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 \(^3\)Hthymidine 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 content during active cell proliferation when compared with control. The decreased content 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<0.001) in 72 hrs pancreatectomised rats when compared with control. The increased 5-HIAA content and the turnover of 5-HIAA/5-HT were reversed by 7 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 NE (p<0.05) levels in 72 hrs pancreatectomised rats compared with control. The plasma 5-HT level increased significantly (p<0.01). The decreased NE and EPI levels and the increased 5-HT level reversed to normal levels by 7 days after partial 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 during pancreatic regeneration. The decreased NE and EPI reversed to control levels at 7 days after partial pancreatectomy (Table - 5).
Receptor Alterations in the Brain Regions of Experimental Rats

5-HT₁A Receptor Analysis

Cerebral Cortex

[^3H]8-OH DPAT Binding Parameters

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

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

The competition curve for 5-HT against[^3H]8-OH DPAT fitted for two-sited model in all the groups with Hill slope value away from Unity. The Kᵢₒ(H) increased in 72 hrs pancreatectomised rats along with an increase in the log (EC₅₀)-1 indicating a shift in high affinity towards low affinity. Kᵢₒ(L) also showed an increase in 72 hrs pancreatectomised rats with an increase in log (EC₅₀)-2 denoting a shift in the low affinity site towards much lower affinity (Figure -5 & Table -8).
Brain Stem

**lHJ8-OH DPAT Binding Parameters**

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

**Displacement Analysis of lHJ8-OH DPAT by 5-HT**

The competition curve for 5-HT against $[^3H]J8-OH$ DPAT fitted for two-site model in all the groups with Hill slope value away from Unity. The $K_i(1)$ increased in 72 hrs with an increased log ($EC_{50}$)-1. This indicates a shift of high affinity towards low affinity. $K_i(2)$ and the log ($EC_{50}$)-2 value showed no change (Figure - 7 & Table - 12).

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

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

**[^H]8-OH DPAT binding parameters**

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

**Displacement Analysis of $[^3H]8$-OH DPAT by 5-HT**

The competition curve for 5-HT against $[^3H]8$-OH DPAT fitted for two-sited model in all the groups with Hill slope value away from unity. The $K_i(H)$ 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. $K_i(L)$ 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\textsubscript{2C} Receptor Analysis

Cerebral cortex

\textit{\textsuperscript{3}H}\textsuperscript{}Mesulergine Binding Parameters

There was a significant decrease (p<0.05) in the B\textsubscript{max} of \textsuperscript{3}H\textsuperscript{}Mesulergine binding without any change in K\textsubscript{d} in 72 hrs pancreatectomised rats compared with control. The decreased B\textsubscript{max} reversed to control level by 7 days after partial pancreatectomy (Figure - 15 & Table - 18).

\textit{Displacement Analysis of \textsuperscript{3}H}\textsuperscript{}Mesulergine by 5-HT}

The competition curve for 5-HT against \textsuperscript{3}H\textsuperscript{}Mesulergine fitted for one-site model in all the groups with Unity as Hill slope value. The K\textsubscript{i} and log (EC\textsubscript{50}) values showed no change in 72 hrs pancreatectomised rats compared with control indicating no shift in affinity (Figure - 16 & Table - 19).

\textit{RT-PCR Analysis of 5-HT\textsubscript{2C} Receptor}

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

Brain stem

\textit{\textsuperscript{3}H}\textsuperscript{}Mesulergine Binding Parameters

The B\textsubscript{max} of \textsuperscript{3}H\textsuperscript{}Mesulergine binding decreased significantly (p<0.01). K\textsubscript{d} of the receptor binding showed a significant increase (p<0.01) in 72 hrs pancreatectomised rats compared with control. The altered parameters reversed near normal in 7 days pancreatectomised rats (Figure - 18 & Table - 21).
**Displacement Analysis of [³H]Mesulergine by 5-HT**

The competition curve for 5-HT against [³H]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 (EC₅₀) in 72 hrs pancreatectomised rats (Figure - 19 & Table - 22).

**RT-PCR analysis of 5-HT₂C receptor:** RT-PCR analysis revealed a decreased mRNA in 72 hrs pancreatectomised rats (Figure - 20 & Table 23).

**Hypothalamus**

[³H]Mesulergine Binding Parameters

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

**Displacement Analysis of [³H]Mesulergine by 5-HT**

The competition curve for 5-HT against [³H]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 (EC₅₀) 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-HT₂C Receptor:** 5-HT₂C 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\textsubscript{1A} Receptor Analysis

\textit{\textsuperscript{3}H/8-OH DPAT Binding Parameters}

There was a significant increase (p<0.01) in the $B_{\text{max}}$ of \textsuperscript{3}H8-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.

\textit{Displacement Analysis of \textsuperscript{3}H 8-OH DPAT by 5-HT}

The competition curve for 5-HT against \textsuperscript{3}H8-OH DPAT fitted for one-sited model in all the groups with Unity as the Hill slope value. The $K_i$ and log ($EC_{50}$) value showed no change in 72 hrs pancreatectomised rats compared with control indicating no shift in affinity. While the $K_i$ and log ($EC_{50}$) value of 7 days pancreatectomised rats increased significantly (Figure - 25 & Table - 28).

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

5-HT\textsubscript{2C} receptor analysis in the pancreatic islets

\textit{\textsuperscript{3}H/Mesulergine Binding Parameters}

There was a significant increase (p<0.01) in the $B_{\text{max}}$ of the \textsuperscript{3}Hmesulergine 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 $[^3H]$Mesulergine by 5-HT

The competition curve for 5-HT against $[^3H]$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-HT$_{1A}$ receptor agonist, 8-OH DPAT at lower concentrations, $10^{-7}$ & $10^{-4}$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 $10^{-6}$ $(P<0.01)$, $10^{-4}$
(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 vitro culture. 5-HT increased insulin secretion significantly at 10^{-4}M (p<0.05), 10^{-3} (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 secretion stimulated by 4mM glucose. There was no significant effect at 10^{-5}M concentration (Figure - 35). 5-HT significantly inhibited glucose induced (20mM) insulin secretion at 10^{-7} (p<0.01), 10^{-6} (p<0.01), 10^{-5} (p<0.05) and 10^{-4}M (p<0.01) concentrations (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 study. 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 significant at 10^{-7} and 10^{-6}M concentrations (Figure - 38).

Effect of Mesulergine on Glucose Induced Insulin Secretion in vitro

Mesulergine (10^{-4}M) significantly decreased 5-HT induced insulin secretion at 10^{-7} (p<0.05), 10^{-6} (p<0.05), 10^{-5} (p<0.05) and 10^{-4}M (p<0.01) concentrations in the presence of 4mM glucose (Figure - 39). A significant decrease in insulin 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^{-5} M, 5-HT significantly (p<0.01) increased DNA synthesis. Addition of 10^{-4} M and 10^{-3} M 5-HT along with EGF caused a significant increase (p<0.01) in DNA synthesis when compared with EGF alone group. Addition of TGF\(\beta\)I (1ng/ml) caused no significant change in the basal level of DNA synthesis, while addition of 10^{-4} M and 10^{-3} M 5-HT along with TGF\(\beta\)I caused a significant increase (p<0.05) in DNA synthesis when compared with TGF\(\beta\)I alone group. Addition of TGF\(\beta\)I 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. TGF\(\beta\)I 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).