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Hypothalamic GABA receptor functional regulation and liver cell proliferation

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Abstract

GABAergic alterations in hypothalamus during compensatory hyperplasia after partial hepatectomy (PH), lead nitrate (LN) induced direct hyperplasia and *N*-nitrosodiethylamine (NDEA) induced neoplasia in liver were investigated. Serum GABA levels were increased in all 3 experimental groups compared with the control. GABA content decreased in hypothalamus of PH and NDEA treated rats, while it increased in LN treated rats. $GABA_A$ receptor number and affinity in hypothalamic membrane preparations of rats showed a significant decrease in PH and NDEA treated rats, while in LN treated rats the affinity increased without any change in the receptor number. The $GABA_B$ receptor number increased in PH and NDEA treated rats, while it decreased in LN treated rats. The affinity of the receptor also increased in NDEA treated rats. Plasma NE levels showed significant increase in PH and NDEA rats compared with the control while it decreased in LN treated rats. The results of the present study suggests that liver cell proliferation is influencing the hypothalamic GABAergic neurotransmission and these changes regulate the hepatic proliferation through the sympathetic stimulation. (Mol Cell Biochem **216**: 65–70, 2001)

Key words: hypothalamus, GABA receptor, liver regeneration, hyperplasia, liver cancer

Introduction

Brain plays an important regulatory role in hepatic function [1]. The relationship between the functional status of the liver and that of the brain has been known for centuries [2]. In hepatic encephalopathy and other liver diseases neurotransmission in the brain is reported to be altered [3–5]. The liver is richly innervated [6] and autonomic nervous system has an important role in the process of hepatic cell proliferation [7]. Lateral lesions of hypothalamus cause an increase in DNA synthesis during liver regeneration and sympathectomy and vagotomy block this effect [8]. Central thyrotropin releasing hormone has been identified as one of the chemical messengers involved in brain regulation of hepatic proliferation [9]. The autonomic nervous system links the hepatic parenchyma to the autonomic centers in the hypothalamus [10]. Hence, the hypothalamus plays a crucial role in hepatic cell proliferation by direct innervation [8].

GABA, a non-protein associated neutral amino acid, is the principal inhibitory neurotransmitter of the mammalian brain. Advanced liver disease of either acute or chronic nature can be associated with a significant impairment in hepatic regenerative activity and GABA neurotransmission in brain [11]. Elevated intra-cerebral concentrations of GABA significantly decreased ornithine decarboxylase activity in the liver [12], which is an index for decreased hepatic proliferation. Although there are several studies regarding the brain control on hepatic proliferation how the hypothalamus responds to the hepatic cell proliferation is not well studied. In the present study GABAergic alterations in hypothalamus during com-

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pensatory hyperplasia after partial hepatectomy (PH), lead nitrate (LN) induced direct hyperplasia and *N*-nirosodiethylamine (NDEA) induced neoplasia in liver were analyzed.

Materials and methods

Animals

Adult male Wistar rats weighing 200–300 g were obtained from Central Institute of Fisheries Technology, Cochin, India and used for all experiments. They were fed lab chow and water *ad libitum* and were maintained under a 12 h light/12 h dark cycle and controlled temperature. All animal care and procedures were in accordance with institutional and National Institute of Health guidelines.

Materials

γ-Aminobutyric acid (GABA), (±)Norepinephrine, Sodium octyl sulfonate, Ethylenediamine tetra acetic acid (EDTA), baclofen and bicuculline methochloride were purchased from Sigma Chemical Co., St. Louis, MO, USA. Baclofen, (–)-[butyl-4-^{3}H(N)] (specific activity – 42.9 Ci/mmol), bicuculline methyl chloride, (–)-[methyl-^{3}H] (specific activity – 82.9 Ci/mmol) were purchased from NEN Life Sciences products Inc., Boston, MA, USA. All the other biochemicals used were of analytical grade.

Partial hepatectomy

Two-thirds of the liver constituting the median and left lateral lobes were surgically excised under light ether anesthesia, following a 16 h fast [13]. Sham operations involved median excision of the body wall followed by all manipulations except removal of the lobes. All the surgeries were done between 7 a.m. and 9 a.m. to avoid diurnal variations in responses.

Lead nitrate administration

Rats received a single intravenous injection of lead nitrate (100 μmol/kg of body wt) while the control rats received distilled water only [14].

N-*Nitrosodiethylamine treatment*

Liver cancer was induced using NDEA [15]. Animals received 0.02% NDEA in distilled water (2.5 ml/animal by gavage, 5 days a week for 20 weeks). Rats treated only with distilled water served as control. After 20 weeks all the rats were kept without any treatment for 1 week and sacrificed at 22nd week. Neoplasia was confirmed by histological techniques.

Sacrifice of rats

The rats were sacrificed by decapitation and the hypothalamus was rapidly dissected out [16] and immediately immersed into liquid nitrogen and stored at –70°C for various experiments.

GABA HPLC determinations

GABA was quantified by HPLC using electrochemical detection after derivatization [17]. A 10% homogenate of the tissue/serum was made in 0.15 M sodium acetate buffer. Ten microliters of homogenate was mixed with 4 μl of derivatization reagent, (27 mg of *o*-phthaldialdehyde in 10 ml of 50% 0.1 M carbonate buffer (pH 9.6), 50% methanol, and 45 μl of *t*-butylthiol), exactly 3 min before injection into a Shimadzu HPLC system with electrochemical detector fitted with C18-CLC-ODS reverse phase column. Mobile phase was 0.15 M sodium acetate buffer (pH 5.4) containing 1 mmol/l EDTA and 50% (v/v) acetonitrile delivered at a flow rate of 1.0 ml/min. Quantitation was by electrochemical detection, using a glass carbon electrode set at +0.70 V. The peaks were identified by relative retention time compared with standards and concentrations were determined using a Shimadzu integrator interfaced with the detector.

Analysis of circulating norepinephrine

The blood samples were obtained by decapitation [18]. Plasma norepinephrine was extracted from 1 ml of plasma and diluted twice with distilled water. To it, 50 μl of 5 mM sodium bisulphite was added, followed by 250 μl of 1 M Tris buffer, pH 8.6. Acid alumina (20 mg) was added, shaken in the cold for 20 min and was washed with 5 mM sodium bisulphite. Norepinephrine was extracted from the final pellet of alumina with 0.1 N perchloric acid, mixed well, and 20 μl of filtered sample was analyzed [19].

$GABA_A$ receptor binding studies

[^{3}H]Bicuculline methochloride binding to the $GABA_A$ receptor was assayed in synaptic membranes from hypothalamus [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 of [^{3}H]bicuculline methochloride incubated with and without excess of unlabelled bicuculline methochloride (100 μM) and in competition binding experiments the incubation mixture contained 5 nM of [^{3}H]bicuculline methochloride with and without bicuculline methochloride at a concentration range of 10^{-9}–10^{-4} M. The incubation was continued for 30 min at 0–4°C and terminated by centrifugation at 35,000 g for 20 min. [^{3}H]Bicuculline methochloride in the pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting non-specific binding from the total binding. The non-specific binding determined was 30–40% of the total binding in all our experiments.

$GABA_B$ receptor binding studies

[^{3}H]Baclofen binding to $GABA_B$ receptor in the hypothalamic synaptic membrane preparations were assayed as previously described [21]. Crude synaptic membrane preparation was suspended in 50 mM Tris-HCl buffer (pH 7.4) containing 2 mM $CaCl_2$ and 0.3–0.4 mg protein. In saturation binding experiments, 10–100 nM of [^{3}H]baclofen was incubated with and without excess of unlabelled baclofen (100 μM) and in competition binding experiments the incubation mixture contained 10 nM of [^{3}H]baclofen with and without baclofen at a concentration range of 10^{-9}–10^{-4} M. The incubations were carried out at 20°C for 30 min. The binding reactions were terminated by centrifugation at 7,500 g for 10 min. [^{3}H]Baclofen in the pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting non-specific binding from total binding. The non-specific binding determined was 30–40% of the total binding in all our experiments.

Receptor binding parameters analysis

The receptor binding parameters were 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 software (version 2.0, Jandel GmbH, Erkrath, Germany). Competitive binding data were analyzed using non-linear regression curve-fitting procedure (GraphPad PRISM™, San Diego, CA, 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 Ki 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 were represented graphically with the negative log of concentration of the competing drug on x-axis and percentage of the radioligand bound on the y-axis. The Hill slope was used to indicate a one or two-site model of curve-fitting.

Results

Serum GABA levels showed a significant increase ($p < 0.05$ to $p < 0.001$) in all 3 experimental groups compared with the control. NDEA treated rats showed maximum increase in serum GABA level ($p < 0.001$) compared with control (Table 1). Plasma NE levels showed significant increase in PH and NDEA rats ($p < 0.001$ and $p < 0.01$) compared with the control while it decreased significantly ($p < 0.05$) in LN treated rats (Table 1). GABA content significantly decreased in hypothalamus ($p < 0.01$) of PH and NDEA treated rats while it increased ($p < 0.01$) in LN treated rats. (Table 2).

Scatchard analysis of [^{3}H]bicuculline binding to hypothalamic synaptic membrane preparations of rats showed a significant decrease in B_{max} in PH and NDEA treated rats ($p < 0.01$ and $p < 0.001$) while it remained unaltered in LN treated rats compared with the control. K_d showed a significant increase in NDEA treated ($p < 0.01$) and PH ($p < 0.05$) groups while it decreased in ($p < 0.05$) LN treated rats compared with control (Table 3). The competition curve for bicuculline

Table 1. Circulating GABA and norepinephrine concentration in rats

Experimental groups	Serum GABA (nmoles/ml serum)	Norepinephrine (nmoles/ml plasma)
Control¶	4.25 ± 0.18¶	0.30 ± 0.10¶
Partial hepatectomised	6.59 ± 0.19**	1.86 ± 0.03***
N-Nitrosodiethylamine treated	8.22 ± 0.35***	1.24 ± 0.09**
Lead nitrate treated	6.24 ± 0.31*	0.15 ± 0.08*

Values are mean ± S.E.M. of 4-6 separate experiments. ¶Control value given is a pooled data from different control experiments since there was no significant difference in values among groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ with respect to control.

Table 2. GABA content in the hypothalamus of rats

Experimental groups	GABA (μmoles/g wet wt. of tissue)
Control¶	3.16 ± 0.11¶
Partial hepatectomised	2.34 ± 0.24*
N-Nitrosodiethylamine treated	2.30 ± 0.12*
Lead nitrate treated	4.19 ± 0.08**

Values are mean ± S.E.M. of 4–6 separate experiments. ¶Control value given is a pooled data from different control experiments since there was no significant difference in values among groups. *$p < 0.05$, **$p < 0.01$ with respect to control.

against [^{3}H]bicuculline fitted a two-site model in all the groups with Hill slope value away from unity. Although the log(EC_{50})-1 value did not show any change, the $Ki_{(H)}$ increased significantly in NDEA and PH rats compared with control. This shows a shift from the high-affinity site to low-affinity. In LN rats log(EC_{50})-1 and $Ki_{(H)}$ decreased indicating a shift in affinity to high-affinity (Table 4, Fig. 1).

The B_{max} of [^{3}H]baclofen binding increased significantly in PH and NDEA ($p < 0.01$ and $p < 0.001$) while it remained unaltered in LN treated rats. The K_d value decreased significantly ($p < 0.01$) in NDEA treated rats while in PH and LN treated rats it remained unaffected (Table 3). The competition curve for unlabelled baclofen against [^{3}H]baclofen binding fitted for one site model in control and experimental group with a Hill slope value near unity. The log-(EC_{50}) and Ki of NDEA treated rats decreased compared with control indicating a shift in affinity to high-affinity. The binding parameters remained unaltered in LN treated and PH rats (Table 5, Fig. 2).

Discussion

The animals were sacrificed at the time of peak DNA synthesis in liver based on previous reports [14, 24] and by [^{3}H]thymidine incorporation studies (data not shown). The results clearly show that hypothalamic GABA content is independent of serum GABA levels. The decreased GABA content in hypothalamus of PH rats was observed during active hepatic proliferation. PH induces the remnant liver to re-enter into cell cycle from quiescent state to compensate the lost mass of liver. Sympathetic innervation is important for liver regeneration [25]. The decrease in GABA content may be a homeostatic feed back adjustment by the hypothalamus to trigger the sympathetic innervation and thereby DNA synthesis in the liver. In LN treated rats the increased hepatic proliferation has to be suppressed in order to restore the normal liver mass [26]. The increased hypothalamic GABA content in LN induced hepatic hyperplasia may be a mechanism to bring back the original liver mass by decreasing the liver DNA synthesis through the sympathetic activity regulation. Increased hepatic DNA synthesis was also observed in NDEA treated rats but the changes in hypothalamic GABA content was reciprocal to that of LN treated rats. This suggests that the hypothalamic GABAergic adaptive adjustment that was observed in the LN treated rats to suppress the excess hepatic proliferation was not seen in NDEA treated rats leading to hepatic neoplasia. Hypothalamus is the center of autonomic nervous system reinforcement. Lateral lesions of hypothalamus caused an increase in DNA synthesis during liver regeneration through autonomic nervous system [8, 25]. We have analyzed the receptors of GABA to study whether is there any co-relation between these hypothalamic receptors, hepatic DNA synthesis and sympathetic stimulation.

The $GABA_A$ receptor binding parameters as determined by [^{3}H]bicuculline against bicuculline indicate a decrease in number and affinity of the receptor in PH and NDEA treated rats. The decrease in activity of the receptor was more pro-

Table 3. GABA receptor binding parameters in the hypothalamus of rats

Experimental groups	[^{3}H]Bicuculline binding		[^{3}H]Baclofen binding	
	B_{max} (pmol/mg protein)	K_d (nM)	B_{max} (pmol/mg protein)	K_d (nM)
Control¶	2.84 ± 0.17¶	49.62 ± 2.45	2.46 ± 0.20¶	67.48 ± 3.33
Partial hepatectomised	1.90 ± 0.15**	62.33 ± 4.02*	3.46 ± 0.24**	65.78 ± 4.10
N-Nitrosodiethylamine treated	1.45 ± 0.12***	66.67 ± 2.44**	4.60 ± 0.20***	46.49 ± 4.68**
Lead nitrate treated	2.74 ± 0.13	40.44 ± 2.62*	2.45 ± 0.10	73.27 ± 3.14

Scatchard analysis of [^{3}H]Bicuculline and [^{3}H]baclofen binding against baclofen in hypothalamus of control, partial hepatectomised, lead nitrate treated or *N*-nitrosodiethylamine treated rats. Experimental procedures are given under 'Materials and methods'. Values are mean ± S.E.M. of 4–6 separate experiments. B_{max} – binding maximum; K_d – dissociation constant. ¶Control value given is a pooled data from different control experiments since there was no significant difference in values among groups. **$p < 0.01$, ***$p < 0.001$ with respect to control.

Table 4. Binding parameters of [^{3}H]bicuculline against bicuculline in hypothalamus of rats

Experimental groups	Best-fit model	log(EC_{50})-1	log(EC_{50})-2	$Ki_{(H)}$	$Ki_{(L)}$	Hill slopes
Control¶	Two-site	–7.98	–5.76	9.91×10^{-9}	1.62×10^{-6}	–0.52
Partial hepatectomised	Two-site	–7.47	–5.70	3.17×10^{-8}	1.87×10^{-6}	–0.65
N-Nitrosodiethylamine treated	Two-site	–7.28	–4.79	4.86×10^{-8}	1.51×10^{-5}	–0.58
Lead nitrate treated	Two-site	–8.36	–5.95	4.07×10^{-9}	1.06×10^{-6}	–0.47

Data were fitted with an iterative nonlinear regression software (Prism, GraphPad, San Diego, CA, USA). 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. Values are mean of 4–6 separate experiments. ¶Control value given is a pooled data from different control experiments since there was no significant difference in values among groups.

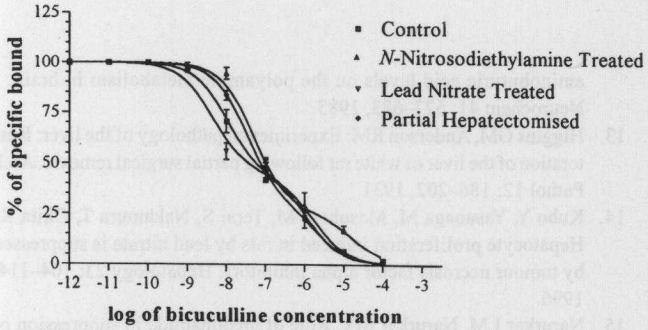

Fig. 1. Displacement of [^{3}H]bicuculline methochloride with bicuculline methochloride in hypothalamic synaptic membrane preparations of control, partial hepatectomised, lead nitrate treated and *N*-nitrosodiethylamine treated rats. Incubation was done at 0–2°C for 30 min with 5 nM [^{3}H]-bicuculline methochloride in each tube and cold concentration varying from 10^{-12}–10^{-3} M. Reaction was stopped by centrifugation at 35,000 g for 20 min. [^{3}H]Bicuculline methochloride in the pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting nonspecific binding from total binding. Values are representation of 4–6 separate experiments.

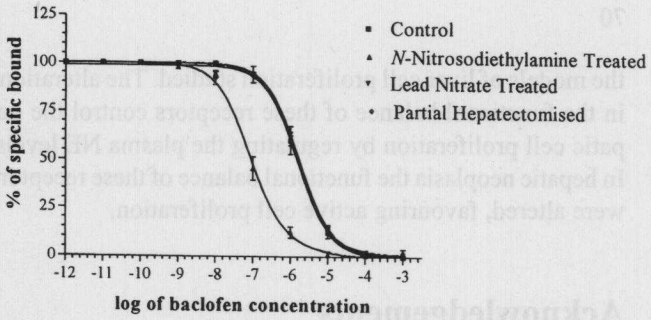

Fig. 2. Displacement of [^{3}H]baclofen with baclofen in hypothalamic synaptic membrane preparations of control, partial hepatectomised, lead nitrate treated and *N*-nitrosodiethylamine treated rats. Incubation was done at 20°C for 30 min with 10 nM [^{3}H]baclofen in each tube and cold concentration varying from 10^{-12}–10^{-3} M. Reaction was stopped by centrifugation at 7,500 g for 20 min. [^{3}H]Baclofen in the pellet was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting nonspecific binding from total binding. Values are representation of 4–6 separate experiments.

nounced in NDEA treated group. Displacement analysis showed a shift in the high-affinity site to low-affinity site in PH and NDEA groups indicating a decreased functioning of the receptor. In LN treated group the shift in this site was more towards the high-affinity indicating an increased $GABA_A$ receptor function. The feedback changes in hypothalamic GABAergic system in LN treated rats, to prevent excess liver cell proliferation, was not seen in NDEA treated rats. In NDEA treated rats, the changes were similar to that of PH rats facilitating active hepatic proliferation. The results showed that hypothalamic $GABA_A$ receptor activity decreased during liver regeneration and neoplasia. It is already reported that intra-hypothalamic administration of $GABA_A$ receptor antagonist bicuculline methoiodide decreased the sympathetic innervation and blood pressure in a dose dependent manner [27]. The decreased $GABA_A$ receptor activity observed may be facilitating the sympathetic innervation.

$GABA_B$ receptor density was increased in the PH and NDEA treated rats. In NDEA treated rats the affinity of the receptor to baclofen also increased significantly. The affinity change in NDEA treated rats was confirmed by displacement analysis where we have observed a shift in affinity towards high-affinity indicating the increased $GABA_B$ receptor function. Hypothalamic $GABA_B$ergic innervation is reported to have a stimulatory effect on sympathetic nervous system [28]. $GABA_B$ receptor activity was increased in PH and NDEA treated rats. The differential functioning of $GABA_A$ and $GABA_B$ receptor system and its importance in sympathetic innervation is already reported [29]. Plasma NE levels of different experimental groups in the present study are also in accordance with the differential functioning of these $GABA_A$ and $GABA_B$ receptors.

In PH and LN hypothalamic GABAergic changes were opposite and in NDEA treated rats the changes are similar to that of PH. In PH, the hypothalamic GABAergic mechanism is suggested to involve in the regeneration of the lost liver mass by activating the cell proliferation through sympathetic stimulation. These changes get normalised when the whole liver mass has regained. But in the case of NDEA these changes remained similar to that of PH rats because of the uncontrolled liver cell proliferation.

We conclude from our studies that $GABA_A$ and $GABA_B$ receptors of hypothalamus were regulated differentially in all

Table 5. Binding parameters of [^{3}H]baclofen against baclofen in hypothalamus of rats

Experimental groups	Best-fit model	log(EC_{50})	Ki	Hill slopes
Control¶	One-site	–5.82	1.18×10^{-6}	–1.03
Partial hepatectomised	One-site	–5.81	1.20×10^{-6}	–0.94
N-Nitrosodiethylamine treated	One-site	–7.05	6.90×10^{-8}	–1.00
Lead nitrate treated	One-site	–5.76	1.34×10^{-6}	–1.00

Data were fitted with an iterative nonlinear regression software (Prism, GraphPad, San Diego, CA, USA). Ki – The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding. Values are mean of 4–6 separate experiments. ¶Control value given is a pooled data from different control experiments since there was no significant difference in values among groups.

the models of liver cell proliferation studied. The alterations in the functional balance of these receptors control the hepatic cell proliferation by regulating the plasma NE levels. In hepatic neoplasia the functional balance of these receptors were altered, favouring active cell proliferation.

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References

1. Lautt WW: Afferent and efferent neural roles in liver function. Prog Neurobiol 21: 323–348, 1983
2. Frerichs FT: A clinical treatise on diseases of the liver. Vol 1. In: The New Sydenham Society. Translated by C. Murchison, London, 1860, pp 193–246
3. Basile AS, Jones EA, Skolnick P: The pathogenesis and treatment of hepatic encephalopathy: Evidence for the involvement of benzodiazepine receptor ligands. Pharmacol Rev 43: 27–71, 1991
4. Butterworth RF: The neurobiology of hepatic encephalopathy. Semin Liver Dis 16: 235–244, 1995
5. Jones EA, Yurdaydin C: Is fatigue associated with cholestasis mediated by altered central neurotransmission? Hepatology 25: 492–494, 1997
6. Rogers RC, Hermann GE: Central connections of the hepatic branch of the vagus nerve: A horseradish peroxidase histochemical study. J Auto Nerv Sys 7: 165–174, 1983
7. Tanaka K, Ohkawa S, Nishino T, Niijima A, Inoue S: Role of the hepatic branch of the vagus nerve in liver regeneration in rats. Am J Physiol 253: G439–G444, 1987
8. Kiba T, Tanaka K, Inoue S: Lateral hypothalamic lesions facilitate hepatic regeneration after partial hepatectomy in rats. Pflügers Arch 430: 666–671, 1995
9. Yoneda M, Tamori K, Sato Y, Yokohama S, Nakamura K, Kono T, Makino I: Central thyrotropin-releasing hormone stimulates hepatic DNA synthesis in rats. Hepatology 26: 1203–1208, 1997
10. Nobin A, Baumarten HG, Flack B, Ingemansson S, Moghimzadeh E, Rosengren E: Organisation of sympathetic innervation in liver tissues from monkey and man. Cell Tissue Res 195: 371–380, 1978
11. Jones EA: Fatigue associated with chronic liver disease: A riddle wrapped in a mystery inside an enigma. Hepatology 22: 1606–1608, 1995
12. Lapinjoki SP, Pulkka AE, Laitinen SI, Pajunen AEI: Possible involvement of humoral regulation in the effects of elevated cerebral 4-aminobutyric acid levels on the polyamine metabolism in brain. J Neurochem 41: 677–683, 1983
13. Higgins GM, Anderson RM: Experimental pathology of the liver: Restoration of the liver of white rat following partial surgical removal. Arch Pathol 12: 186–202, 1931
14. Kubo Y, Yasunaga M, Masuhara M, Terai S, Nakamura T, Okita K: Hepatocyte proliferation induced in rats by lead nitrate is suppressed by tumour necrosis factor alpha inhibitors. Hepatology 23: 104–114, 1996
15. Narurkar LM, Narurkar MV: Role of nicotinamide in suppression of diethylnitrosamine hepatocarcinogenesis in rats. In: S.V. Bhide, G.B. Maru (eds). Chemoprevention of Cancer. Omega Scientific Publishers, New Delhi, 1989, pp 162–177
16. Glowinski J, Iversen LL: Regional studies of catecholamines in the rat brain. The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]DOPA in various regions of the brain. J Neurochem 13: 655–669, 1966
17. Gaskins HR, Baldeon ME, Selassi L, Beverly JL: Glucose modulates gamma-aminobutyric acid release from the pancreatic beta TC6 cell line. J Biol Chem 270: 30286–30289, 1995
18. Paulose CS, Dakshinamurti K: Chronic catheterization using vascular-access-port in rats: Blood sampling with minimal stress for plasma catecholamine determination. J Neurosci 22: 141–146, 1987
19. Jackson J, Pius SP, Thomas P, Paulose CS: Platelet monoamine changes in diabetic patients and streptozotocin induced diabetic rats. Curr Sci 72: 137–139, 1997
20. Mohler H: GABA receptor binding with [^{3}H]GABA and [^{3}H]bicuculline-methiodide: An improved method. In: P. Mandel, F.V. DeFeudis (eds). GABA-Biochemistry and CNS Functions. Plenum Press, New York, 1978, pp 355–362
21. Hill DR, Bowery NG, Hudson AL: Inhibition of GABA-B receptor binding by guanyl nucleotides. J Neurochem 42: 652–657, 1984
22. Scatchard G: The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51: 660–672, 1949
23. Cheng Y, Prusoff WH: Relationship between the inhibition constant and the concentration of an inhibitor that cause a 50% inhibition of an enzymatic reaction. Biochem Pharmacol 22: 3099–3108, 1973
24. Grisham JW: A morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating liver, autoradiography with thymidine-H3. Cancer Res 22: 842–849, 1962
25. Kiba T, Tanaka K, Numata K, Hoshino M, Inoue S: Facilitation of liver regeneration after partial hepatectomy by ventromedial hypothalamic lesions in rats. Pflügers Arch 428: 26–29, 1994
26. Columbano A, Shinozuka H: Liver regeneration versus direct hyperplasia. FASEB J 10: 1118–1128, 1996
27. Tellioglu T, Akin S, Ozkutlu U, Oktay S, Onat F: The role of brain acetylcholine in $GABA_A$ receptor antagonist-induced blood-pressure changes in rat. Eur J Pharmacol 317: 301–307, 1996
28. Nonogaki K, Kotomi M, Nobuo S, Akihisa I: Effect of central GABA receptors activation on catecholamine secretion in rats. Life Sci 55: PL239–PL243, 1994
29. Takenaka K, Sasaki S, Uchida A, Fujita H, Ichida T, Itoh H, Nakata T, Takeda K, Nakagawa M: Hypothalamic and medullary $GABA_A$ and $GABA_B$-ergic systems differentially regulate sympathetic and cardiovascular systems. Clin Exp Pharmacol Physiol 22: S48–S50, 1995