Neurochem Res
DOI 10.1007/s11064-007-9283-3

ORIGINAL PAPER

AUTHOR PROOF

Decreased $GABA_A$ Receptor Function in the Brain Stem during Pancreatic Regeneration in Rats

S. Balarama Kaimal · G. Gireesh · C. S. Paulose

Accepted: 4 January 2007

Abstract Gamma amino butyric acid is a major inhibitory neurotransmitter in the central nervous system. In the present study we have investigated the alteration of GABA receptors in the brain stem of rats during pancreatic regeneration. Three groups of rats were used for the study: sham operated, 72 h and 7 days partially pancreatectomised. GABA was quantified by [^{3}H]GABA receptor displacement method. GABA receptor kinetic parameters were studied by using the binding of [^{3}H]GABA as ligand to the Triton X-100 treated membranes and displacement with unlabelled GABA. $GABA_A$ receptor activity was studied by using the [^{3}H]bicuculline and displacement with unlabelled bicuculline. GABA content significantly decreased ($P < 0.001$) in the brain stem during the regeneration of pancreas. The high affinity GABA receptor binding showed a significant decrease in B_{max} ($P < 0.01$) and K_d ($P < 0.05$) in 72 h and 7 days after partial pancreatectomy. [^{3}H]bicuculline binding showed a significant decrease in B_{max} and K_d ($P < 0.001$) in 72 h pancreatectomised rats when compared with sham where as B_{max} and K_d reversed to near sham after 7 days of pancreatectomy. The results suggests that GABA through GABA receptors in brain stem has a regulatory role during active regeneration of pancreas which will have immense clinical significance in the treatment of diabetes.

Keywords GABA · GABA receptors · Brain stem · Bicuculline · Pancreatectomy

S. Balarama Kaimal · G. Gireesh · C. S. Paulose (✉)
Molecular Neurobiology and Cell Biology Unit, Centre for Neuroscience, Department of Biotechnology, Cochin University of Science and Technology, Cochin 682022, India
e-mail: cspaulose@cusat.ac.in

Introduction

The brain neurotransmitters' receptor activity and hormonal pathways control many physiological functions in the body. γ- aminobutyric acid, also known as GABA was discovered over 40 years ago as a key inhibitory neurotransmitter in the brain [1, 2]. GABA has been implicated in cell growth during differentiation in the cultures in at least certain neuron types [3]. GABA was reported to be present in the pancreas in comparable concentrations with those in the central nervous system during the early seventies [4, 5]. Prolonged binding to peripheral benzodiazepine receptors is hypothesized to cause human β-cells functional damage and apoptosis [6]. Cytokines produced by immune system cells infiltrating pancreatic islets are candidate mediators of islet β-cells destruction in autoimmune insulin-dependent diabetes mellitus. Peripheral benzodiazepine receptors constitute the aspecific mitochondrial permeability transition pore, and that it has been suggested to be involved in cytokine-induced cell death [7]. In the CNS, GABA affects neuronal activity through both the ligand-gated $GABA_A$ receptor channel and the G protein-coupled $GABA_B$ receptor. In the mature nervous system, both receptor subtypes decrease neural excitability, whereas in most neurons during development, the $GABA_A$ receptor increases neural excitability and raises cytosolic Ca^{2+} levels. Changes in cytosolic Ca^{2+} during early neural development would, in turn, profoundly affect a wide array of physiological processes, such as gene expression, neurite outgrowth, transmitter release and synaptogenesis [8].

The endocrine part of the pancreas plays a central role in blood-glucose regulation. GABA released from

Journal : 11064 | Dispatch : 18-1-2007 | Pages : 10
Article No : 9283 | LE | TYPESET
MS Code : 9283 | CP | DISK

β-cells is considered as an inhibitor of insulin secretion in pancreatic islets and that the effect is principally due to direct suppression of exocytosis [9]. GABA has been proposed to function as a paracrine signaling molecule in islets of Langerhans and the Glucose inhibition of glucagon secretion from rat alpha-cells is mediated by GABA released from neighboring β-cells [10].

The natural source for new pancreatic β-cells is an important issue both for understanding the pathogenesis of diabetes, and for possibly curing diabetes by increasing the number of β-cells. Transplantation of pancreatic islets can now be applied successfully to treat diabetes, but its widespread use is hampered by a shortage of donor organs. Since insulin-producing β-cells cannot be expanded significantly in vitro, efforts are under way to identify stem or progenitor cells that potentially could be grown and differentiated into β-cells in vitro. Such cells could provide an ample supply of transplantable tissue. Current research in this field focuses mainly on pluripotential embryonic stem cells and on pancreas-specific adult progenitor cells. β-cell replication is the only source for new β-cells without contributions from stem cells or other non- β-cells. The pancreatic gland has an enormous potential for growth and regeneration, mainly in rodents. Animal models of pancreatic regeneration can be easily established in weanling rats. Partial pancreatectomy is an established model to study the pancreatic regeneration.

In addition to its presence in the central nervous system, GABA has been demonstrated in the pancreatic β-cells of normal rat [11]. GABA is present in large number in the islet cells in the pancreas. The concentration of GABA in the endocrine pancreas is comparable to that measured in the in the central nervous system [12]. It is known that the β-cells can produce and release GABA in response to glucose [5, 13, 14]. It is possible that GABA and Glutamate mediate a paracrine signaling pathway whereby α and β-cells communicate within the islets [12, 14–16].

In the present study, we have investigated the changes in the GABA content and GABA receptor activity in brain stem during active regeneration following partial pancreatectomy.

Experimental procedure

Chemicals

All biochemicals used were of analytical grade. GABA and bicuculline were purchased from Sigma Chemical Co. USA. [^{3}H]GABA was purchased from Amersham Biosciences, USA and [^{3}H]bicuculline from NEN, USA. Tris HCL and other chemicals for buffer solutions were obtained from SRL and MERCK.

Animals

Weanling rats of Wistar strain weighing 80–100 g purchased from Amrita Institute of Medical Sciences and Research Centre, Cochin were used in all experiments. They were housed in separate cages in 12 h light and 12 h dark periods and maintained on food and water ad libitum. All animal care and procedures were in accordance with the CPCSEA 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 [17]. The removal of most of the pancreas was done by gentle abrasion with cotton applications, leaving the major blood vessels supplying the other organs intact [18]. 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 7.00am and 9.00am to avoid diurnal variation 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 Glowinski and Iversen, 1966 [19]. The tissues were stored at −70°C for various experiments.

GABA receptor binding assays

[^{3}H]GABA binding to the GABA receptor was assayed in Triton X-100 treated synaptic membranes [20]. Crude synaptic membranes were prepared using sodium-free 10 mM tris buffer (pH 7.4). Each assay tube contained a protein concentration of 0.3-0.4 mg. In saturation binding experiments, 1-10 nM of [^{3}H]GABA incubated with and without excess of unlabelled GABA (100 μM) and in competition binding experiments the incubation mixture contained 2 nM of [^{3}H]GABA with and without muscimol at a concentration range of 10^{-9} M to 10^{-4} M. The incubation was continued for 20 min at 0–4°C and terminated by centrifugation at 35,000g for 20 min. [^{3}H]GABA in

Journal : 11064 | Dispatch : 18-1-2007 | Pages : 10
Article No : 9283 | LE | TYPESET
MS Code : 9283 | CP | DISK

pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting non-specific binding from the total binding.

$GABA_A$ receptor binding assays

[^{3}H]bicuculline binding to the GABA receptor was assayed in Triton X-100 treated synaptic membranes [20]. Crude synaptic membranes were prepared using sodium-free 10 mM tris buffer (pH 7.4). Each assay tube contained a protein concentration of 0.3-0.4 mg. In saturation binding experiments, 5–75 nM concentrations of [^{3}H]bicuculline incubated with and without excess of unlabelled bicuculline (100 µM) and in competition binding experiments the incubation mixture contained 2 nM of [^{3}H]bicuculline with and without bicuculline at a concentration range of 10^{-9} – 10^{-4} M. The incubation was continued for 20 min at 0–4°C and terminated by centrifugation at 35,000g for 20 min. [^{3}H]bicuculline in the pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting non-specific binding from the total binding.

Quantification of GABA using [^{3}H]radioligand

GABA content in the brain stem of the sham and experimental rat groups was quantified by displacement method [20] where the incubation mixture contained 1 nM [^{3}H]GABA with and without GABA at a concentration range of 10^{-9}–10^{-4} M. The unknown concentrations were determined from the standard displacement curve using appropriate dilutions and calculated for µmoles/g wt. of the tissue.

Protein determination

Protein was measured by the method of Lowry et al. 1951 [21] using bovine serum albumin as standard.

Reverse transcription polymerase chain reaction (RT-PCR)

Isolation of mRNA

About 25–50 mg tissue was homogenized in 0.5 ml Tri Reagent. The homogenate was centrifuged at 12,000g for 10 min at 4°C. The clear supernatant was transferred to a fresh tube and it was allowed to stand at room temperature for 5 min. 100 µl of chloroform was added to it, shaken vigorously for 15 s and allowed to stand at room temperature for 15 min. The tube was centrifuged at 12,000g for 15 min at 4°C. Three distinct phases appear after centrifugation. The bottom red organic phase contained protein, interphase contained DNA and a colorless upper aqueous phase contained RNA. The upper aqueous phase was transferred to a fresh tube and 250 µl of isopropanol was added and the tubes allowed to stand at room temperature for 10 min. The tubes were centrifuged at 12,000g for 10 min at 4°C. RNA precipitate forms a pellet on the sides and bottom of the tube. The supernatant was removed and the RNA pellet was washed with 500 µl of 75% ethanol, vortexed and centrifuged at 12,000g for 5 min at 4°C. The pellet was semi dried and dissolved in minimum volume of DEPC-treated water. 2 µl of RNA was made up to 1 ml and absorbance was measured at 260 nm and 280 nm. For pure RNA preparation the ratio of absorbance at 260/280 was ≥1.7. The concentration of RNA was calculated as one absorbance $_{260}$ = 42 µg.

RT PCR Primers	
5′ ACA AGA AGC CAG AGA ACA AGC CAG 3′	α_2 GABA
5′ GAG GTC TAC TGG TAA GCT CTA CCA 3′	
5′TGA GAT GGC CAC ATC AGA AGC AGT 3′	β_2
5′ TCA TGG GAG GCT GGA GTT TAG TTC 3′	GABA
5′ CAG AGA CAG GAA GCT GAA AAG CAA 3′	γ_1 GABA
5′ CGA AGT GAT TAT ATT GGA CTA AGC 3′	
5′-TGT GAG CAA CCG GAA ACC AAG CAA-3′	γ_2 GABA
5′ CGT GTG ATT CAG CGA ATA AGA CCC 3′	

RT-PCR of $GABA_A$ receptor subunits

RT-PCR was carried out in a total reaction volume of 20 µl in 0.2 ml tubes. RT-PCR was performed on an Eppendorf Personal Thermocycler. cDNA synthesis of 2 µg RNA was performed in a reaction mixture containing MuMLV reverse transcriptase (40 units/reaction), 2 mM dithiothreitol, 4 units of human placental RNAse inhibitor, 0.5 µg of random hexamer and 0.25 mM dNTPs (dATP, dCTP, dGTP and dTTP). The tubes were then incubated at 42°C for one hour. After incubation heating at a temperature of 95°C inactivated the reverse transcriptase enzyme, MuMLV.

Receptor data analysis

The receptor binding parameters determined using Scatchard analysis [22]. 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 computer software. This is called a Scatchard plot. The B_{max} is

Journal : 11064 Dispatch : 18-1-2007 Pages : 10
Article No : 9283 LE TYPESET
MS Code : 9283 CP DISK

a measure of the total number of receptors present in the tissue and the K_d represents affinity of the receptors for the radioligand. The K_d is inversely related to receptor affinity or the "strength" of binding. Competitive binding data were analyzed using non-linear 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 [23].

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 steepness of the binding curve can be quantified with a slope factor, often called a Hill slope. A one-site competitive binding curve that follows the law of mass action has a slope of 1.0. If the curve is more shallow, the slope factor will be a negative fraction (i.e., –0.85 or –0.60). The slope factor is negative because curve goes downhill. If slope factor differs significantly from 1.0, then the binding does not follow the law of mass action with a single site, suggesting a two-site model of curve fitting.

Statistics

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

Results

In the brain stem the GABA content was decreased significantly ($P < 0.001$) at 72 h after partial pancrea-

Table 1 GABA content in the brain stem of the sham and experimental rats during partial pancreatectomy (μmole/gm wt of the tissue)

Region	Sham	72 h after pancreatectomy	7 days after pancreatectomy
Brainstem	2.45 ± 0.12	0.84 ± 0.04***	1.69 ± 0.016***

Values are mean ± S.E.M. of 4–6 separate experiments
* $P < 0.05$ when compared with 72 h after pancreatectomy
** $P < 0.01$ when compared with control
*** $P < 0.001$ when compared with control

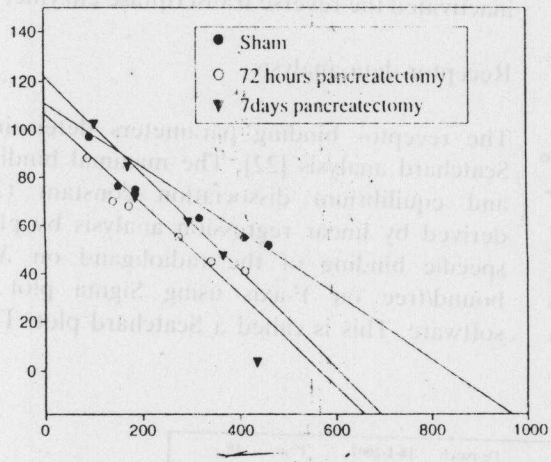

Fig. 1 Scatchard analysis of GABA receptor using [^{3}H]GABA against GABA in the brainstem of rats

Table 2 [^{3}H]GABA binding parameters in the brainstem of rats

Experimental group	B_{max} (fmoles/mg protein)	K_d
Sham	983.33 ± 14.53	8.93 ± 0.72
72 h pancreatectomy	640.26 ± 15.26***	5.13 ± 0.46**
7 days pancreatectomy	717.58 ± 10.14***,*	6.07 ± 0.32*

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
*** $P < 0.001$ when compared with Sham

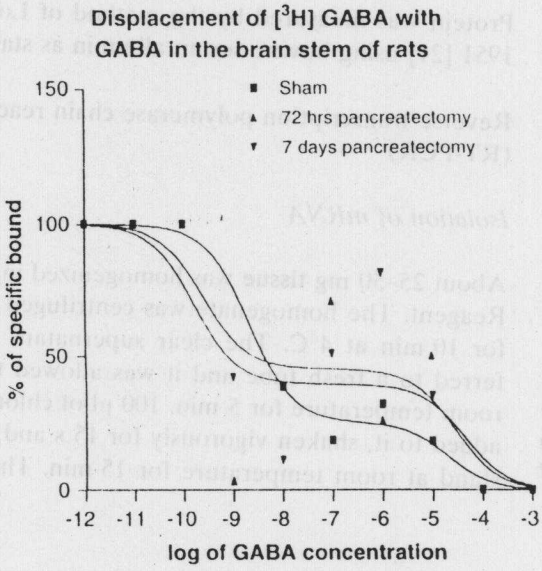

Fig. 2 Displacement of [^{3}H] GABA with GABA in the brain stem of rats

Journal : 11064 Dispatch : 18-1-2007 Pages : 10
Article No : 9283 LE TYPESET
MS Code : 9283 CP DISK

Table 3 Binding parameters of [^{3}H]GABA against GABA in the brain stem of experimental rats

Experimental Group	Best fit model	log (EC$_{50}$) 1	log (EC$_{50}$) 2	$Ki_{(H)}$	$Ki_{(L)}$	Hill slope
Sham	Two-site	−8.66	−9.77	1.4×10^{-10}	3.5×10^{-5}	−0.41
72 h pancreatectomy	Two-site	−4.69	−4.36	2.1×10^{-10}	2.2×10^{-5}	−0.21
7 days pancreatectomy	Two-site	−9.59	−4.56	2.1×10^{-10}	2.3×10^{-5}	−0.20

Values are mean of 4–6 separate 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. The affinity for the first and second site of the competing drug are designated as $Ki_{(H)}$ (for high affinity) and $Ki_{(L)}$ (for low affinity). EC$_{50}$ is the concentration of the competitor that competes for half the specific binding

AUTHOR PROOF

tectomy when compared with sham. The decreased content was reversed to normal near sham value (Table 1).

Scatchard analysis of [^{3}H]GABA to synaptic membrane preparations of brain stem showed a significant decrease ($P < 0.001$) in B_{max} and K_d in 72 h pancreatectomised rats when compared with sham. The decreased B_{max} and K_d showed a tendency to reverse to near normal level by 7 days (Fig. 1, Table 2). The competition curve for GABA against [^{3}H]GABA fitted for two-sited model in all the groups with Hill slope value away from Unity. The $Ki_{(H)}$ increased in 72 h 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 h pancreatectomised rats with an increase in log (EC$_{50}$)-2 denoting a shift in the low affinity site towards much lower affinity (Fig. 2, Table 3).

Scatchard analysis of [^{3}H]bicuculline showed that the B_{max} and K_d decreased significantly ($P < 0.001$) in 72 h pancreatectomised rats when compared with sham. During 7 days the B_{max} and K_d increased significantly ($P < 0.001$ and $P < 0.05$ respectively) when compared with 72 h pancreatectomised rats. This means that the altered parameters tend to reverse to the normal level (Fig. 3 Table 4).

The competition curve for bicuculline against [^{3}H]bicuculline fitted for two-sited model in all the groups with Hill slope value away from Unity. The $Ki_{(H)}$ increased in 72 h pancreatectomised rats along

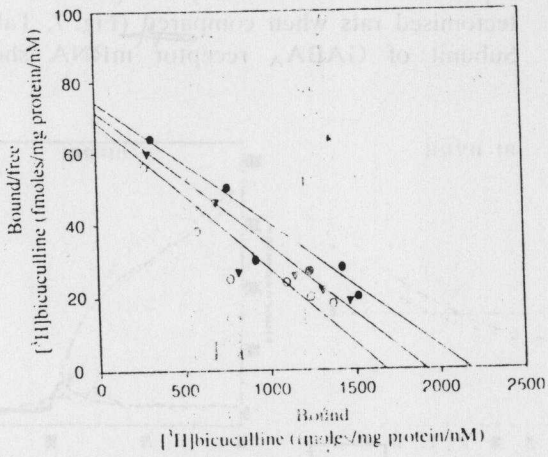

Fig. 3 Scatchard analysis of GABA$_A^1$ receptor using [^{3}H]bicuculline against bicuculline in the brain stem of rats

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

Experimental group	B_{max} (fmoles/mg protein)	K_d
Sham	2.18 + 11.55	29.30 + 0.92
72 h pancreatectomy	1.69 + 20.28**	24.53 + 0.80**
7 days pancreatectomy	1.90 + 14.53***,†††	26.67 + 0.43†

Values are mean + S.E.M. of 4–6 separate experiments

* $P < 0.05$ when compared with Sham, † $P < 0.05$ when compared with Sham

** $P < 0.01$ when compared with Sham, *** $P < 0.001$ when compared with Sham

††† $P < 0.001$ when compared with 72 h after pancreatectomy

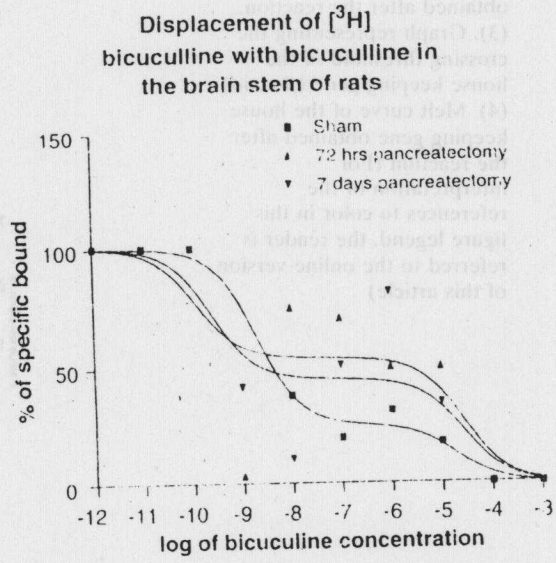

Fig. 4 Displacement of [^{3}H] bicuculline with bicuculline in the brain stem of rats

Journal : 11064 Dispatch : 18-1-2007 Pages : 10
Article No : 9283 LE TYPESET
MS Code : 9283 CP DISK

Table 5 Binding parameters of [^{3}H]bicuculline against bicuculline in the brain stem of experimental rats

Experimental Group	Best-fit model	log (EC_{50})-1	log (EC_{50})-2	$Ki_{(1)}$	$Ki_{(L)}$	Hill slope
Sham	Two-site	−8.68	−4.72	1.54×10^{-9}	1.38×10^{-5}	−0.40
72 h pancreatectomy	Two-site	−9.90	−4.55	9.28×10^{-11}	2.04×10^{-5}	−0.20
7 days pancreatectomy	Two-site	−9.59	−4.56	1.89×10^{-10}	2.02×10^{-5}	−0.20

Values are mean of 4–6 separate 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. The affinity for the first and second site of the competing drug are designated as $Ki_{(1)}$ (for high affinity) and $Ki_{(L)}$ (for low affinity). EC_{50} is the concentration of the competitor that competes for half the specific binding

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 h pancreatectomised rats with an increase in log (EC_{50})-2 denoting a shift in the low affinity site towards much lower affinity (Fig. 4, Table 5).

Real time-PCR analysis of $GABA_A$ receptor

α_2 Subunit of $GABA_A$ receptor mRNA showed an increase in Ct value showing decreased expression in 72 h pancreatectomised rats. The Ct value of the P 7d decreased showing an increased expression in mRNA synthesis (Fig. 5, Table 6). β_2 Subunit of $GABA_A$ receptor mRNA showed an increase in Ct value showing decreased expression in 72 h pancreatectomised rats. The Ct value of the P 7d decreased showing an increased expression in mRNA synthesis (Fig. 6, Table 7). γ_1 Subunit of $GABA_A$ receptor mRNA expression did not show any change in 72 h pancreatectomised rats when compared (Fig. 7, Table 8). γ_2 Subunit of $GABA_A$ receptor mRNA showed an

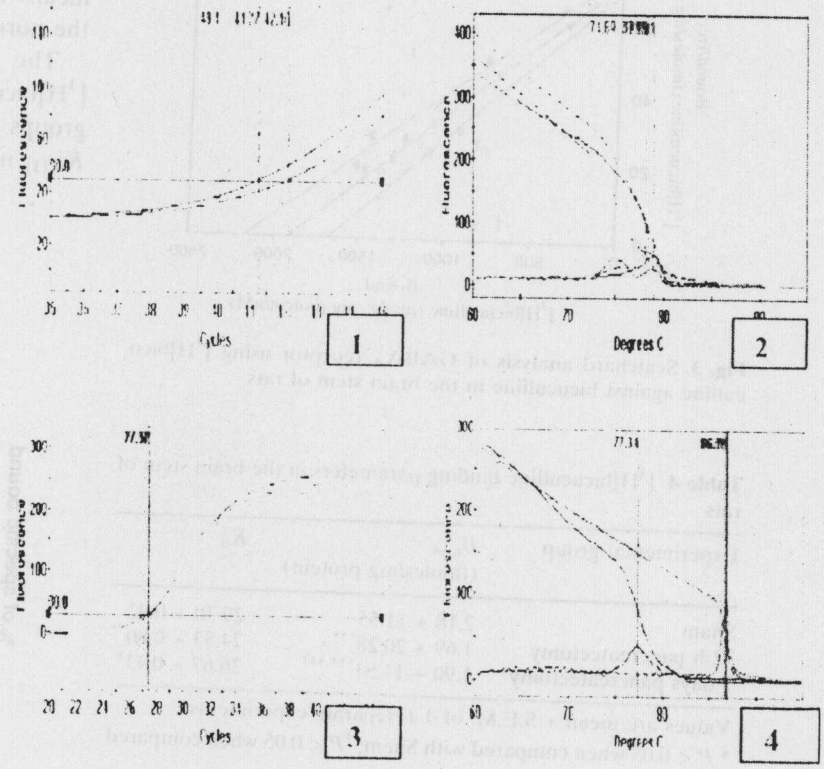

Fig. 5 Real Time PCR amplification of the α_2 sub unit of $GABA_A$ receptor mRNA from the brain stem of experimental rats. (1).Graph representing the crossing threshold (Ct) of sample, (2). Melt curve of the sample of the amplicon obtained after the reaction. (3). Graph representing the crossing threshold of the house keeping gene (β-actin). (4). Melt curve of the house keeping gene obtained after the reaction (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this article)

Table 6

No.	Experimental group	Ct Value
1	Sham	40.10
2	72 hrs pancreatectomy	42.16
3	7 days pancreatectomy	41.27

Journal 11064 Dispatch 18-1-2007 Pages 10
Article No. 9283 LE TYPESET
MS Code 9283 CP DISK

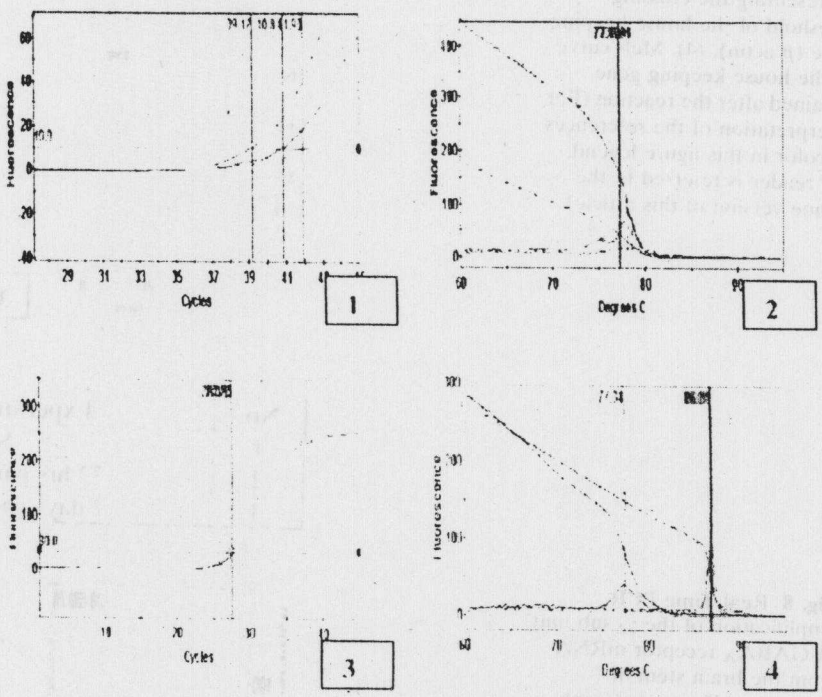

Fig. 6 Real Time PCR amplification of the β_2 sub unit of $GABA_A$ receptor mRNA from the brain stem of experimental rats (1).Graph representing the crossing threshold (Ct) of sample, (2). Melt curve of the sample of the amplicon obtained after the reaction, (3). Graph representing the crossing threshold of the house keeping gene (β-actin), (4). Melt curve of the house keeping gene obtained after the reaction (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this article)

Table 7

No.	Experimental group	Ct Value
1	Sham	39.17
2	72 hrs pancreatectomy	41.93
3	7 days pancreatectomy	40.81

increase in Ct value showing decreased expression in 72 h pancreatectomised rats. The Ct value of the P 7d decreased showing an increased expression in mRNA synthesis (Fig. 8, Table 9).

Discussion

Functional pancreatic β-cell mass is dynamic and although fully differentiated, β-cells are capable of re-entering the cell cycle upon appropriate stimuli. Stimulating regeneration-competent cells in situ is clearly the most desirable way to restore damaged tissue. A large number of growth factors and growth-stimulating peptides are expressed in or have stimulatory effect in the growing islets [24]. The presence of GABA in the cells of the islets of Langerhans is well documented in various species, particularly rats, on the basis of immunohistochemical and biochemical data [5, 11, 13, 25–31].

GABA is one of the most abundant neurotransmitters in the vertebrate central nervous system and is involved in neuroendocrine processes such as development, reproduction, feeding and stress [32]. A decrease in GABA content was observed during active pancreatic proliferation in brain stem. The decreased content in the brain stem was reversed to basal level when pancreatic DNA synthesis declined to control level. The effect of regeneration in the peripheral tissues to the hypothalamic GABA content was already reported during the regeneration of liver [33]. This indicates the decrease in brain GABA content is important in the DNA synthesis in pancreas. It may be a homeostatic feedback adjustment by the hypothalamus to trigger the sympathetic innervation and thereby DNA synthesis. The pancreas enhances the insulin secretion to compensate the insulin demand in the body during the loss of the cells. Brain GABAergic functional alterations are reported to regulate autonomic nerve function in rats [34]. GABA has been known to function as an autocrine/paracrine signal molecule in addition to its well-known inhibitory neurotransmitter function. Studies on the developing brain and on primary brain cell cultures showed that neuron formation was facilitated by GABA through $GABA_A$ ion channels during postmitotic differentiation, but not earlier during the phases of cell fate commitment [35]. These

Journal : 11064 Dispatch : 18-1-2007 Pages : 10
Article No. : 9283
MS Code : 9283
LE
CP
TYPESET
DISK

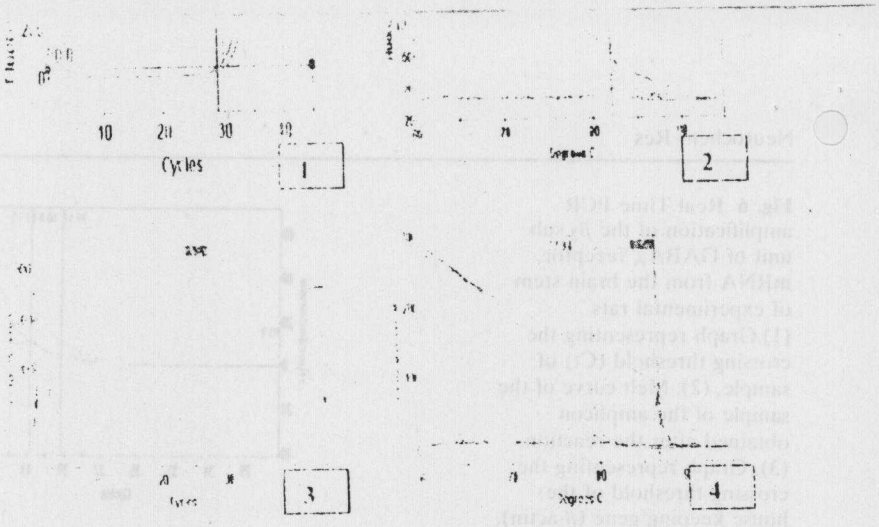

Table 8

No.	Experimental group	CT Value
1	Sham	29.07
2	72 hrs pancreatectomy	29.24
3	7 days pancreatectomy	30.66

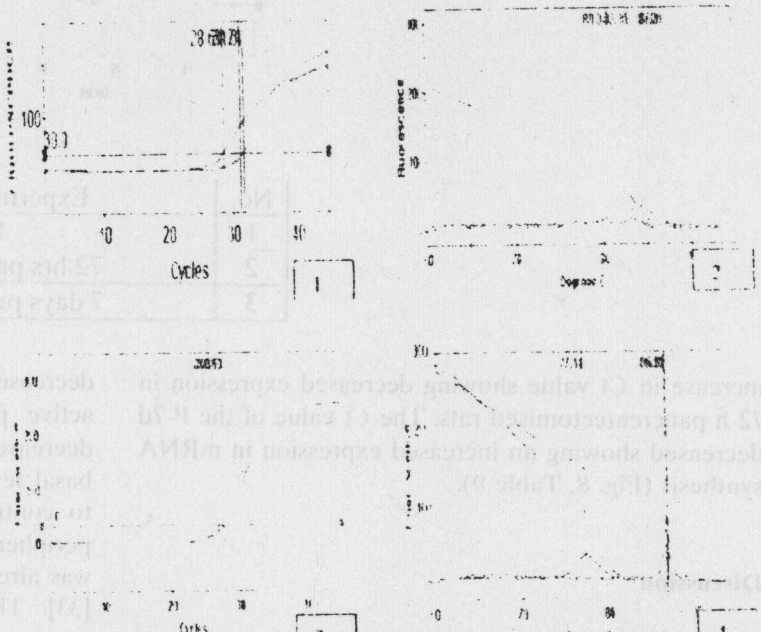

Table 9

No.	Experimental group	CT Value
1	Sham	30.30
2	72 hrs pancreatectomy	31.74
3	7 days pancreatectomy	28.86

Fig. 8 Real Time PCR amplification of the γ_2 sub unit of $GABA_A$ receptor mRNA from the brain stem of experimental rats (1).Graph representing the crossing threshold (CT) of sample, (2). Melt curve of the sample of the amplicon obtained after the reaction, (3). Graph representing the crossing threshold of the house keeping gene (β-actin), (4). Melt curve of the house keeping gene obtained after the reaction (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this article)

Journal 11064 Article No. 9283 MS Code 9283 Dispatch 18-1-2008 Pages 10 ED TYPESET CP DISK

indicate that a decrease in the brain GABA content is important in the DNA synthesis in pancreas. Brain GABAergic changes are reported to regulate autonomic nerve functions in rats [34]. So the results show that a reduction in the GABA content in the brain regions may enhance DNA synthesis in pancreas by facilitating the sympathetic tone.

Previous studies in the regeneration of liver have showed significant alterations in the $GABA_A$ receptor function in brain regions [33, 35]. So we have studied the $GABA_A$ receptor alterations during the regeneration of pancreas of which the endocrine and exocrine secretions have a strong influence from the brain signals. Many gastrointestinal and pancreatic functions are under strong modulatory control by the brain via the vagus nerve [37]. Pancreatic polypeptide when microinjected into the dorsal vagal complex potentiates glucose-stimulated insulin secretion [38]. Some of the neurons of dorsal motor nucleus of the vagus are presumed to play a role in the brain stem neural control of glycemic homeostasis [39]. Targeted pharmacological lesion of the adrenergic innervation of dorsal motor nucleus of the vagus nerve causes hypersecretion by pancreatic β-cells, an effect, which requires an intact vagus nerve [40, 41]. Also, the hypothalamic neurons producing oxytocin that densely project to the dorsal vagal complex are proposed to involve in an inhibitory control of the vagal preganglionic neurons that innervate the pancreas [42]. These all suggest the control of brain from hypothalamus and brain stem over pancreas by the vagal innervation. GABA and the hormonal functional studies will elucidate the functional integrity of their control on peripheral tissues including pancreas. A study in our lab in the regeneration of liver has already explained the importance of the GABAergic receptor function and gene expression [33, 35].

It is well established that the autonomic fibres supplying the pancreas travel via the vagus and splanchnic nerves. These nerves are clearly related to the ventral hypothalamus. The ventro-medial hypothalamic nuclei are considered as the sympathetic centre and the stimulation of this area decreases insulin secretion [43]. Studies of in vivo pancreatic nerve activity after VMH lesions show increased parasympathetic and decreased sympathetic nerve firing rates [44]. Decreased $GABA_A$ receptor binding observed in the hypothalamus reduces the sympathetic nerve stimulation thus reducing the inhibitory effect of EPI on insulin secretion

Pancreatic β-cells express glutamate decarboxylase (GAD), which is responsible for the production and release of GABA. Increased cytoplasmic ATP levels can suppress GAD activity in β-cells, and hence GABA production and release, is compatible with previous findings on ATP suppression of brain GAD activity [45].

Our studies have revealed the significance of GABA and $GABA_A$ receptors functional regulation during pancreatic regeneration and insulin secretion in rats. The decreased binding of $GABA_A$ receptors observed in the brain stem during pancreatic regeneration has a stimulatory role on insulin secretion mediated through sympathetic system.

Acknowledgment This work is supported by the research grant from ICMR, Government of India to Dr. C. S. Paulose. S. Balarama Kaimal thanks ICMR for SRF.

References

1. Bazemore AW, Elliott KAC, Florey E (1957) Isolation of factor I. J Neurochem 1:334–339
2. Krnjevic K, Phillis JW (1963) Iontophoretic studies of neurones in the mammalian cerebral cortex. J Physiol (Lond) 165:274–304
3. Spoerri PE (1982) Neurotrophic effects of GABA in cultures of embryonic chick brain and retina. Synapse 2(1):11–22
4. Briel G, Gylfe E, Hellman B, Newhoff V (1975) Microdetermination of free amino acids in pancreatic islets isolated from obese-hyperglycemic mice. Acta Physiol Scand 84(2):247–253
5. Okada Y, Taniguchi H, Shiamada S (1976) High concentration of GABA and high glutamate decarboxylase activity in the rat pancreatic islets and human insulinoma. Science 194:620–622
6. Marselli L, Trincavelli L, Santangelo C, Lupi R, Del Guerra S, Boggi U, Falleni A, Gremigni V, Mosca F, Martini C, Dotta F, Di Mario U, Del Prato S, Marchetti P (2004) The role of peripheral benzodiazepine receptors on the function and survival of isolated human pancreatic islets. Eur J Endocrinol 151(2):207–214
7. Trincavelli ML, Falleni A, Gremigni V, Ragge E, Dotta F, Santangelo C, Marchetti P, Lucacchini A, Martini C (2000) Upregulation of mitochondrial peripheral benzodiazepine receptor expression by cytokine-induced damage of human pancreatic islets. J Cell Biochem 84(3):636–644
8. Karl Obrietan, Anthony N, van den Pol (1998) GABAB receptor-mediated inhibition of GABAA receptor calcium elevations in developing hypothalamic neurons. J Neurophysiol 79:1360–1370
9. Braun M, Wendt A, Buschard K, Salehi A, Sewing S, Gromada J, Rorsman P (2004) GABAB receptor activation inhibits exocytosis in rat pancreatic beta-cells by G-protein-dependent activation of calcineurin. J Physiol 559:397–409
10. Wendt A, Birnir B, Buschard K, Gromada J, Salehi A, Sewing S, Rorsman P, Braun M (2004) Glucose inhibition of glucagon secretion from rat alpha-cells is mediated by GABA released from neighboring beta-cells. Diabetes 53(4):1038–1045
11. Garry DJ, Sorenson RL, Elde RP, Maley BE, Madsen A 1986 Immunohistochemical co-localization of GABA and insulin in beta cells of rat islet. Diabetes 35:1090–1095

Journal : 11064 Dispatch : 18-1-2007 Pages : 10
Article No : 9283 TYPESET

12. Rorsman P, Berggren PO, Bokvist K, Ericson H, Mohler H, Ostenson CG, Smith PA (1989) Glucose-inhibition of glucagon secretion involves activation of GABAA-receptor chloride channels. Nature 341:233–236
13. Gilon P, Bertrand G, Loubatieres-Marini MM, Remacle C, Henquin JC (1991) The influence of gamma-aminobutyric acid on hormone-release by the mouse and rat endocrine pancreas. Endocrinology 129:2521–2529
14. Satin LS, Kinard TA (1998) Neurotransmitters and their receptors ion the islets of Langerhans of the pancreas. Endocrine 8:213–223
15. Skerry TM, Genever PG (2001) Glutamate signaling in non-neuronal tissues. Trends Pharmacol Sci (22):174–181
16. Gill SS, Pulido OM (2001) Glutamate receptors in peripheral tissues:current knowledge, future research and implications for toxicology. Toxicol Pathol 29:208–223
17. Pearson KW, Scott D, Torrance B (1977) Effects of partial surgical pancreatectomy in rats. Gastroenterology 72:469–473
18. Zangen DH, Bonner-Weir S, Lee CH, Latimer JB, Miller CP, Habener JF, Wen GC (1997) Reduced insulin, GLUT2 and IDX-1 in b-cells after partial pancreatectomy. Diabetes (46):258–264
19. Glowinski J, Iverson LL (1966) Regional studies of catecholamines in the rat brain: the disposition of [3H]Norepinephrine, [3H]DOPA, in various regions of the brain. J Neurochem 13:655–669
20. Kurioka Susumu, Toshiaki Kim L.a, Makoto Matsuda (1981) Effects of sodium and bicarbonate ions on gamma amino butyric acid receptor binding in synaptic membranes of rat brain. J Neurochem 37:418–421
21. Lowry OH, Rosenbrough NJ, Farr AL, Randall J (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265–275
22. Scatchard G (1949) The attractions of proteins for small molecules and ions. Ann N.Y. Acad Sci 51:660–672
23. Chen Y, Prusoff WH (1973) Relationship between the inhibition constant and the concentration of an inhibitor that cause a 50% inhibition of an enzyme reaction. Biochem Pharmacol 22:3099–3108
24. Corbett J, Serup P, Bonner Weir S, Nielsen J H (1997) B-Cell ontogeny: growth and death. Diabetologia 40:B27–B32
25. Haber EP, Ximenes LM, Procopio J, Carvalho CR, Curi R, Carpinelli AR (2003) Pleiotropic effects of fatty acids on pancreatic beta-cells. J Cell Physiol 194(1):1–12
26. Vincent SR, Hokfelt T, Wu JY, Elde RP, Morgan LM, Kimmel JR (1983) Immunohistochemical studies of the GABA system in the pancreas. Neuroendocrinology 36:197–204
27. Sakaue M, Saito N, Tanaka C (1987) Immunohistochemical localization of gamma-aminobutyric acid (GABA) in the rat pancreas. Histochemistry 86(4):365–369
28. Garry DJ, Appel NM, Garry MG, Sorenson RL (1988) Cellular and sub-cellular immunolocalization of L-glutamate decarboxylase in rat pancreatic islets. J Histochem. Cytochem 36:573–580
29. Reetz A, Solimena M, Matteoli M, Folli F, Takei K, De Camilli P (1991) GABA and pancreatic beta-cells: colocalization of glutamic acid decarboxylase (GAD) and GABA with synaptic like microvesicles suggests their role in GABA storage and secretion. Embo J 10:1275–1284
30. Michalik M, Erecinska M (1992) GABA in pancreatic islets: metabolism and function. Biochem Pharmacol 44:1–9
31. Michalik M, Nelson J, Erecinska M (1993) GABA production in rat islets of Langerhans. Diabetes 42(10):1506–1513
32. Martyniuk CJ, Crawford AB, Hogan NS, Trudeau VL (2005) GABAergic modulation of the expression of genes involved in GABA synaptic transmission and stress in the hypothalamus and telencephalon of the female goldfish (Carassius auratus). J Neuroendocrinol 17(5):269–275
33. Biju MP, Pyroja S, Rajeshkumar NV, Paulose CS (2001) Hypothalamic GABA receptor functional regulation and liver cell proliferation. Mol Cell Biochem 216:65–70
34. Martin DS, Haywood JR (1998) Reduced GABA inhibition of sympathetic function in renal wrapped hypertensive rats. Am J Physiol 275
35. Biju MP, Pyroja S, Rajeshkumar NV, Paulose CS (2002) Enhanced GABA(B) receptor in neoplastic rat liver: induction of DNA synthesis by baclofen in hepatocyte cultures. J Biochem Mol Biol Biophys 6(3):209–214
36. Jelitai M, Anderova M, Marko K, Kekesi K, Koncz P, Sykova E, Madarasz E (2004) Role of gamma-aminobutyric acid in early neuronal development: studies with an embryonic neuroectodermal stem cell clone. J Neurosci Res 76(6):801–811
37. Berthoud HR, Patterson LM, Zheng H (2001) Vagal-enteric interface: vagal activation-induced expression of c-Fos and p-CREB in neurons of the upper gastrointestinal tract and pancreas. Anat Rec 262:29–40
38. Krowicki ZK (1996) Role of selected peptides in the vagal regulation of gastric motor and endocrine pancreatic function. J Physiol Pharmacol 47(3):399–409
39. Adachi A, Kobashi M, Funahashi M (1995) Glucose-responsive neurons in the brainstem. Obes Res 3:735S–740S
40. Siaud P, Mekaouche M, Givalois L, Balmefrezol M, Marcilhac A, Ixart G (1995) Effects of pharmacological lesion of adrenergic innervation of the dorsal vagal nucleus on pancreatic insulin secretion in normal and vagotomized rats. Physiol Res 44(4):227–231
41. Siaud P, Puech R, Assenmacher I, Alonso G (1990) Adrenergic innervation of the dorsal vagal motor nucleus: possible involvement in inhibitory control of gastric acid and pancreatic insulin secretion. Cell Tissue Res 259(3):535–542
42. Siaud P, Puech R, Assenmacher I, Alonso G (1991) Microinjection of oxytocin into the dorsal vagal complex decreases pancreatic insulin secretion. Brain Res 546(2):190–194
43. Helman Marre M, Bobbioni E, Poussier P, Reach G, Assan R 1982 The brain-islet axis: the nervous control of the endocrine pancreas. Diabete Metab 8(1):53–64
44. Yoshimatsu H, Niijima A, Oomura Y, Yamabe K, Katafuchi T 1984 Effects of hypothalamic lesion on pancreatic autonomic nerve activity in the rat. Brain Res 1984 303(1):147–52
45. Winnock FLZ, De Proft R, Dejonghe S, Schuit F, Gorus F, Pipeleers D 2002 Correlation between GABA release from rat islet beta-cells and their metabolic state. Am J Physiol Endocrinol Metab 282(4):E937–42

12. Rorsman P, Berggren PO, Bokvist K, Ericson H, Mohler H, Ostenson CG, Smith PA (1989) Glucose-inhibition of glucagon secretion involves activation of GABAA-receptor chloride channels. Nature 341:233–236
13. Gilon P, Bertrand G, Loubatieres-Marini MM, Remacle C, Henquin JC (1991) The influence of gamma-aminobutyric acid on hormone-release by the mouse and rat endocrine pancreas. Endocrinology 129:2521–2529
14. Satin LS, Kinard TA (1998) Neurotransmitters and their receptors ion the islets of Langerhans of the pancreas. Endocrine 8:213–223
15. Skerry TM, Genever PG (2001) Glutamate signaling in non-neuronal tissues. Trends Pharmacol Sci (22):174–181
16. Gill SS, Pulido OM (2001) Glutamate receptors in peripheral tissues:current knowledge, future research and implications for toxicology. Toxicol Pathol 29:208–223
17. Pearson KW, Scott D, Torrance B (1977) Effects of partial surgical pancreatectomy in rats. Gastroenterology 72:469–473
18. Zangen DH, Bonner-Weir S, Lee CH, Latimer JB, Miller CP, Habener JF, Wen GC (1997) Reduced insulin, GLUT2 and IDX-1 in b-cells after partial pancreatectomy. Diabetes (46):258–264
19. Glowinski J, Iverson LL (1966) Regional studies of catecholamines in the rat brain: the disposition of [3H]Norepinephrine, [3H]DOPA, in various regions of the brain. J Neurochem 13:655–669
20. Kurioka Susumu, Toshiaki Kimura, Makoto Matsuda (1981) Effects of sodium and bicarbonate ions on gamma amino butyric acid receptor binding in synaptic membranes of rat brain. J Neurochem 37:418–421
21. Lowry OH, Rosenbrough NJ, Farr AL, Randall J (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265–275
22. Scatchard G (1949) The attractions of proteins for small molecules and ions. Ann N.Y. Acad Sci 51:660–672
23. Chen Y, Prusoff WH (1973) Relationship between the inhibition constant and the concentration of an inhibitor that cause a 50% inhibition of an enzyme reaction. Biochem Pharmacol 22:3099–3108
24. Corbett J, Serup P, Bonner-Weir S, Nielsen J H (1997) B Cell ontogeny: growth and death. Diabetologia 40:B27–B32
25. Haber EP, Ximenes LM, Procopio J, Carvalho CR, Curi R, Carpinelli AR (2003) Pleiotropic effects of fatty acids on pancreatic beta-cells. J Cell Physiol 194(1):1–12
26. Vincent SR, Hokfelt T, Wu JY, Hide RP, Morgan LM, Kimmel JR (1983) Immunohistochemical studies of the GABA system in the pancreas. Neuroendocrinology 36:197–204
27. Sakaue M, Saito N, Tanaka C (1987) Immunohistochemical localization of gamma-aminobutyric acid (GABA) in the rat pancreas. Histochemistry 86(4):365–369
28. Garry DJ, Appel NM, Garry MG, Sorenson RL (1988) Cellular and sub-cellular immunolocalization of L-glutamate decarboxylase in rat pancreatic islets. J Histochem. Cytochem 36:573–580
29. Reetz A, Solimena M, Matteoli M, Folli F, Takei K, De Camilli P (1991) GAD and pancreatic beta-cells: colocalization of glutamic acid decarboxylase (GAD) and GABA with synaptic like microvesicles suggests their role in GABA storage and secretion. Embo J 10:1275–1284
30. Michalik M, Erecinska M (1992) GABA in pancreatic islets: metabolism and function. Biochem Pharmacol 44:1–9
31. Michalik M, Nelson J, Erecinska M (1993) GABA production in rat islets of Langerhans. Diabetes 42(10):1506–1513
32. Martyniuk CJ, Crawford AB, Hogan NS, Trudeau VL (2005) GABAergic modulation of the expression of genes involved in GABA synaptic transmission and stress in the hypothalamus and telencephalon of the female goldfish (Carassius auratus). J Neuroendocrinol 17(5):269–275
33. Biju MP, Pyroja S, Rajeshkumar NV, Paulose CS (2001) Hypothalamic GABA receptor functional regulation and liver cell proliferation. Mol Cell Biochem 216:65–70
34. Martin DS, Haywood JR (1998) Reduced GABA inhibition of sympathetic function in renal wrapped hypertensive rats. Am J Physiol 275
35. Biju MP, Pyroja S, Rajeshkumar NV, Paulose CS (2002) Enhanced GABA(B) receptor in neoplastic rat liver: induction of DNA synthesis by baclofen in hepatocyte cultures. J Biochem Mol Biol Biophys 6(3):209–214
36. Jelitai M, Anderova M, Marko K, Kekesi K, Koncz P, Sykova E, Madarasz E (2004) Role of gamma-aminobutyric acid in early neuronal development: studies with an embryonic neuroectodermal stem cell clone. J Neurosci Res 76(6):801–811
37. Berthoud HR, Patterson LM, Zheng H (2001) Vagal-enteric interface: vagal activation-induced expression of c-Fos and p-CREB in neurons of the upper gastrointestinal tract and pancreas. Anat Rec 262:29–40
38. Krowicki ZK (1996) Role of selected peptides in the vagal regulation of gastric motor and endocrine pancreatic function. J Physiol Pharmacol 47(3):399–409
39. Adachi A, Kobashi M, Funahashi M (1995) Glucose-responsive neurons in the brainstem. Obes Res 3:735S–740S
40. Siaud P, Mekaouche M, Givalois L, Balmefrezol M, Marcilhac A, Ixart G (1995) Effects of pharmacological lesion of adrenergic innervation of the dorsal vagal nucleus on pancreatic insulin secretion in normal and vagotomized rats. Physiol Res 44(4):227–231
41. Siaud P, Puech R, Assenmacher I, Alonso G (1990) Adrenergic innervation of the dorsal vagal motor nucleus: possible involvement in inhibitory control of gastric acid and pancreatic insulin secretion. Cell Tissue Res 259(3):535–542
42. Siaud P, Puech R, Assenmacher I, Alonso G (1991) Microinjection of oxytocin into the dorsal vagal complex decreases pancreatic insulin secretion. Brain Res 546(2):190–194
43. Helman Marre M, Bobbioni E, Poussier P, Reach G, Assan R 1982 The brain-islet axis: the nervous control of the endocrine pancreas. Diabete Metab 8(1):53–64
44. Yoshimatsu H, Niijima A, Oomura Y, Yamabe K, Katafuchi T 1984 Effects of hypothalamic lesion on pancreatic autonomic nerve activity in the rat. Brain Res 1984 303(1):147–52
45. Winnock FLZ, De Proft R, Dejonghe S, Schuit F, Gorus F, Pipeleers D 2002 Correlation between GABA release from rat islet beta-cells and their metabolic state. Am J Physiol Endocrinol Metab 282(4):E937–42

Journal : 11064 Dispatch : 18-1-2007 Pages : 10
Article No : 9283 LE TYPESET