INSULIN SECRETION STUDIES IN PANCREATIC ISLETS

One hour *in vitro* culture

Effect of 5-HT on Glucose Induced Insulin Secretion in vitro

The isolated islets incubated for 24 hrs with 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4}M concentrations of 5-HT and with two different concentrations of glucose, 4mM and 20mM. 5-HT at lower concentrations (10^{-8}, 10^{-7} and 10^{-6}M) significantly increased ($p<0.01$, $p<0.001$, $p<0.01$ respectively) insulin secretion in the presence of 4mM glucose. But the insulin secretion significantly decreased at higher concentration (10^{-4}M) (Figure - 29). 5-HT dose dependently inhibited ($p<0.01$) insulin secretion from 10^{-7} to 10^{-4}M concentration in the presence of 20mM glucose (Figure - 30).

Effect of 8-OH DPAT on Glucose induced Insulin Secretion in vitro

The $5\text{-}HT_{1A}$ receptor agonist, 8-OH DPAT at lower concentrations, 10^{-7} & 10^{-6}M, significantly increased ($p<0.01$) glucose (4mM) induced insulin secretion. But at higher concentration (10^{-4}M) insulin secretion was significantly ($p<0.05$) inhibited (Figure - 31). 8-OH DPAT dose dependently inhibited ($p<0.01$, $p<0.05$) insulin secretion in the presence of 20mM glucose (Figure - 32).

Effect of Mesulergine on Glucose Induced Insulin Secretion in vitro

Mesulergine (10^{-4}M) decreased insulin secretion mediated by 5-HT. A significant decrease in insulin secretion was observed at 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4}M ($p<0.05$) concentrations of 5-HT in the presence of 4mM glucose (Figure - 33). There was also a significant decrease in insulin secretion at 10^{-6} ($P<0.01$), 10^{-5}

($p<0.05$) and 10^{-4}M ($p<0.01$) concentrations of 5-HT when incubated with 10^{-4} mesulergine in the presence of 20mM glucose (Figure - 34).

24 hrs *in vitro* culture

Effect of 5-HT on Glucose induced Insulin Secretion in 24 hrs Islet Cultures

Islets were incubated with 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M concentrations 5-HT and two different concentrations of glucose, 4mM and 20mM in 24 hrs *in vit* culture. 5-HT increased insulin secretion significantly at 10^{-8}M ($p<0.05$), 10^{-7} ($p<0.01$) and 10^{-6}M ($p<0.05$) concentration in the presence of 4mM glucose. But 10^{-4}M concentration 5-HT significantly inhibited ($p<0.01$) insulin secreti stimulated by 4mM glucose. There was no significant effect at 10^{-5}M concentrati (Figure - 35). 5-HT significantly inhibited glucose induced (20mM) insulin secreti at 10^{-7} ($p<0.01$), 10^{-6} ($p<0.01$), 10^{-5} ($p<0.05$) and 10^{-4}M ($p<0.01$) concentratio (Figure - 36).

Effect of 8-OH DPAT on Glucose induced Insulin Secretion in vitro

8-OH DPAT at 10^{-7} & 10^{-6}M concentrations significantly ($p<0.05$ & $p<0.0$ increased glucose (4mM) induced insulin secretion in the long term incubation stud Insulin secretion was slightly inhibited at 10^{-4}M concentration (Figure - 37). significant decrease in insulin secretion was observed at 10^{-6}, 10^{-5} and 10^{-4}M ($p<0.0$ concentrations in the presence of 20mM glucose. The inhibitory effect was n significant at 10^{-7} and 10^{-8}M concentrations (Figure - 38).

Effect of Mesulergine on Glucose Induced Insulin Secretion in vitro

Mesulergine (10^{-4}M) significantly decreased 5-HT induced insulin secreti at 10^{-7} ($p<0.05$), 10^{-6} ($p<0.05$), 10^{-5} ($p<0.05$) and 10^{-4}M ($p<0.01$) concentrations the presence of 4mM glucose (Figure - 39). A significant decrease in insul secretion was also observed at 10^{-7} ($p<0.01$), 10^{-6} ($P<0.01$), 10^{-5} ($p<0.01$) and 10^{-4}

($p<0.01$) concentrations of 5-HT with mesulergine (10^{-4}M) in the presence of 20mM glucose (Figure - 40).

IN VITRO DNA SYNTHESIS STUDIES IN PANCREATIC ISLETS

Effect of 5-HT on Islet DNA Synthesis

Isolated islets in culture medium exhibited very low levels of [^{3}H]thymidine incorporation into DNA. Addition of EGF (10ng) caused a significant increase ($p<0.01$) in the islet DNA synthesis. 5-HT at 10^{-4}M concentration caused no significant change in the DNA synthesis from basal level. But at lower concentration, 10^{-8}M, 5-HT significantly ($p<0.01$) increased DNA synthesis. Addition of 10^{-4}M and 10^{-8}M 5-HT along with EGF caused a significant increase ($p<0.01$) in DNA synthesis when compared with EGF alone group. Addition of TGFβ1 (1ng/ml) caused no significant change in the basal level of DNA synthesis, while addition of 10^{-4}M and 10^{-8}M 5-HT along with TGFβ1 caused a significant increase ($p<0.05$) in DNA synthesis when compared with TGFβ1 alone group. Addition of TGFβ1 along with EGF caused no significant change in DNA synthesis (Figure - 41).

Effect of 8-OH DPAT on Islet DNA Synthesis

Addition of 8-OH DPAT (10^{-4}M) caused a significant decrease ($p<0.01$) in the DNA synthesis when compared with control. Addition of 10^{-4}M 8-OH DPAT along with EGF caused a significant increase ($p<0.01$) in DNA synthesis when compared with EGF alone group. TGFβ1 mediated islet DNA synthesis was increased significantly ($p<0.05$) by the addition of 10^{-4}M 8-OH DPAT to the primary islet culture (Figure - 42).

Dose-dependent Response of Islet DNA Synthesis to 8-OH DPAT

8-OH DPAT at lower concentrations, 10^{-8} and 10^{-6}M, significantly increased ($p<0.01$ & $p<0.001$) the DNA synthesis of primary islet in culture. There was a significant decrease ($p<0.01$) in DNA synthesis at higher concentration (10^{-4}M) of 8-OH DPAT (Figure - 43).

Dose-dependent Response of EGF Induced islet DNA synthesis to 8-OH DPAT

Addition of 8-OH DPAT at a concentration from 10^{-8}M to 10^{-4}M significantly increased ($p<0.01$ & $p<0.001$) the EGF mediated DNA synthesis of cultured islets. Maximum DNA synthesis was observed at 10^{-6} M 8-OH DPAT (Figure - 44).

Dose-dependent Response of TGFβ1 Induced islet DNA Synthesis to 8-OH DPAT

TGFβ1 mediated DNA synthesis was increased significantly at 10^{-8} ($p<0.001$), 10^{-6} ($p<0.001$), 10^{-5} ($p<0.01$) and 10^{-4}M ($p<0.01$) 8-OH DPAT. Maximum DNA synthesis was found at 10^{-8}M 8-OH DPAT (Figure - 45).

Effect of pertussis toxin on 8-OH DPAT mediated DNA synthesis: Pertussis toxin significantly inhibited potentiation of EGF effect induced by 8-OH DPAT at 10^{-8}M ($p<0.001$) and 10^{-4}M ($p<0.01$) (Figure - 46).

Effect of Mesulergine on islet DNA Synthesis

Addition of 5-HT (10^{-4}M) with mesulergine (10^{-4}M) and EGF 1ng/ml with mesulergine (10^{-4}M) caused a significant decrease ($p<0.001$) in the basal and EGF mediated DNA synthesis. TGFβ1 mediated islet DNA synthesis decreased significantly ($p<0.01$) by the addition of mesulergine to the primary islet culture (Figure - 47).

Dose-dependent Response of islet DNA Synthesis to Mesulergine

Mesulergine inhibited significantly the DNA synthesis of primary islets in culture induced by 5-HT from 10^{-8}M to 10^{-4}M ($p<0.01$ & $p<0.05$) (Figure - 48).

Dose-dependent Response of EGF Induced Islet DNA Synthesis to Mesulergine

Addition of mesulergine at a concentration from 10^{-8}M to 10^{-4}M dose dependently suppressed ($p<0.01$ & $p<0.001$) the EGF mediated DNA synthesis of cultured islets (Figure - 49).

Dose-dependent Response of TGFβ1 Induced islet DNA Synthesis to Mesulergine

Mesulergine at a concentration of 10^{-6}, 10^{-5} and 10^{-4}M significantly ($p<0.05$, $p<0.01$) decreased TGFβ1 mediated DNA synthesis in primary islet culture (Figure - 50).