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Hepatic $GABA_A$ receptor functional regulation during rat liver cell proliferation

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Abstract

Gamma aminobutyric acid ($GABA_A$) receptor functional status was analysed in partial hepatectomised (PH), lead nitrate (LN) induced hyperplastic and *N*-nitrosodiethylamine (NDEA) treated neoplastic rat livers during peak DNA synthesis. The high-affinity [^{3}H]GABA binding significantly decreased in PH and NDEA rats and the receptor affinity decreased in NDEA and increased in LN rats compared with control. In NDEA, displacement analysis of [^{3}H]GABA with muscimol showed loss of low-affinity site and a shift of high-affinity site towards low-affinity. The affinity sites shifted towards high-affinity in LN rats. The number of low-affinity [^{3}H]bicuculline receptors decreased significantly in NDEA and PH whereas it increased in LN rats. $GABA_A$ receptor agonist, muscimol, dose dependently inhibited epidermal growth factor (EGF) induced DNA synthesis and enhanced the transforming growth factor β1 (TGF-β1) mediated DNA synthesis suppression in primary hepatocyte cultures. Our results suggest that $GABA_A$ receptor act as an inhibitory signal for hepatic cell proliferation.

Keywords: GABA receptor; Liver regeneration; Hyperplasia; Liver cancer; Hepatocyte

1. Introduction

Gamma aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian brain. GABA has also been demonstrated to be present in peripheral tissues and functionally active throughout the body [1]. GABA possesses growth-regulatory properties. The growth of murine squamous cell carcinoma and HeLa cell lines was shown to be inhibited by GABA [2]. Gliomas with highest malignancy grade IV according to World Health Organisation (WHO) classification and with high proliferation rate lack expression of functional $GABA_A$ receptors [3]. GABA also plays an important role in terminating the growth of rapidly developing tissues in utero [4,5].

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Sodium-independent, bicuculline-sensitive $GABA_A$ receptor sites were identified to be present in the mammalian liver. Innervation of the receptor causes marked hyperpolarisation of the hepatocyte transmembrane potential [6]. $GABA_A$ mediated growth inhibition of the liver following partial hepatectomy and during recovery from various forms of hepatic injury is already reported [7–9]. A recent study has shown that increased $GABA_A$ receptor activity inhibits proliferation activity of the HepG2, human hepatocellular carcinoma cell line. The inhibitory effect was prolonged in the cell line co-transfected with $GABA_A$ receptor β_2 and γ_2 subunit genes [10]. The liver regeneration after partial hepatectomise (PH), lead nitrate (LN) induced hepatic proliferation and *N*-nitrosodiethylamine (NDEA) induced hepatocellular carcinoma in rats are established models to study the normal and neoplastic cell proliferation [11–13]. Studies with replicating hepatocytes in culture can be used to investigate the trophic factors that control the proliferation of hepatocytes [14]. In primary cultures of hepatocytes, epidermal growth factor (EGF) induces DNA synthesis [15] and this was abolished by transforming growth factor β1 (TGFβ1) [16]. In the present study $GABA_A$ receptor functional regulation during compensatory hyperplasia after PH, LN induced direct hyperplasia and NDEA induced neoplasia in the rat liver were investigated. The effect of $GABA_A$ receptor agonist on EGF induced DNA synthesis and TGFβ1 mediated DNA synthesis suppression was also studied in the primary cultures of rat hepatocytes.

2. Materials and methods

2.1. Animals

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

2.2. Materials

GABA, collagenase type IV, muscimol, bicuculline methoiodide, Collagen from rat-tail, William's medium E, epidermal growth factor (EGF), transforming growth factorβ1 (TGFβ1) and insulin were purchased from Sigma Chemical Co., St. Louis, USA. 4-Amino-n-[2,3-^{3}H]butyric acid (specific activity, 84.0 Ci/mmol) was purchased from Amersham Life Science, Buckinghamshire, UK. Bicuculline methyl chloride, (-)-[methyl-^{3}H] (specific activity, 82.9 Ci/mmol) was purchased from NEN Life Sciences products, Inc., Boston, USA and [^{3}H]thymidine (specific activity 18 Ci/mmol) was from Bhabha Atomic Research Centre, Mumbai, India.

2.3. Partial hepatectomy

Two-thirds of the liver constituting the median and left lateral lobes were surgically excised under light ether anaesthesia, following a 16 h fast [17]. Sham operations involved median excision of the body wall followed by all manipulations except removal of the lobes. All the surgeries were done between 07:00 and 09:00 h to avoid diurnal variations in responses.

2.4. Lead nitrate administration

Rats received a single intravenous injection of LN (100 μmol/kg of body weight) while the control rats received distilled water only[18].

2.5. *N*-nitrosodiethylamine treatment

Liver cancer was induced using NDEA [19]. Animals received 0.02% NDEA in distilled water (2.5 ml per 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.

2.6. Sacrifice of rats

The rats were sacrificed by decapitation and the iver was dissected and stored at −70°C after mmediate freezing in liquid nitrogen.

2.7. Preparation of isolated cells for receptor studies

Suspensions of liver cells were generated by perfusing the livers in situ with 0.05% collagenase ollowing the modified procedure described by Minuk et al. [6]. The hepatocyte preparation, aving a viability of >90% as assessed by trypan blue exclusion, was chosen for receptor assay. Contamination of hepatocyte with other cells was ess than 1%.

2.8. $GABA_A$ receptor binding assay

[^{3}H]GABA binding to the GABA receptors of hepatocytes was assayed as previously described 7]. In brief, incubations were carried out in sodium-free complete Hank's balanced salt solution (pH 7.4). Each assay tube contained 1×10^6 cells. 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 [^{3}H]GABA with and without muscimol at a concentration range of 10^{-9}–10^{-4} M. The incubation was continued for 20 min at 0–2°C and terminated by centrifugation at 35 000 × g for 20 min. [^{3}H]GABA in the pellet was determined using Wallac liquid scintillation counter. Specific binding was determined by subtracting non-specific binding from the total binding. The non-specific binding determined was less than 40% of the total binding in all our experiments. Protein concentrations were estimated [20] using bovine serum albumin as the standard after digesting the hepatocytes with 1 N NaOH.

[^{3}H]bicuculline binding to the GABA receptor was assayed in the same way as described for [^{3}H]GABA binding. In saturation binding experiments, 5–75 nM of [^{3}H]bicuculline was incubated with and without excess of unlabelled bicuculline (100 μM) and in competition binding experiments the incubation mixture contained 5 nM of [^{3}H]bicuculline with and without bicuculline at a concentration range of 10^{-9}–10^{-4} M. The non-specific binding determined was less than 40% of the total binding in all our experiments.

2.9. Receptor binding parameters analysis

The receptor binding parameters were determined using Scatchard analysis [21]. 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 analysed 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 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 [22].

2.10. 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 steep 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 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.

2.11. Hepatocyte culture

Hepatocytes were isolated from adult male Wistar rats by collagenase perfusion, filtration and low speed centrifugation [23]. Livers were perfused with a Ca^{2+}-free HEPES buffer (pH 7.4),

lowed by the same buffer (pH 7.6) containing 5 M $CaCl_2$ and 0.05% collagenase. The hepato- e preparation, having a viability of >90% as essed by trypan blue exclusion, was chosen for lture. Hepatocytes were plated on rat tail colla- n coated dishes at a density of 10^6 cells per 35 n culture dish in 1 ml of William's medium E plemented with 10% fetal calf serum, 10^{-7} M ulin and 50 μg/ml gentamycin sulphate. After 3 (zero time of assay), the plating media was placed by serum-free media containing 2.5 μCi/ of [^{3}H]thymidine. Dose response of hepatocyte NA synthesis to muscimol was studied by ding varying concentrations of muscimol 10^{-8}–10^{-4} M) to primary cultures of rat hepa- ytes in presence of fixed concentrations of GF (10 ng/ml) and insulin (10^{-7} M). Combined ect of GABA, muscimol and TGFβ1 on EGF uced hepatocyte DNA synthesis was studied ng TGFβ1 at a concentration of 1 ng/ml. The tures were incubated for 48 h at 37°C in 5% O_2 in Shellab CO_2 incubator (Sheldon Manufac- ing Inc., Oregon, USA).

2.2. DNA synthesis assays in cultured rat patocytes

Hepatocytes were washed twice in the cold osphate buffered saline after 48 h of incubation d 1 ml of cold 10% TCA was added. The patocytes were solubilised by incubation at °C for 30 min in 0.5 ml of 1 N NaOH and then d 100% TCA was added to the solution to get inal concentration of 15%. The precipitate was washed with 1 ml of 5% TCA. DNA was hydrolysed by heating the precipitate at 90°C for 15 min in 0.5 ml of 10% TCA. DNA synthesis was measured by [^{3}H]thymidine incorporation and expressed as dpm of [^{3}H]thymidine incorporated per mg protein [24].

Table 1
[^{3}H]GABA binding parameters in the liver of rats[a]

Experimental groups	B_{max} (fmol/mg protein)	K_d (nM)
Control¶	175.39 ± 5.175	11.84 ± 0.87
	135.13 ± 8.45**	11.13 ± 1.13
PH treated	168.24 ± 10.56	5.11 ± 0.19**
NDEA treated	127.50 ± 9.54**	19.62 ± 1.02**

[a] Values are mean ± S.E.M. of four to six separate experiments. **, $P<0.01$ with respect to control; ¶, control value given is a pooled data from different control experiments since there was no significant difference in values among groups.

2.3. Statistical analysis

The equality of all the groups is tested by the analysis of variance (ANOVA) technique for different values of P. Further the pair wise comparisons of all the experimental groups are studied using Students t-test at different significance levels. The testing is performed using GraphPad Instat (version 2.04a, San Diego, USA). The significance level is expressed taking $P<0.05$ as the threshold value. P value <0.05 is considered as significant, P value <0.01 is very significant and $P<0.001$ is considered extremely significant.

3. Results

A significant decrease in the B_{max} of [^{3}H]GABA binding ($P<0.01$) to hepatocytes of NDEA treated and PH rats was observed compared with control while it remained unaltered in LN treated rats. The K_d of the receptor in NDEA treated rats significantly increased ($P<0.01$) while it significantly decreased ($P<0.01$) in LN treated rats compared with control. In PH rats the K_d remained unaltered (Table 1).

The competition curve for muscimol against [^{3}H]GABA fitted for two-site model in control, PH and LN treated groups with Hill slope values away from unity. In NDEA treated rats the curve fitted for a one-site model with Hill slope value near unity. In LN treated rats both $\log(EC_{50})-1$, $\log(EC_{50})-2$, $Ki_{(H)}$ and $Ki_{(L)}$ decreased compared with the control indicating a shift in both high-affinity and low-affinity sites to respective high-affinity regions. The high-affinity site was shifted to low-affinity and low-affinity site was completely lost in NDEA treated rats (Table 2 and Fig. 1).

Scatchard analysis of [^{3}H]bicuculline binding to isolated hepatocytes of rats showed a significant

Table 2
Binding parameters of [^{3}H]GABA against muscimol in liver of rats[a]

Experimental groups	Best-fit model	Log (EC_{50}) – 1	Log (EC_{50}) – 2	$Ki_{(H)}$	$Ki_{(L)}$	Hill slopes
Control[¶]	Two-site	–7.84	–5.32	1.21×10^{-8}	4.01×10^{-6}	–0.48
PH	Two-site	–7.91	–5.46	1.02×10^{-8}	2.91×10^{-6}	–0.46
NDEA treated	One-site	–6.09		6.79×10^{-7}		–1.01
LN treated	Two-site	–8.58	–7.23	2.21×10^{-9}	4.90×10^{-8}	–0.71

[a] Values are mean of four to six separate experiments, data were fitted with an iterative non-linear 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. ¶, Control value given is a pooled data from different control experiments since there was no significant difference in values among groups.

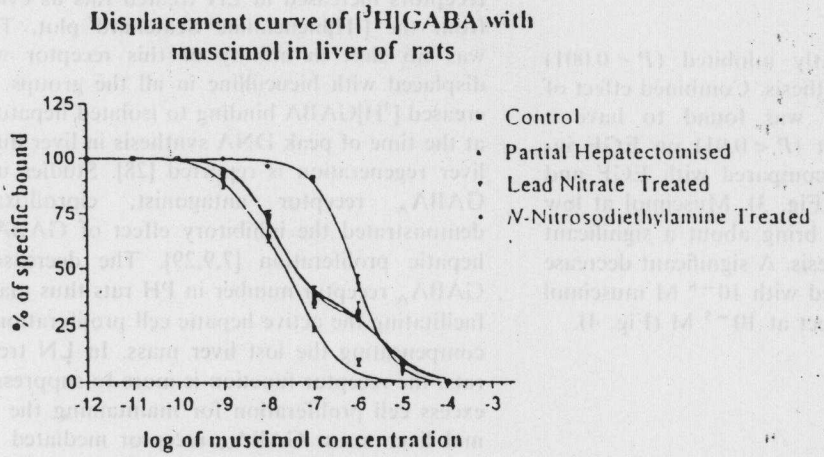

Fig. 1. Displacement analysis of [^{3}H]GABA by muscimol in hepatocyte preparations of control, PH, LN treated and NDEA treated rats, assayed in vitro. Competitive binding studies were done with 2 nm [^{3}H]GABA and 10^{-12}–10^{-3} M cold muscimol. Values are mean ± S.E.M. of four to six separate experiments and plotted at different concentrations.

decrease ($P < 0.001$) in B_{max} in PH and NDEA treated rats while it increased ($P < 0.05$) in LN treated rats compared with the control. K_d remained unaffected in all the experimental groups compared with control (Table 3). The competition curve for bicuculline against [^{3}H]bicuculline fitted for two-site model in all the groups with Hill slope value away from unity. The binding parameters remained unaltered in all the experimental groups compared with control (Table 4).

Isolated hepatocytes in serum-free culture medium exhibited very low levels of [^{3}H]thymidine incorporation into DNA. Addition of EGF caused a significant increase ($P < 0.001$) in the hepatocyte DNA synthesis. When GABA (100 μM) alone was added to cultured hepatocytes there was no significant change in the DNA synthesis from basal level. However, addition of GABA (100 μM) in hepatocyte cultures caused a significant inhibition ($P < 0.001$) on EGF induced DNA synthesis (Fig. 2). TGFβ1 and GABA combination and TGFβ1 alone did not show any significant change compared with control.

Addition of muscimol caused a significant decrease ($P < 0.001$) in the EGF induced DNA synthesis. When muscimol (100 μM) alone was added to cultured hepatocytes there was no significant change in the DNA synthesis compared with con-

Table 3
[^{3}H]bicuculline binding parameters in the liver of rats[a]

Experimental groups	B_{max} (fmol/mg protein)	K_d (nM)
Control¶	324.76 ± 10.78	40.84 ± 1.87
PH	197.54 ± 13.24***	45.13 ± 2.13
LN treated	398.45 ± 9.45*	43.24 ± 0.19
NDEA treated	148.65 ± 11.23***	39.62 ± 1.02

[a] Values are mean ± S.E.M. of four to six separate experiments. ***, $P<0.001$; *, $P<0.05$ with respect to control; ¶, control value given is a pooled data from different control experiments since there was no significant difference in values among groups.

trol. TGFβ1 significantly inhibited ($P<0.001$) EGF induced DNA synthesis. Combined effect of TGFβ1 and muscimol was found to have a greater inhibitory effect ($P<0.01$) on EGF induced DNA synthesis compared with EGF and TGFβ1 treated group (Fig. 3). Muscimol at low concentrations did not bring about a significant decrease in DNA synthesis. A significant decrease ($P<0.001$) was observed with 10^{-6} M muscimol reaching a maximal effect at 10^{-5} M (Fig. 4).

4. Discussion

[^{3}H]GABA acts as a $GABA_A$ receptor high-affinity agonist [25] and bicuculline has a higher affinity for rapidly dissociating low-affinity $GABA_A$ sites [26,27]. The binding studies revealed that both high-affinity and low-affinity $GABA_A$ receptors are present in hepatocytes. The animals were sacrificed at the time of peak DNA synthesis in liver based on previous reports [11,18] and by [^{3}H]thymidine incorporation studies done. There was a significant decrease in the number of [^{3}H]GABA binding high-affinity $GABA_A$ receptors in PH and NDEA treated rats. In NDEA treated rats the low-affinity site for [^{3}H]GABA binding completely lost and the high-affinity site shifted to low-affinity. The low-affinity [^{3}H]bicuculline binding $GABA_A$ receptor also showed a decrease in the number of receptors in PH and NDEA treated rats while the number of receptors increased in LN treated rats as evident from the [^{3}H]bicuculline Scatchard plot. There was no shift in affinity of this receptor when displaced with bicuculline in all the groups. Decreased [^{3}H]GABA binding to isolated hepatocyte at the time of peak DNA synthesis in liver during liver regeneration is reported [28]. Studies using $GABA_A$ receptor antagonist, ciprofloxacin, demonstrated the inhibitory effect of GABA on hepatic proliferation [7,9,29]. The decrease in $GABA_A$ receptor number in PH rats thus may be facilitating the active hepatic cell proliferation for compensating the lost liver mass. In LN treated rats, the receptor function is more to suppress the excess cell proliferation for maintaining the normal liver mass. $GABA_A$ receptor mediated neuronal apoptosis is reported [30]. In LN induced direct hyperplasia the removal of excess liver mass by apoptosis is already described [31]. So increased $GABA_A$ receptor function may be responsible for maintaining normal liver mass. Increasing malignancy of gliomas is correlated to

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

Experimental groups	Best-fit model	log(EC_{50})−1	log(EC_{50})−2	$Ki_{(H)}$	$Ki_{(L)}$	Hill slopes
Control¶	Two-site	−8.02	−5.75	8.95×10^{-9}	1.67×10^{-6}	−0.38
PH	Two-site	−7.11	−5.00	7.26×10^{-8}	9.35×10^{-6}	−0.34
NDEA treated	Two-site	−7.03	−5.20	8.72×10^{-8}	1.86×10^{-6}	−0.38
LN treated	Two-site	−8.12	−6.02	7.19×10^{-9}	9.00×10^{-7}	−0.37

[a] Values are mean of four to six separate experiments. Data were fitted with an iterative non-linear 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; ¶, control value given is a pooled data from different control experiments since there was no significant difference in values among groups.

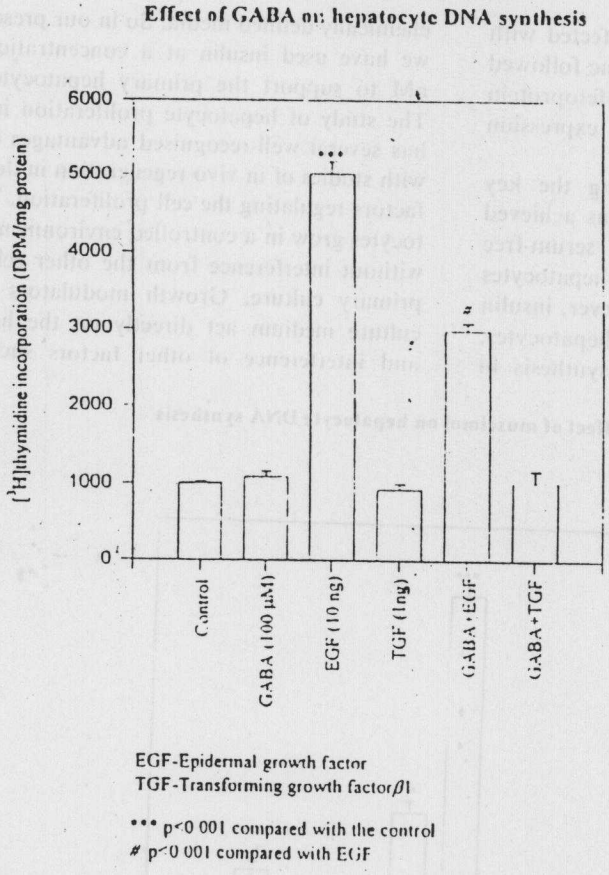

Fig. 2. Effect of GABA on DNA synthesis in primary cultures of hepatocytes. GABA (10^{-4} M) was added to cultured hepatocytes and [³H]thymidine incorporation was determined in the presence of insulin (10^{-7} M), EGF (10 ng) and TGFβ1(1 ng). Values are mean ± S.E.M. of four to six separate experiments.

decrease of GABA binding sites [32]. So a decrease in both number and affinity of [³H]GABA binding with loss in one of the affinity sites may be responsible for the malignant transformation and hepatocellular carcinoma observed in NDEA treated rats. [³H]bicuculline binding sites were also decreased in NDEA treated rats. This denotes that the $GABA_A$ergic mechanism to suppress cell proliferation observed in LN treated rats is absent in NDEA treated rats. Thus, $GABA_A$ receptor functional alteration may be one of the contributing factors for hepatocarcinogenesis in NDEA administered rats.

$GABA_A$ receptors are found to disappear in malignant tumours and immortal cell lines [3]. Our radio-receptor data clearly indicate that the functional status of these receptors are significantly decreased in controlled and cancerous hepatic cell proliferation. The alterations of the receptor function were more in neoplastic liver than in regenerating liver. Also, our result is supported by the recently reported study on human hepatocellular carcinoma cell line, HepG2, over expressing β_2 and γ_2 subunits of $GABA_A$ receptor [10]. $GABA_A$ receptor activity is markedly down regulated in malignant hepato-

cytes. Studies in HepG2 cells co-transfected with $GABA_A$ receptor β_2 and γ_2 subunit gene followed by exposure to muscimol inhibited α-fetoprotein (a hepatic malignant marker) mRNA expression and also the cell proliferation [10].

The important progress in defining the key factors in hepatic cell proliferation was achieved by using hepatocyte cultures in serum-free medium. In the absence of insulin, hepatocytes degenerate within 24–48 h [16]. However, insulin despite its strong trophic effects on hepatocytes, does not by itself stimulate DNA synthesis in chemically defined media. So in our present study, we have used insulin at a concentration of 100 nM to support the primary hepatocyte culture. The study of hepatocyte proliferation in cultures has several well-recognised advantages compared with studies of in vivo regeneration in defining the factors regulating the cell proliferation. The hepatocytes grow in a controlled environment virtually without interference from the other cell types in primary culture. Growth modulators added to culture medium act directly on the hepatocytes and interference of other factors such as hor-

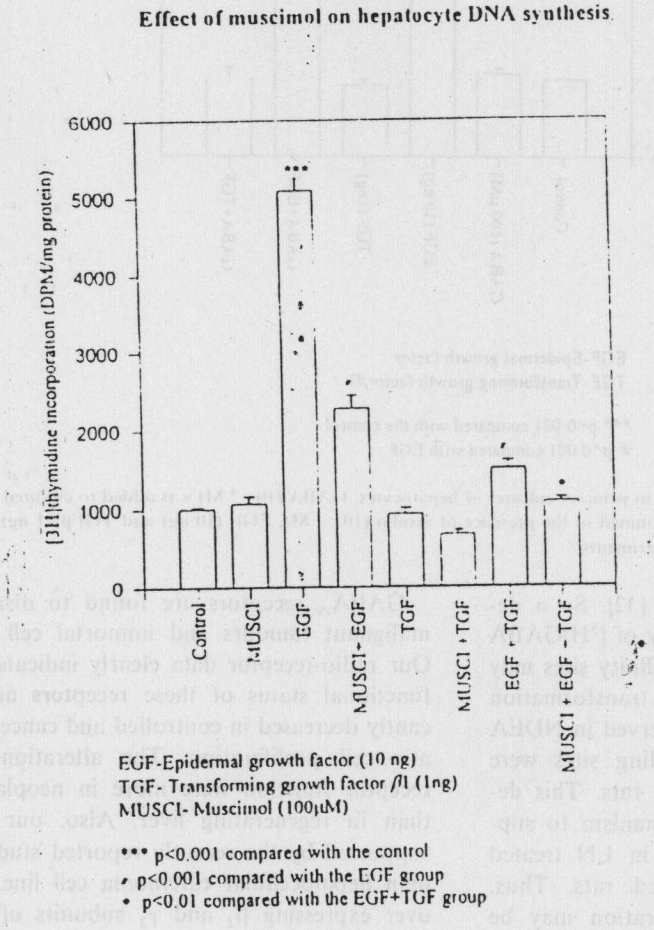

Fig. 3. Effect of muscimol on DNA synthesis in primary cultures of hepatocytes. Muscimol (10^{-4} M) was added to cultured hepatocytes and [^{3}H]thymidine incorporation was determined in the presence of insulin (10^{-7} M), EGF (10 ng) and TGFβ1 (1 ng). Values are mean ± S.E.M. of four to six separate experiments.

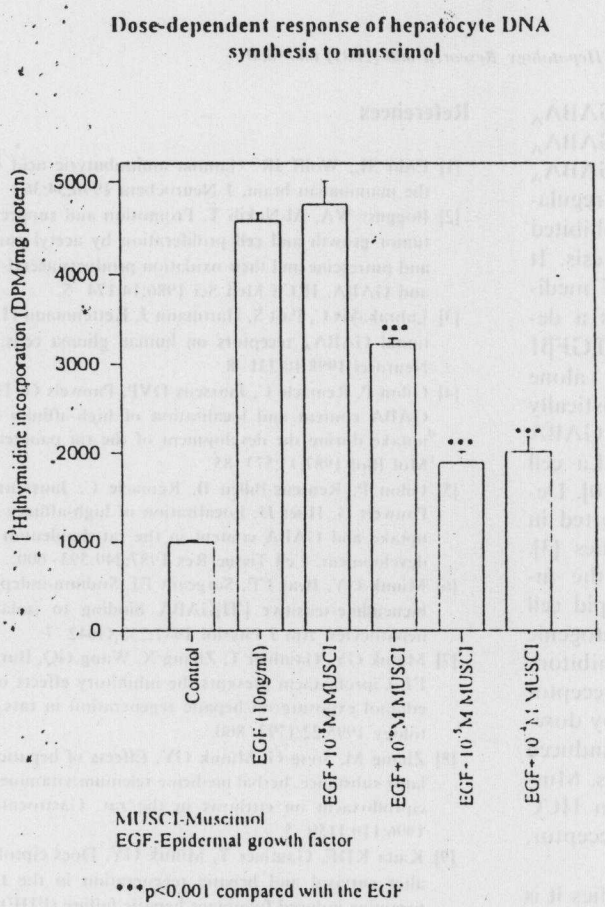

Fig. 4. Dose-dependent response of DNA synthesis in primary cultures of hepatocytes to muscimol. Different concentrations of muscimol (10^{-4}–10^{-8} M) were added to cultured hepatocytes and [^{3}H]thymidine incorporation was determined in the presence of insulin (10^{-7} M), EGF (10 ng). Values are mean ± S.E.M. of four to six separate experiments.

mones can be excluded [16]. Most of the replicating hepatocytes enter into multiple consecutive rounds of DNA synthesis in culture and this replicating system of hepatocytes can be used to investigate the trophic factors that control growth of normal and neoplastic hepatocytes. Hepatocytes do not replicate when kept in chemically defined media even supplemented with fetal bovine serum. However, addition of EGF caused a marked increase in DNA synthesis from basal level [15]. When EGF is added to cultures of freshly isolated hepatocytes, DNA synthesis did not start for 24 h. The active DNA synthesis occurred from 48 to 72 h [33] and hence we chose 48 h of culture to study the effect of growth factors and GABA. Addition of GABA (100 μM) alone did not elicit any significant change in hepatocyte DNA synthesis but it significantly inhibited the EGF mediated DNA synthesis. This clearly demonstrates the inhibitory effect of GABA on hepatocyte proliferation. This result supports the already demonstrated effect of GABA on terminating rapid growth in developing tissue in utero [4,5,34]. Since $GABA_A$ and $GABA_B$ receptors are present in liver [6,35], we studied the effect of these receptors on hepatocyte DNA synthesis in-

dependently by using specific agonists for $GABA_A$ receptor. Muscimol, a specific agonist for $GABA_A$ receptor [26], was used to study the $GABA_A$ receptor mediated hepatocyte proliferation regulation. Muscimol (100 μM) significantly inhibited the EGF induced hepatocyte DNA synthesis. It also enhanced TGFβ1 inhibition on EGF mediated DNA synthesis. Although there was a decrease in DNA synthesis in muscimol and TGFβ1 treated groups compared with TGFβ1 alone treated group, the changes were not statistically significant. Growth inhibitory property of GABA is reported in HCC cell line-HepG2, HeLa cell lines and squamous murine carcinoma [2,10]. Decreased $GABA_A$ receptor function is reported in malignant tumours and immortal cell lines [3]. These reports and our results confirm the inhibitory effect of $GABA_A$ receptor on rapid cell proliferation by reducing the activity of mitogenic growth factors and enhancing the inhibitory power of growth inhibitors. The $GABA_A$ receptor mediated inhibitory effect was confirmed by dose-dependent study of muscimol on EGF induced mitogenicity in primary hepatocyte cultures. Muscimol functions as an inhibitor of human HCC cell line co-transfected with $GABA_A$ receptor, which supports our finding [10].

Thus, from our in vivo and in vitro studies it is clear that $GABA_A$ receptor function is important for inhibition of DNA synthesis in hepatocytes. This receptor function is highly altered in chemically induced hepatocellular carcinoma facilitating abnormal cell proliferation. The results also show that the $GABA_A$ receptor functional regulation is necessary to maintain the normal hepatic DNA synthesis and normal liver mass.

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