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Effect of chronic administration of ethanol on the regulation of the δ-subunit of $GABA_A$ receptors in the rat brain

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Abstract

In the present study, we investigated the effect of chronic ethanol (CE) administration on the polypeptide levels of the δ-subunit of $GABA_A$ receptors and [^{3}H]muscimol binding to the immunoprecipitated δ-subunit-containing $GABA_A$ receptor assemblies in the rat brain. CE administration resulted a down-regulation of polypeptide levels of the δ-subunit of $GABA_A$ receptors in the rat cerebellum and hippocampus, whereas there were no changes in the δ-subunit polypeptide levels in the rat cerebral cortex. Further, CE administration caused a down-regulation of native δ-subunit-containing $GABA_A$ receptor assemblies in the rat cerebellum as determined by [^{3}H] muscimol binding to the immunoprecipitated receptor assemblies. These results indicate that the δ-subunit-containing $GABA_A$ receptors may play a role in chronic ethanol-induced tolerance and dependence.

Keywords

Chronic ethanol; $GABA_A$ receptors; δ-Subunit; Polypeptide levels; Immunoprecipitation; Radioligand binding

1. Introduction

Extensive structural heterogeneity exists among various subtypes of $GABA_A$ receptor since native pentameric receptor assemblies are derived from several subunits such as $\alpha_{1–6}$, $\beta_{1–3}$, $\gamma_{1–3}$, δ, ε, π, and θ (see Mehta and Ticku, 1999a; Sieghart et al., 1999; Whiting, 1999). Most common subunits-combination for $GABA_A$ receptors in the brain is $\alpha_1\beta_2\gamma_2$ (Fritschy et al., 1992). $GABA_A$ receptors gene expression is affected by physiological and pathological processes as well as by the drugs that modulate $GABA_A$ receptors (Aguayo et al., 2002; Cagetti et al., 2003; Follesa et al., 2003; Sanna et al., 2003). Ethanol is known to modulate the activity of a variety of receptors and ion channels (Lovinger et al., 1989; Lovinger and White 1991; Lovinger, 1999; Lewohl et al., 1999; Mihic, 1999; Narahashi et al., 1999; Woodward, 1999; Walter and Messing, 1999; Weight et al., 1999; Lei et al., 2000; Aguayo et al., 2002; Carta et al., 2003; Davies et al., 2003; Roberto et al., 2003; Gehlert et al., 2007), and role of $GABA_A$ receptors in the behavioral effects of ethanol is well documented (see Ticku and Mehta, 1995).

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Chronic ethanol (CE) as well as chronic intermittent ethanol (CIE) treatment is known to modulate the expression of the major subunits of $GABA_A$ receptors in the brain (Montpied et al., 1991; Mhatre and Ticku, 1992, 1994; Devaud et al., 1995, 1997; see Mehta and Ticku, 1999a; Cagetti et al., 2003; Marutha Ravindran and Ticku, 2006a, 2006b; Marutha Ravindran et al., 2007). Recently, it has been suggested that the $GABA_A$ receptor derived from δ-subunit is important site for the pharmacological actions of ethanol (Mihalek et al., 2001; Sundstrom-Poromaa et al., 2002; Cagetti et al., 2003; Wallner et al., 2003; Wei et al., 2004; Hanchar et al., 2004; Glykys et al., 2007). On the other hand, there are also reports contradicting the involvement of the δ-subunit-containing $GABA_A$ receptors in the pharmacology of clinically relevant low concentrations of ethanol (Borghese et al., 2006; Yamashita et al., 2006; Casagrande et al., 2007). Recently, we have reported that ethanol (up to 50 mM) does not affect [^{3}H]muscimol binding to the immunoprecipitated δ-subunit-containing $GABA_A$ receptor assemblies in the rat cerebellum and hippocampus, thereby suggesting that the native δ-subunit-containing $GABA_A$ receptors do not play a major role in the pharmacology of clinically relevant low concentrations of ethanol (Mehta et al., 2007). In the present study, we investigated the effect of CE administration on the polypeptide levels of the δ-subunit of $GABA_A$ receptors in the rat cerebellum, hippocampus and cerebral cortex. Further, we investigated the effect of chronic administration of ethanol on the regulation of native δ-subunit-containing $GABA_A$ receptor assemblies in the rat cerebellum so as to examine whether δ-subunit-containing $GABA_A$ receptors are a potential target site for the chronic ethanol-induced phenomena.

2. Results

2.1. Effect of CE administration on the polypeptide levels of the δ-subunit of $GABA_A$ receptors in the rat brain

Western blot experiments revealed that chronic administration of ethanol causes down-regulation of the polypeptide levels of the δ-subunit of $GABA_A$ receptors in the rat cerebellum (Fig. 1A–a,c; ANOVA $F_{2,6} = 43.59$, $p = 0.0003$; Dunnett's multiple comparison $p < 0.01$) and hippocampus (Fig. 1B–a,c; ANOVA $F_{2,6} = 29.79$, $p = 0.0008$; Dunnett's multiple comparison $p < 0.01$). These changes in the polypeptide levels of δ-subunit reverted back to control levels in the ethanol-withdrawn group (48 h) as shown in Fig. 1A and Fig. 1B. However, chronic administration of ethanol did not result any change in the polypeptide levels of the δ-subunit of $GABA_A$ receptors in the rat cerebral cortex (Fig. 1C–a,c; ANOVA $F_{2,6} = 1.104$, $p = 0.39$; Dunnett's multiple comparison $p > 0.05$). Notably, there was equal intensity of the protein actin band following probing with actin antibody by Western blotting (Fig. 1A–b, Fig. 1B–b and Fig. 1C–b).

2.2. Effect of CE administration on the $GABA_A$ receptor assemblies derived from δ-subunit in the rat cerebellum

Antiserum for the rat $GABA_A$ receptors δ-subunit immunoprecipitated 18.0 ± 1.1% of the $GABA_A$ receptor assemblies in the rat cerebellum as determined with [^{3}H]muscimol (Table 1). Chronic administration of ethanol to the rats decreased the percentage immunoprecipitation of the binding activity for the $GABA_A$ receptor assemblies derived from δ-subunit in the rat cerebellum as determined with [^{3}H]muscimol (11.1 ± 0.7% vs. 18.0 ± 1.1%, ANOVA $F_{2,15} = 16.67$, $p = 0.0002$, Dunnett's multiple comparison $p < 0.01$, Table 1), and this effect reverted to control level following withdrawal of chronic ethanol treatment (48 h) as shown in Table 1.

3. Discussion

Role of $GABA_A$ receptors in the pharmacology of ethanol has been investigated in detail (see Ticku and Mehta, 1995; Mehta and Ticku, 1999a; Sieghart et al., 1999; Whiting, 1999; Hanchar et al., 2004). The subunit composition and stoichiometry of native $GABA_A$ receptors are

currently unknown, but the most abundant population of native $GABA_A$ receptors in the mammalian brain is believed to be the $\alpha_1\beta_2\gamma_2$ subunit combination (Benke et al., 1991; Fritschy et al., 1992; McKernan and Whiting 1996), and these subunits are present in abundant amount in almost every region of the brain (see Mehta and Ticku 1999a). Chronic administration of ethanol is reported to reduce the levels of α_1-, α_2-, and α_5-subunits mRNA and polypeptide levels in cerebellum and cerebral cortex (Mhatre and Ticku 1992; Devaud et al., 1997; Marutha Ravindran et al., 2007). However, the α_4-, γ_1-, γ_{2S}- (Devaud et al., 1995), α_6- (Mhatre and Ticku 1992) and $\beta_{2/3}$-subunits (Mhatre and Ticku 1994) mRNA levels increase in the ethanol-dependent animals. CE as well as CIE exposure of cultured cortical neurons of mice results in up-regulation of the β_2-subunit, down-regulation of the α_1-subunit and no change in the polypeptide levels of the γ_2-subunit of the $GABA_A$ receptors (Marutha Ravindran and Ticku, 2006a, 2006b).

Our present study revealed that chronic administration of ethanol results in a down-regulation of the polypeptide levels of the δ-subunit of the $GABA_A$ receptors in the rat cerebellum and hippocampus. However, chronic administration of ethanol did not elicit any changes in the polypeptide levels of the δ-subunit of $GABA_A$ receptors in the rat cerebral cortex. Our results with CE paradigm are consistent with the report indicating that CIE treatment of rats elicits a decrease in the polypeptide levels of the δ-subunit of the $GABA_A$ receptors in hippocampus (Cagetti et al., 2003). On the other hand, there is also a report indicating that CE treatment of cultured cerebellar granule neurons causes a statistically insignificant reduction in the expression of δ-subunit (Follesa et al., 2005). However, these researchers also observed a statistically significant reduction in the expression of δ-subunit in the cultured cerebellar granule neurons at 6 h of ethanol-withdrawal following CE paradigm, which returned to control values at 12 h of ethanol-withdrawal (Follesa et al., 2005). Further, these researchers found that CE paradigm results in an increase in the expression of δ-subunit in cultured hippocampal neurons (Follesa et al., 2005). These differences may be due to different experimental models (intact rats versus cultured neurons). Notably, CE-induced changes in polypeptide levels of δ-subunit of $GABA_A$ receptors in our present study reverted back to normal levels in the rat cerebellum and hippocampus following 48 h of ethanol withdrawal.

Consistent with chronic ethanol-induced down-regulation of the polypeptide levels of the $GABA_A$ receptors δ-subunit in our study, we also observed a down-regulation of δ-subunit-containing $GABA_A$ receptor assemblies in the rat cerebellum as determined by immunoprecipitation followed by [^{3}H]muscimol binding. It is possible that chronic ethanol-induced down-regulation of native δ-subunit-containing $GABA_A$ receptor assemblies may be due to altered receptor trafficking, as reported recently in the case of α_1-subunit-containing $GABA_A$ receptors (Kumar et al., 2003). Down-regulation of the polypeptide levels of the $GABA_A$ receptors δ-subunit as well as δ-subunit-containing $GABA_A$ receptor assemblies following the chronic administration of ethanol may alter the GABAergic transmission and synaptic responses, thereby leading to tolerance and dependence. Thus our study suggests δ-subunit-containing $GABA_A$ receptors as an important potential target for the treatment of chronic ethanol-induced tolerance and dependence.

In summary, CE administration caused a down-regulation of the polypeptide levels of the $GABA_A$ receptors δ-subunit in the rat cerebellum and hippocampus. Further, chronic administration of ethanol resulted in a down-regulation of native δ-subunit-containing $GABA_A$ receptor assemblies in the rat cerebellum. These CE-induced changes in the δ-subunit-containing $GABA_A$ receptors may have important implications in chronic ethanol-induced dependence and tolerance.

4. Experimental procedures

Adult male Sprague-Dawley rats (Harlan, Indianapolis IN, U.S.A.) weighing 200–250 g were maintained at a constant room temperature (22°C) on a 12-h light/12-h dark cycle. All experiments were conducted in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health. Adequate measures were taken to minimize pain or discomfort to the animals. Food and water were available ad libitum. They were divided into three groups: saline control, ethanol-maintained (chronic ethanol-administered) and ethanol-withdrawn rats.

4.1. Chronic administration of ethanol to the rats

The animals were intoxicated by intragastric intubation method for 6 days as described earlier (Majchrowicz 1975; Mhatre et al., 1988; Mehta and Ticku 1999b; Marutha Ravindran et al., 2007). Briefly, at the beginning of the experiment, a priming dose of ethanol of 5 g/kg (20% v/v in normal saline) followed by 9 g/kg (20% v/v in normal saline) over 24 h period in three divided doses was administered orally to all animals for six days. The control rats received normal saline. Chronic ethanol-maintained rats were sacrificed 1 h after the last dose of ethanol and the ethanol-withdrawn rats were sacrificed 48 h after the last dose of ethanol. Different regions of the rat brain were dissected, and tissues were stored at −80°C until use.

4.2. Electrophoresis and immunoblotting

Tissues were homogenized in ice-cold lysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 1% Triton X 100, 10 mM NaF, 1 mg/ml bacitracin, 50 mM β-glycerophosphate, 1 mM phenyl methyl sulfonyl fluoride (PMSF), 1 mM *N*-ethylmaleimide, 1 mM Na_3VO_4, 10 μg/ml leupeptin). These samples were centrifuged (2300 *g*, 10 min, 4°C) so as to remove the supernatant containing protein (Bjornstrom et al., 2002; Marutha Ravindran and Ticku, 2006a, 2006b; Marutha Ravindran et al., 2007). Fifty micrograms of protein was boiled in a boiling water-bath for 5 min to denature the proteins, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) and transferred to polyvinylidene difluoride membrane followed by treatment with 5% nonfat dry milk (Nestle, Ohio, USA) in Tris-buffered saline containing 0.1% Tween 20. Following this, the membrane was incubated with 1:100 dilution of the antiserum for the $GABA_A$ receptors δ-subunit since preliminary experiments revealed that 1:100 dilution of the δ-subunit antiserum yields the intensity of its protein band in a linear range. The membrane was washed several times and peroxidase-coupled secondary antibody (anti-mouse IgG/anti-rabbit IgG; New England Biolabs, USA) was added and incubated for 1 h. Following this, the membrane was washed and specific bands were visualized using super signal west pico chemiluminescent substrate kit (Pierce Biotechnology Inc, USA). Intensities of the bands on the membrane were scanned using a densitometer. Average intensity of the bands from three different Western blots of the control group was considered as 100% and the changes in the intensity of the band in the experimental group were determined as detailed earlier (Marutha Ravindran and Ticku, 2004, 2006a, 2006b; Marutha Ravindran et al., 2007). The data are expressed as % mean ± S.E.M. from three different Western blots performed on different days using pooled tissue from 10 rats in each group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett test. Antiserum for the rat $GABA_A$ receptors δ-subunit (AA 1–11) was procured from Alpha Diagnostics (San Antonio, TX, USA). We have reported the characterization of this antiserum for the $GABA_A$ receptors δ-subunit recently (Mehta et al., 2007). Pre-incubation with the antigenic peptide (AA 1–11; 20 μg/ml) for the δ-subunit of $GABA_A$ receptors antibody blocked the immunoprecipitation in control experiments, and molecular mass of the δ-subunit of $GABA_A$ receptors was found to be 54 kDa in Western blot analysis (Mehta et al., 2007).

4.3. Immunoprecipitation and [^{3}H]muscimol binding

The rat cerebellum membrane preparation, the $GABA_A$ receptors solubilization, immunoprecipitation and [^{3}H]muscimol binding assays were performed as reported in the literature (Khan et al., 1993; Mehta and Ticku, 1999b, 2005). Briefly, the frozen tissue was thawed, homogenized in ice-cold 0.32 M sucrose solution (pH 7.4) and it was then centrifuged at 78,000 *g* for 30 min at 4°C. The pellet was suspended in 0.32 M sucrose solution (pH 7.4) and kept frozen overnight at −80°C. After thawing, Tris-HCl (50 mM, pH 7.4) was added to the tissue, and it was centrifuged at 30,000 *g* for 10 min at 4°C. The pellet was resuspended in Tris-HCl buffer (50 mM, pH 7.4), and centrifuged at 30,000 *g* for 10 min at 4°C. The last step was repeated four more times. The pellet was resuspended in Tris-HCl buffer, and kept frozen overnight at −80°C. After thawing, Tris-HCl buffer was added to the tissue, and the mixture was centrifuged at 78,000 *g* for 30 min at 4°C. The membranes were then suspended in Tris-HCl (50 mM, pH 7.4), distributed in aliquots, and kept frozen at −80°C until use. $GABA_A$ receptors were solubilized in modified radioimmune precipitation assay buffer (RIPA), i.e., solubilization buffer (pH 7.4) containing NaCl (0.137 M), sodium deoxycholate (1% w/v), Triton X-100 (1% v/v), sodium dodecyl sulfate, i.e, SDS (0.1% w/v), Tris (10 mM) and a cocktail of protease inhibitors containing EDTA (1 mM), EGTA (1 mM), benzamidine HCl (2 mM), trypsin inhibitor type 1-S (0.1 mg/ml), bacitracin (0.1 mg/ml) and phenylmethylsulfonyl fluoride (0.3 mM). After incubation for 1 h at 4°C, insoluble material was removed by centrifugation at 100,000 *g* for 1 h at 4°C. A sample of 400 µl (≈300 µg protein) of the solubilized receptors was incubated overnight at 4°C with 30 µl of the antiserum for the rat $GABA_A$ receptors δ-subunit since preliminary experiments revealed that 30 µl of the antiserum elicits maximal immunoprecipitation of the δ-subunit-containing $GABA_A$ receptor assemblies. The receptor-antibody complexes were recovered by incubation with protein A-agarose suspension (60 µl of 40% v/v) followed by centrifugation. Immunoprecipitation was quantified by determining the binding of [^{3}H]muscimol (36 nM) to the immunoprecipitated pellet and supernatant. Non-specific radioligand binding was determined with GABA (100 µM). Protein was estimated using Micro BCA™ Protein Assay Kit (Pierce, Rockford IL U.S.A.). The data are expressed as mean ± S.E.M. of six individual experiments, each performed in triplicate using the pooled cerebellum from ten rats in each group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett test.

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REFERENCES

Aguayo LG, Peoples RW, Yeh HH, Yevenes GE. $GABA_A$ receptors as molecular sites of ethanol action. Direct or indirect actions? Curr. Top. Med. Chem 2002;2:869–885. [PubMed: 12171577]

Benke D, Mertens S, Trzeciak A, Gillessen D, Mohler H. $GABA_A$ receptors display association of γ2-subunit with α1- and β2/3-subunits. J. Biol. Chem 1991;266:4478–4483. [PubMed: 1847922]

Bjornstrom K, Sjolander A, Schippert A, Eintrei C. A tyrosine kinase regulates propofol-induced modulation of the β-subunit of the $GABA_A$ receptor and release of intracellular calcium in cortical rat neurones. Acta Physiol. Scand 2002;175:227–235. [PubMed: 12100362]

Borghese CM, Storustovu SI, Ebert B, Herd MB, Belelli D, Lambert JJ, Marshall G, Wafford KA, Harris RA. The δ subunit of γ-aminobutyric acid type A receptors does not confer sensitivity to low concentrations of ethanol. J. Pharmacol. Exp. Ther 2006;316:1360–1368. [PubMed: 16272217]

Cagetti E, Liang J, Spigelman I, Olsen RW. Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of $GABA_A$ receptors. Mol. Pharmacol 2003;63:53–64. [PubMed: 12488536]

Carta M, Ariwodola OJ, Weiner JL, Valenzuela CF. Alcohol potently inhibits the kainate receptor-dependent excitatory drive of hippocampal interneurons. Proc. Natl. Acad. Sci. U.S.A 2003;100:6813–6818. [PubMed: 12732711]

Casagrande S, Cupello A, Pellistri F, Robello M. Only high concentrations of ethanol affect $GABA_A$ receptors of rat cerebellum granule cells in culture. Neurosci. Lett 2007;414:273–276. [PubMed: 17234340]

Davies AG, Pierce-Shimomura JT, Kim H, Van Hoven MK, Thiele TR, Bonci A, Bargmann CI, McIntire SL. A central role of the BK potassium channel in behavioral response to ethanol in *C. elegans*. Cell 2003;115:655–666. [PubMed: 14675531]

Devaud LL, Smith FD, Grayson DR, Morrow AL. Chronic ethanol consumption differentially alters the expression of γ-aminobutyric $acid_A$ receptor subunit mRNAs in rat cerebral cortex: competitive, quantitative reverse transcriptase-polymerase chain reaction analysis. Mol. Pharmacol 1995;48:861–868. [PubMed: 7476917]

Devaud LL, Fritschy JM, Sieghart W, Morrow AL. Bidirectional alterations of $GABA_A$ receptor subunit peptide levels in rat cortex during chronic ethanol consumption and withdrawal. J. Neurochem 1997;69:126–130. [PubMed: 9202302]

Follesa P, Mancuso L, Biggio F, Mostallino MC, Manca A, Mascia MP, Busonero F, Talani G, Sanna E, Biggio G. γ-Hydroxybutyric acid and diazepam antagonize a rapid increase in $GABA_A$ receptors α_4 subunit mRNA abundance induced by ethanol withdrawal in cerebellar granule cells. Mol. Pharmacol 2003;63:896–907. [PubMed: 12644591]

Follesa P, Mostallino MC, Biggio F, Gorini G, Caria S, Busonero F, Murru L, Mura ML, Sanna E, Biggio G. Distinct patterns of expression and regulation of $GABA_A$ receptors containing the δ subunit in cerebellar granule and hippocampal neurons. J. Neurochem 2005;94:659–671. [PubMed: 16000147]

Fritschy JM, Benke D, Mertens S, Oertel WH, Bachi T, Mohler H. Five subtypes of type A γ-aminobutyric acid receptors identified in neurons by double and triple immunofluorescence staining with subunit-specific antibodies. Proc. Natl. Acad. Sci. U.S.A 1992;89:6726–6730. [PubMed: 1323116]

Gehlert DR, Cippitelli A, Thorsell A, Lê AD, Hipskind PA, Hamdouchi C, Lu J, Hembre EJ, Cramer J, Song M, McKinzie D, Morin M, Ciccocioppo R, Heilig M. 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism. J. Neurosci 2007;27:2718–2726. [PubMed: 17344409]

Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I. A new naturally occurring $GABA_A$ receptor subunit partnership with high sensitivity to ethanol. Nat. Neurosci 2007;10:40–48. [PubMed: 17159992]

Hanchar HJ, Wallner M, Olsen RW. Alcohol effects on γ-aminobutyric acid type A receptors: are extrasynaptic receptors the answer? Life Sci 2004;76:1–8. [PubMed: 15501475]

Khan ZU, Fernando LP, Escriba P, Busquets X, Mallet J, Miralles CP, Filla M, De Blas AL. Antibodies to the human γ_2 subunit of the γ-aminobutyric $acid_A$/benzodiazepine receptor. J. Neurochem 1993;60:961–971. [PubMed: 8382267]

Kumar S, Kralic JE, O'Buckley TK, Grobin AC, Morrow AL. Chronic ethanol consumption enhances internalization of α1 subunit-containing $GABA_A$ receptors in cerebral cortex. J. Neurochem 2003;86:700–708. [PubMed: 12859683]

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–685. [PubMed: 5432063]

Lei Q, Jones MB, Talley EM, Schrier AD, McIntire WE, Garrison JC, Bayliss DA. Activation and inhibition of G protein-coupled inwardly rectifying potassium (kir3) channels by G protein beta gamma subunits. Proc. Natl. Acad. Sci. U.S.A 2000;97:9771–9776. [PubMed: 10944236]

Lewohl JM, Wilson WR, Mayfield RD, Brozowski SJ, Morrisett RA, Harris RA. G-protein-coupled inwardly rectifying potassium channels are targets of alcohol action. Nat. Neurosci 1999;2:1084–1090. [PubMed: 10570485]

Lovinger DM. $5\text{-}HT_3$ receptor and the neural actions of alcohols: an increasingly exciting topic. Neurochem. Int 1999;35:125–130. [PubMed: 10405996]

Lovinger DM, White G. Ethanol potentiation of 5-hydroxytryptamine$_3$ receptor-mediated ion current in neuroblastoma cells and isolated adult mammalian neurons. Mol. Pharmacol 1991;40:263–270. [PubMed: 1715016]

Lovinger DM, White G, Weight FF. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science 1989;243:1721–1724. [PubMed: 2467382]

Majchrowicz E. Induction of physical dependence upon ethanol and the associated behavioral changes in rats. Psychopharmacology 1975;43:245–254.

Marutha Ravindran CR, Ticku MK. Changes in methylation pattern of NMDA receptor NR2B gene in cortical neurons after chronic ethanol treatment in mice. Mol. Brain Res 2004;121:19–27. [PubMed: 14969733]

Marutha Ravindran CR, Ticku MK. Tyrosine kinase phosphorylation of GABA$_A$ receptor subunits following chronic ethanol exposure of cultured cortical neurons of mice. Brain Res 2006a;1086:35–41. [PubMed: 16580644]

Marutha Ravindran CR, Ticku MK. Tyrosine kinase phosphorylation of GABA$_A$ receptor α_1, β_2, and γ_2 subunits following chronic intermittent ethanol (CIE) exposure of cultured cortical neurons of mice. Neurochem. Res 2006b;31:1111–1118.

Marutha Ravindran CR, Mehta AK, Ticku MK. Effect of chronic administration of ethanol on the regulation of tyrosine kinase phosphorylation of the GABA$_A$ receptor subunits in the rat brain. Neurochem. Res 2007;32:1179–1187. [PubMed: 17404840]

McKernan RM, Whiting PJ. Which GABA$_A$-receptor subtypes really occur in the brain? Trends Neurosci 1996;19:139–143. [PubMed: 8658597]

Mehta AK, Ticku MK. An update on GABA$_A$ receptors. Brain Res. Rev 1999a;29:196–217. [PubMed: 10209232]

Mehta AK, Ticku MK. Prevalence of the GABA$_A$ receptor assemblies containing α_1-subunit in the rat cerebellum and cerebral cortex as determined by immunoprecipitation: lack of modulation by chronic ethanol administration. Mol. Brain Res 1999b;67:194–199. [PubMed: 10101248]

Mehta AK, Ticku MK. Effect of chronic administration of ethanol on GABA$_A$ receptor assemblies derived from α_2-, α_3-, β_2- and γ_2-subunits in the rat cerebral cortex. Brain Res 2005;1031:134–137. [PubMed: 15621022]

Mehta AK, Marutha Ravindran CR, Ticku MK. Low concentrations of ethanol do not affect radioligand binding to the δ-subunit-containing GABA$_A$ receptors in the rat brain. Brain Res 2007;1165:15–20. [PubMed: 17662260]

Mhatre MC, Mehta AK, Ticku MK. Chronic ethanol administration increases the binding of the benzodiazepine inverse agonist and alcohol antagonist [^{3}H]Ro 15-4513 in rat brain. Eur. J. Pharmacol 1988;153:141–145. [PubMed: 2850926]

Mhatre MC, Ticku MK. Chronic ethanol administration alters γ-aminobutyric acid$_A$ receptor gene expression. Mol. Pharmacol 1992;42:415–422. [PubMed: 1383684]

Mhatre MC, Ticku MK. Chronic ethanol treatment upregulates the GABA receptor β subunit expression. Mol. Brain Res 1994;23:246–252. [PubMed: 8057781]

Mihalek RM, Bowers BJ, Wehner JM, Kralic JE, VanDoren MJ, Morrow AL, Homanics GE. GABA$_A$-receptor δ subunit knockout mice have multiple defects in behavioral responses to ethanol. Alcohol. Clin. Exp. Res 2001;25:1708–1718. [PubMed: 11781502]

Mihic SJ. Acute effects of ethanol on GABA$_A$ and glycine receptor function. Neurochem. Int 1999;35:115–123. [PubMed: 10405995]

Montpied P, Morrow AL, Karanian JW, Ginns EI, Martin BM, Paul SM. Prolonged ethanol inhalation decreases γ-aminobutyric acid$_A$ receptor α subunit mRNAs in the rat cerebral cortex. Mol. Pharmacol 1991;39:157–163. [PubMed: 1705000]

Narahashi T, Aistrup GL, Marszalec W, Nagata K. Neuronal nicotinic acetylcholine receptors: a new target site of ethanol. Neurochem. Int 1999;35:131–141. [PubMed: 10405997]

Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR. Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. Proc. Natl. Acad. Sci. U.S.A 2003;100:2053–2058. [PubMed: 12566570]

Sanna E, Mostallino MC, Busonero F, Talani G, Tranquilli S, Mameli M, Spiga S, Follesa P, Biggio G. Changes in GABA$_A$ receptor gene expression associated with selective alterations in receptor

function and pharmacology after ethanol withdrawal. J. Neurosci 2003;23:11711–11724. [PubMed: 14684873]

Sieghart W, Fuchs K, Tretter V, Ebert V, Jechlinger M, Hoger H, Adamiker D. Structure and subunit composition of GABA(A) receptors. Neurochem. Int 1999;34:379–385. [PubMed: 10397365]

Sundstrom-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A, Wiedmann M, Williams K, Smith SS. Hormonally regulated $\alpha_4\beta_2\delta$ $GABA_A$ receptors are a target for alcohol. Nat. Neurosci 2002;5:721–722. [PubMed: 12118257]

Ticku, MK.; Mehta, AK. Effects of alcohol on GABA-mediated neurotransmission. In: Kranzler, HK., editor. Handbook of Experimental Pharmacology: The pharmacology of Alcohol Abuse. Vol. 114. Heidelberg: Springer-Verlag; 1995. p. 103-119.

Wallner M, Hanchar HJ, Olsen RW. Ethanol enhances $\alpha_4\beta_3\delta$ and $\alpha_6\beta_3\delta$ γ-aminobutyric acid type A receptors at low concentrations known to affect humans. Proc. Natl. Acad. Sci. U.S.A 2003;100:15218–15223. [PubMed: 14625373]

Walter HJ, Messing RO. Regulation of neuronal voltage-gated calcium channels by ethanol. Neurochem. Int 1999;35:95–101. [PubMed: 10405992]

Wei W, Faria LC, Mody I. Low ethanol concentrations selectively augment the tonic inhibition mediated by δ subunit-containing $GABA_A$ receptors in hippocampal neurons. J. Neurosci 2004;24:8379–8382. [PubMed: 15385620]

Weight FF, Li C, Peoples RW. Alcohol action on membrane ion channels gated by extracellular ATP (P2X receptors). Neurochem. Int 1999;35:143–152. [PubMed: 10405998]

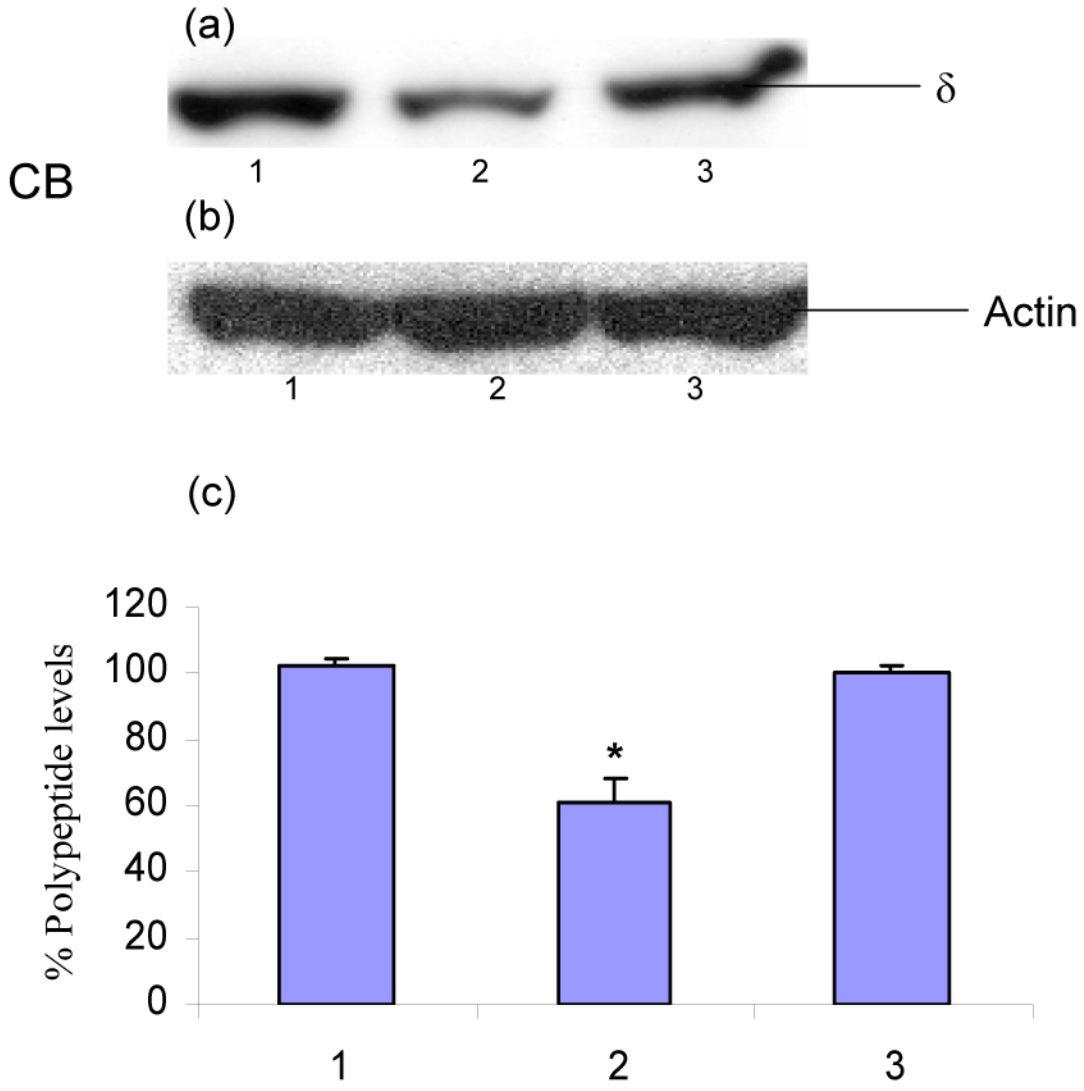
Whiting PJ. The GABA-A receptor gene family: new targets for therapeutic intervention. Neurochem. Int 1999;34:387–390. [PubMed: 10397366]

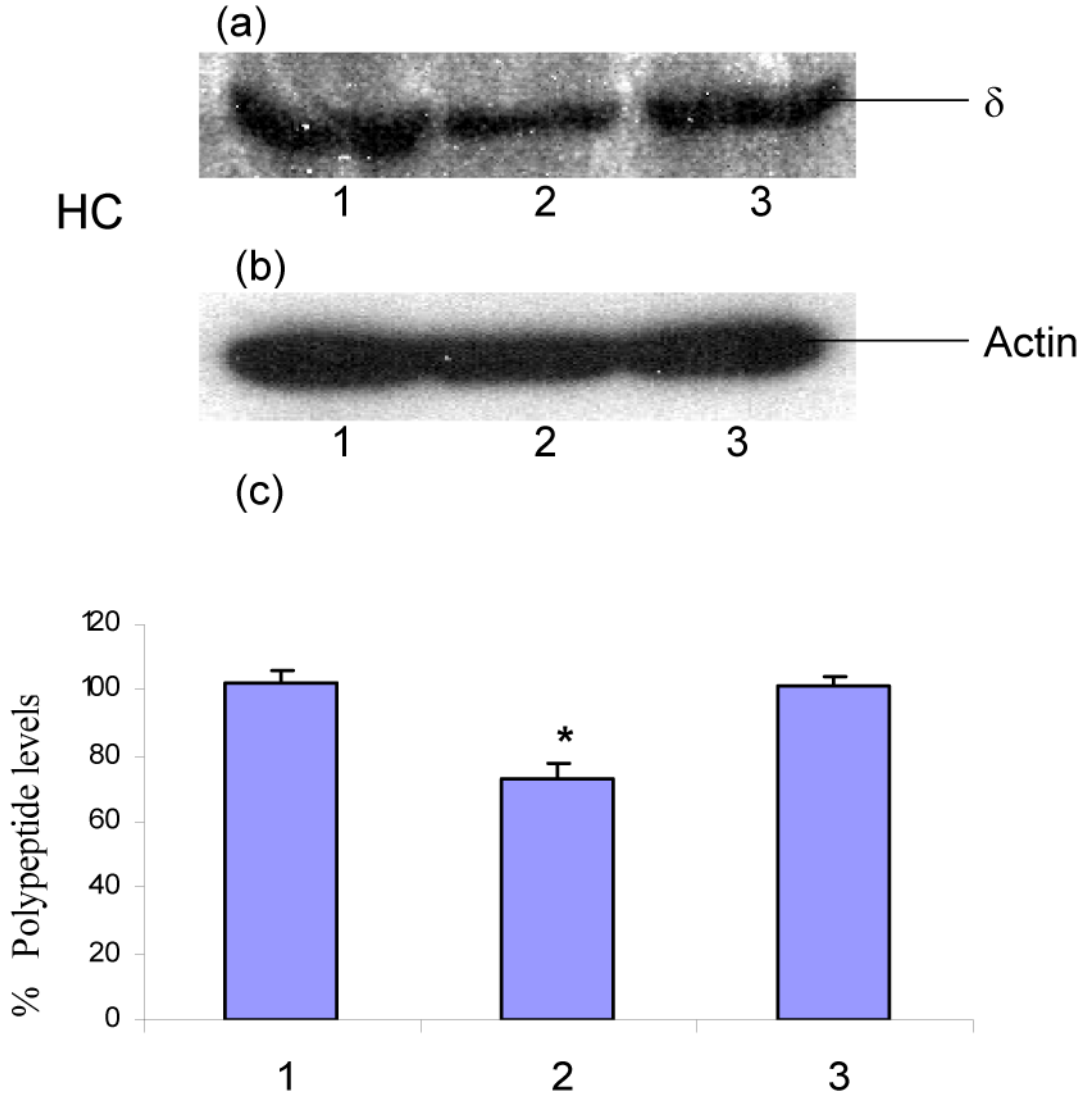
Woodward JJ. Ionotropic glutamate receptors as sites of action for ethanol in the brain. Neurochem. Int 1999;35:107–113. [PubMed: 10405994]

Yamashita M, Marszalec W, Yeh JZ, Narahashi T. Effects of ethanol on tonic GABA currents in cerebellar granule cells and mammalian cells recombinantly expressing $GABA_A$ receptors. J. Pharmacol. Exp. Ther 2006;319:431–438. [PubMed: 16844844]

Abbreviations

CE, chronic ethanol; CIE, chronic intermittent ethanol; CB, cerebellum; HC, hippocampus; CC, cerebral cortex.





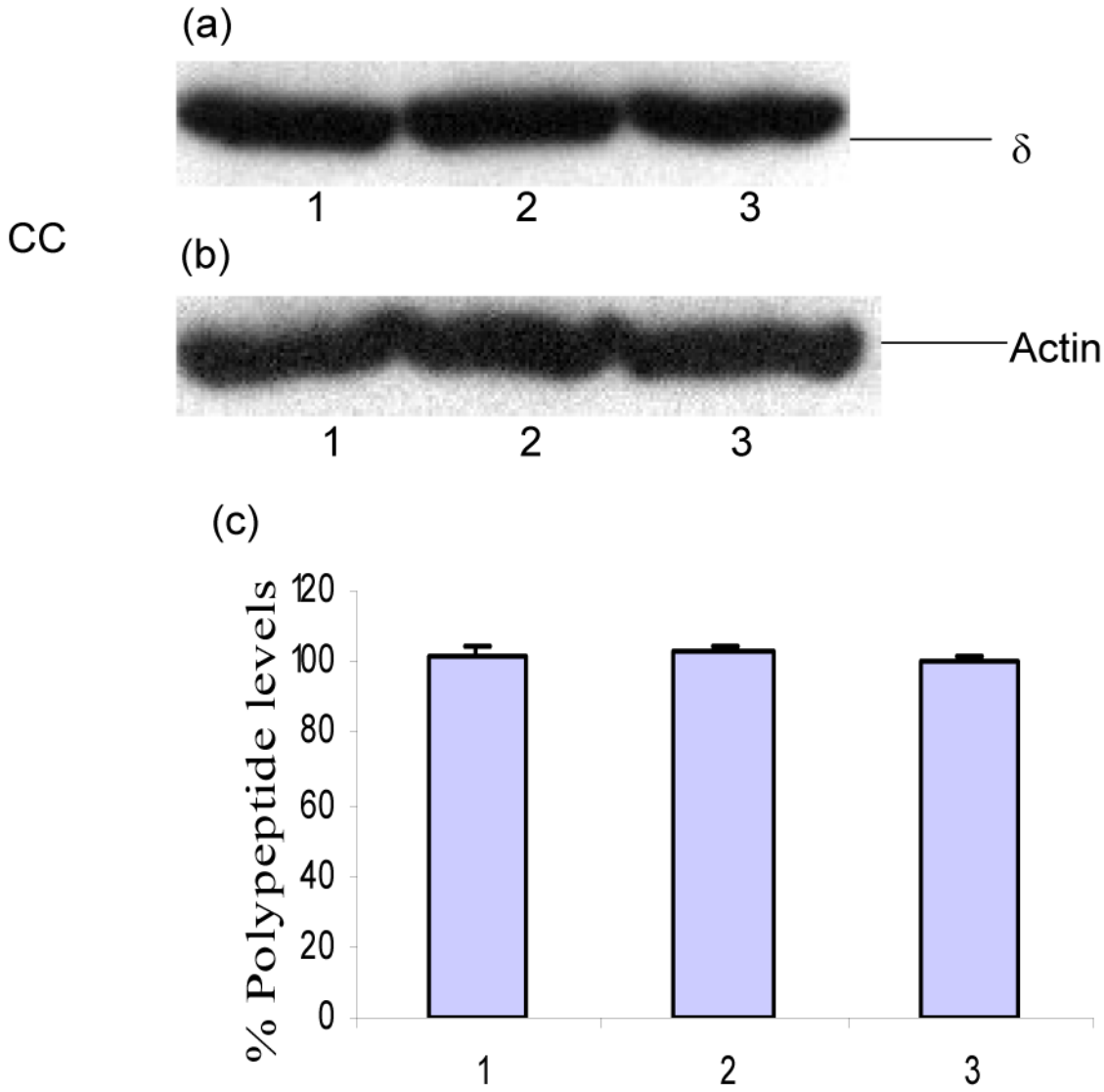


Fig. 1.

Effect of chronic administration of ethanol and its withdrawal on the polypeptide levels of the δ-subunit of $GABA_A$ receptors in the rat (A) cerebellum, i.e., CB, (B) hippocampus, i.e., HC, and (C) cerebral cortex, i.e., CC. Panel (a): Western blot for the $GABA_A$ receptors δ-subunit polypeptide; panel (b): reprobed with actin; and panel (c): percentage polypeptide levels of the δ-subunit of $GABA_A$ receptors. Molecular mass of the δ-subunit of $GABA_A$ receptors was found to be 54 kDa in Western blot analysis. Each value is % mean ± S.E.M derived from three different Western blots performed on different days using pooled tissue from 10 rats in each group. % Polypeptide levels were calculated by comparing the intensity of each band with the average control intensity (100%) from three different Western blots as detailed in materials and methods. Lane 1: proteins extracted from control tissues (saline-administered rats); lane 2: proteins extracted from tissues harvested from chronic ethanol-administered rats; lane 3: proteins extracted from tissues harvested from ethanol-withdrawn rats (48 h).

*$p < 0.01$ as compared to the control group by one-way analysis of variance (ANOVA) followed by Dunnett test.

Table 1

Effect of chronic administration of ethanol on the quantitative immunoprecipitation of the δ-subunit-containing $GABA_A$ receptor assemblies in the rat cerebellum.

Group	% Immunoprecipitation of the binding activity
[³H]Muscimol (36 nM)	
Control	18.0 ± 1.1
Chronic ethanol	11.1 ± 0.7*
Ethanol withdrawn (48 h)	17.6 ± 0.8

The values are mean ± S.E.M. of six individual experiments, each performed in triplicate using the pooled cerebellum from ten rats in each group. Immunoprecipitations were done using 30 μl of the antiserum specific for the rat δ-subunit of $GABA_A$ receptors. [³H]Muscimol (36 nM) binding (100%) to the solubilized receptors (pellet+supernatant) of the rat cerebellum was 0.28 ± 0.02 pmol/mg protein.

* $p < 0.01$ as compared to the control group by one-way analysis of variance (ANOVA) followed by Dunnett test.