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Acute and Chronic Ethanol Treatments Alter GABA Receptor-Operated Chloride Channels[1]

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ALLAN, A. M. AND R. A. HARRIS. *Acute and chronic ethanol treatments alter GABA receptor-operated chloride channels.* PHARMACOL BIOCHEM BEHAV 27(4)665–670, 1987.—The effect of ethanol exposure in vitro on the GABA receptor-operated chloride channel was evaluated by monitoring $^{36}Cl^-$ influx in a membrane vesicle suspension (microsacs) prepared from mouse cerebellum. These experiments directly demonstrate ethanol augmentation of muscimol-stimulated chloride flux. DBA/2J mice were made tolerant to and dependent on ethanol by administration of an ethanol containing liquid diet for 7 days. Exposure to physiologically relevant concentrations of ethanol (10–45 mM) in vitro potentiated muscimol stimulation of $^{36}Cl^-$ uptake in control (pair-fed) membranes, but had no effect on cerebellar microsacs from tolerant/dependent mice. Muscimol stimulation of $^{36}Cl^-$ uptake was not different for pair-fed and ethanol-treated mice. Augmentation of muscimol-induced $^{36}Cl^-$ flux by in vitro ethanol was abolished by a single 4 g/kg injection of ethanol. This "acute tolerance" occurred within 5 min and disappeared within 24 hr after ethanol treatment. The reduced sensitivity of ethanol treated (chronic and acute) mice to ethanol potentiation of muscimol stimulated $^{36}Cl^-$ uptake offers a biochemical correlate to the phenomenon of ethanol tolerance. Moreover, the findings suggest that this biochemical tolerance develops rapidly following a single hypnotic dose of ethanol.

Acute and chronic ethanol GABA Muscimol Mouse cerebellum Chloride channel

THE molecular mechanisms responsible for the actions of ethanol are still unknown, but there is considerable evidence that acute exposure to ethanol enhances the opening of the $GABA_A$ receptor-operated chloride channels [1, 2, 21, 28, 37]. In addition, a number of researchers have examined the role of the GABAergic neurotransmitter system in the development of ethanol tolerance and dependence [3, 5, 9, 11, 19, 22, 29, 32, 36]. In most paradigms, the behavioral effects of GABA agonists are attenuated in ethanol tolerant mice [19,32]. A number of studies show that ethanol withdrawal symptoms are diminished by treatments that increase GABA transmission and exacerbated by treatments that decrease GABA activity [4, 8, 9, 11].

There are surprisingly few electrophysiological studies on the effects of ethanol tolerance and dependence on the GABA neurotransmitter system. Tolerance has been reported to ethanol-induced decreases in striatum radiatum evoked CA1 field potentials of hippocampus [3]. The authors later suggested this effect of ethanol may be mediated by GABA [6]. GABA turnover rates, measured by gabaculine-induced accumulation of GABA, decreased in seven different brain regions following chronic ethanol treatment. In one region, the inferior colliculus, GABA turnover increased by 100% during chronic ethanol exposure and remained high during withdrawal with the other regions returning to normal [29]. It is interesting that this region was identified as a major locus for the initiation of audiogenic seizures in ethanol withdrawn rats and that adaptation to GABAergic inhibitory mechanisms are involved in the production of these seizures [10].

Radioligand binding studies have also detected changes following chronic exposure to ethanol. A decrease in the density of low affinity GABA binding sites was observed in ethanol-tolerant animals [36,40]. In contrast, Unwin and Taberner [39] found an increase in the affinity, but no change in the density of low affinity GABA binding sites in mice chronically treated with ethanol. The development of ethanol tolerance has been reported to have no effect on the inhibition of [^{35}S]t-butylbicyclophosphorothionate (TBPS) (convulsant ligand) binding by either ethanol, GABA or pentobarbital [16].

In general, these studies lend support to the hypothesis that ethanol tolerance results from a compensatory decrease in the sensitivity of the GABA system as a consequence of chronic enhancement of activity by ethanol, and withdrawal symptoms result from unmasking this decrease in GABA

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activity by the removal of ethanol. Techniques are now available to directly monitor functional changes in $GABA_A$ receptor-operated chloride channel [12, 26, 28, 34, 38, 42], and recently it has been reported that low concentrations (10–60 mM) of ethanol increase chloride influx in vitro [1, 2, 30, 31, 37]. Thus far, however, no measures of the effect of ethanol tolerance on GABA mediated $^{36}Cl^-$ have been made. The present experiments were designed to examine the possible functional changes in the mouse cerebellar GABA receptor chloride inonophore following both chronic and acute in vivo ethanol treatments.

METHOD

Drugs

Muscimol HCl was purchased from Research Biochemicals (Wayland, MA), absolute ethanol from Midwest Solvents Company (Pekin, IL), and $^{36}Cl^-$ from ICN (Irvine, CA).

Chronic Ethanol Treatment

Adult (60–90 days) male DBA/2J mice were purchased from Jackson Laboratories (Bar Harbor, ME) and weighed 25–30 g at the beginning of the experiments. Mice were housed singly and given free access to tap water at all times. Mice were chronically fed ethanol for 7 days according to the the method of Harris *et al.* [13]. Briefly, mice were given Slender liquid diet (chocolate flavor, Carnation Company, Los Angeles, CA) ad lib for 3 days. Following this 3 day consumption, a diet containing 88% (v/v) Slender, 5% (v/v) ethanol and 7% (v/v) water was given for 7 days. The control group was pair-fed an equicaloric diet with sucrose substituted for ethanol. This diet regimen has been fully characterized for tolerance and dependence development [13].

Acute Ethanol Treatment

Adult (60–90 days) male DBA/2J mice (Jackson Laboratories, Portland, ME) were injected intraperitoneally with a single 4.0 g/kg dose of ethanol at a volume of 0.02 ml/g body weight. Animals were checked for the loss of the righting reflex and returned to their home cage. Mice were decaptitated and the cerebella removed at either 5 min, 60 min or 24 hr after injection. In another experiment, mice received a single 2 g/kg dose of ethanol (IP) and were decaptiated 30 min following the injection. Control mice were given an equivalent volume of physiologic saline, IP, decaptiated and the cerebella removed at either 5 min, 30 min, 60 min or 24 hr following saline administration.

Membrane Preparation

The cerebellum including both flocculi was dissected and retained. The cerebella from 4 mice were pooled to form one cerebellar membrane suspension. Cerebella from control and ethanol-treated mice were collected and assayed at the same time in a paired fashion.

The procedure for preparation of membrane vesicles (microsacs) was described by Harris and Allan [12]. Cerebella were homogenized by hand (10–12 strokes) in 4.5 ml of ice cold assay buffer [145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM D-Glucose, 1 mM $CaCl_2$ and 10 mM 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid adjusted to pH 7.5 with Tris base], using a glass teflon homogenizer (Thomas, size C). The homogenate was centrifuged at 2800 rpm (900 gav) for 15 minutes, using a Sorvall SA600 rotor. The supernatant was decanted and the pellet resuspended in 8 ml of assay buffer and centrifuged at 2800 rpm (900 gav) for 15 min. The final pellet was suspended in 7 ml of assay buffer, yielding a preparation containing 6–7 mg protein per ml of suspension. Protein content was determined by the method of Lowry *et al.* [18].

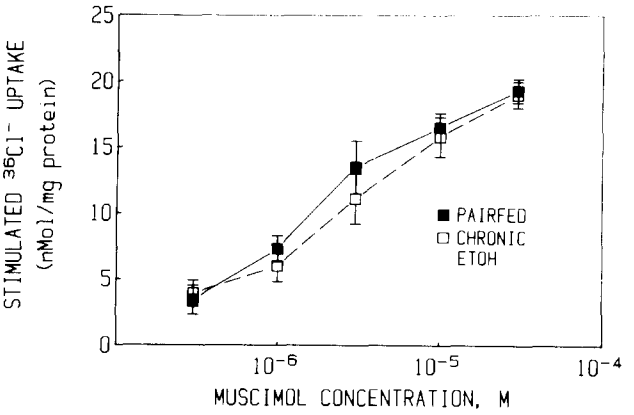

FIG. 1. Effect of muscimol on $^{36}Cl^-$ uptake in cerebellar membranes from chronic ethanol (open squares) and pair-fed control (filled squares) mice. The ordinate represents $^{36}Cl^-$ influx in nmol/mg protein/3 sec. The abcissa represents muscimol concentration in M. Each point represents mean±SEM, n=8. A significant effect of muscimol dose, $F(4,24)=52.4$, $p<0.0001$, was observed.

Procedure for $^{36}Cl^-$ Uptake

Aliquots (200 μl) of membranes were incubated in a shaking water bath at 30°C for a total of 5 min. Following this incubation, uptake was initiated by the addition and immediate vortexing of 200 μl of a solution containing $^{36}Cl^-$ (0.2 μCi/ml of assay buffer). Drugs (muscimol or ethanol) were added only in the $^{36}Cl^-$ solution. Three seconds following the addition of $^{36}Cl^-$, influx was terminated by the addition of 4 ml of ice cold assay buffer and rapid filtration under vacuum (10 in. Hg) onto a 2.4 cm Whatman GF/C glass microfiber filter, using a Hoefer manifold (Hoefer Scientific, San Francisco, CA). The filters were washed with an additional 8 ml of cold assay buffer with the manifold towers removed. The amount of radioactivity on the filters was determined by liquid scintillation spectrometry. The amount of $^{36}Cl^-$ bound to the filters in the absence of membranes (no-tissue blank) was subtracted from all values. Muscimol-dependent or stimulated uptake was defined as the amount of $^{36}Cl^-$ taken up while agonist was present in the medium (total uptake) minus the amount of chloride taken up when agonist was not present (muscimol-independent or nonspecific uptake).

Membranes from control and ethanol-treated mice were prepared on the same day and exposed to the same experimental conditions. Data was analyzed by analysis of variance (ANOVA) and post hoc Tukey A tests where required.

RESULTS

Muscimol-stimulated $^{36}Cl^-$ uptake was examined in ethanol tolerant-dependent and pair-fed control mice. Although chronic ethanol treatment slightly reduced $^{36}Cl^-$ uptake relative to controls, this small difference was not statistically significant (Fig. 1). Maximal levels of uptake and the approximate EC50 were similar in the ethanol and saline-

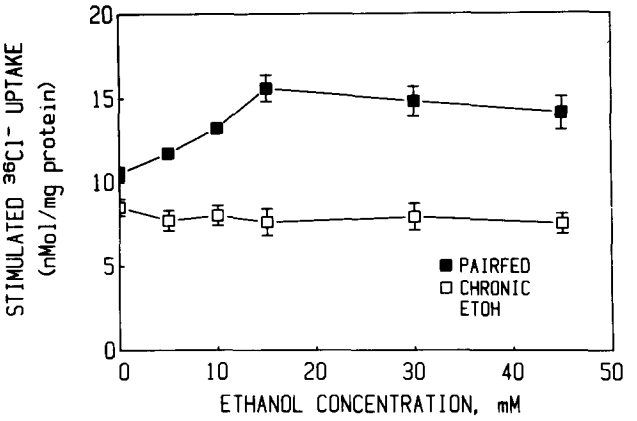

FIG. 2. Effect of in vitro ethanol on muscimol-stimulated $^{36}Cl^-$ uptake. Cerebellar microsacs were prepared from chronic ethanol (open squares) and pair-fed control (filled squares) mice. The ordinate represents $^{36}Cl^-$ influx in nmol/mg protein/3 sec. The concentration of muscimol was 2 μM. The abcissa represents the concentration of ethanol in mM. Each point represents mean±SEM, n=8. A significant effect of in vivo ethanol treatment, $F(1,5)=63.1$, $p<0.0005$, and a significant ethanol concentration × in vivo treatment interaction, $F(5,50)=2.81$, $p<0.02$, was observed.

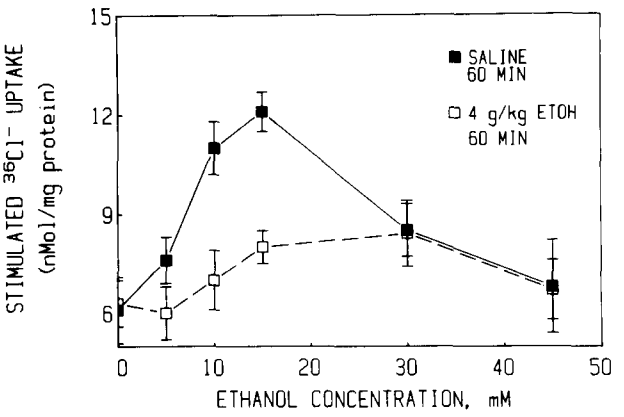

FIG. 3. Effect of in vitro ethanol on muscimol-stimulated $^{36}Cl^-$ uptake 60 min following an injection of 4 g/kg ethanol (open squares) or saline (closed squares) in vivo. The ordinate represents $^{36}Cl^-$ influx in nmol/mg protein/3 sec. The concentration of muscimol was 1 μM. The abcissa represents the concentration of ethanol in mM. Each point represents mean±SEM, n=8. A significant effect of in vivo ethanol treatment, $F(1,5)=32.4$, $p<0.005$, and a significant ethanol concentration × in vivo treatment interaction, $F(5,50)=3.2$, $p<0.01$, was observed.

treated mice. Thus, chronic ethanol treatment did not alter the ability of muscimol to activate the chloride channel. Although it cannot be directly determined from these data, they suggest that chronic ethanol does not affect either affinity or number of GABA-receptors coupled to chloride channels. The uptake of $^{36}Cl^-$ in the absence of muscimol (nonspecific uptake) was not different between the ethanol and control mice (data not shown).

Ethanol augmented muscimol-stimulated $^{36}Cl^-$ uptake in pair-fed cerebella but not in the ethanol tolerant-dependent cerebella (Fig. 2). Concentrations of ethanol ranging from 10–45 mM produced significant increase in the amount of $^{36}Cl^-$ uptake produced by 2 μM muscimol in the pair-fed

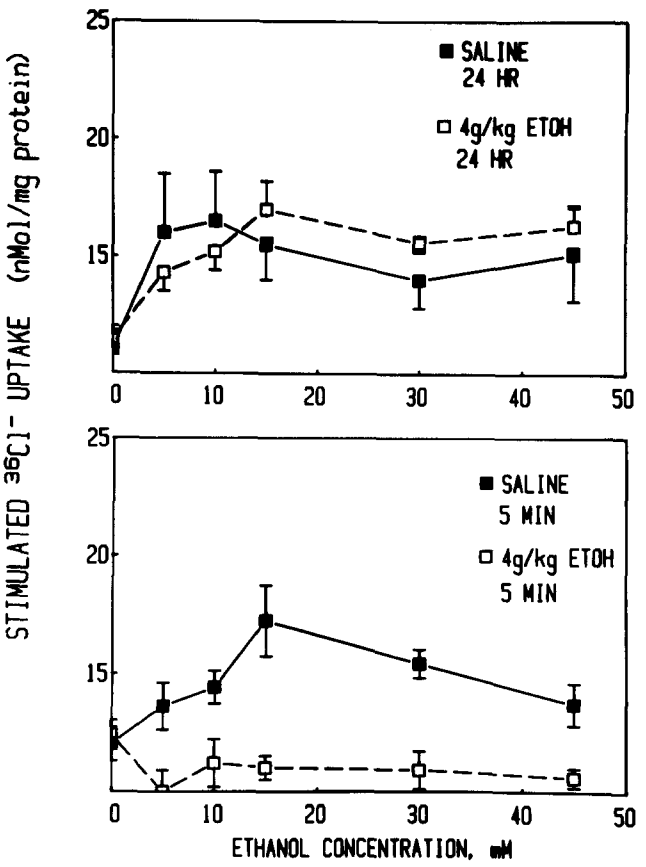

FIG. 4. Effects of in vitro ethanol on muscimol-stimulated $^{36}Cl^-$ uptake 24 hr (upper panel) and 5 min (lower panel) following injection of 4 g/kg ethanol (open squares) or saline (closed squares) in vivo. The ordinate represents $^{36}Cl^-$ influx in nmol/mg protein/3 sec. The concentration of muscimol was 2 μM. The abcissa represents the concentration of ethanol in mM. Each point represents mean±SEM, n=8. A significant effect of ethanol concentration, $F(1,5)=45.2$, $p<0.001$ and $F(1,5)=37.4$, $p<0.001$, was observed following 24 hr and 5 min respectively. A significant ethanol concentration × in vivo treatment interaction, $F(5,50)=3.02$, $p<0.02$, was observed between the ethanol and saline 5 min treatment (lower panel).

(control) cerebella. None of the ethanol concentrations tested produced a significant change in $^{36}Cl^-$ flux in the cerebella from ethanol tolerant-dependent mice. Thus, chronic ethanol exposure virtually eliminated the ability of ethanol to potentiate muscimol-induced $^{36}Cl^-$ flux, resulting in a type of biochemical tolerance. Ethanol did not alter the uptake of $^{36}Cl^-$ in the absence of muscimol (nonspecific uptake) in either treatment condition (data not shown).

The tolerance to in vitro ethanol augmentation of muscimol-stimulated $^{36}Cl^-$ influx was also obtained with cerebellar microsacs prepared 1 hr following a single acute hypnotic dose (4 g/kg) of ethanol (Fig. 3). As with the pair-fed controls in Fig. 2, microsacs from saline treated control mice showed an increase in stimulated chloride flux in response to in vitro addition of ethanol. The acute ethanol treatment, however, produced membranes resistant to the stimulating effects of in vitro ethanol on muscimol-induced $^{36}Cl^-$ flux.

To more fully characterize the development of this rapid form of ethanol tolerance (acute tolerance), we tested the ability of ethanol to potentiate muscimol-induced chloride influx in cerebellar microsacs prepared 5 min (loss of the

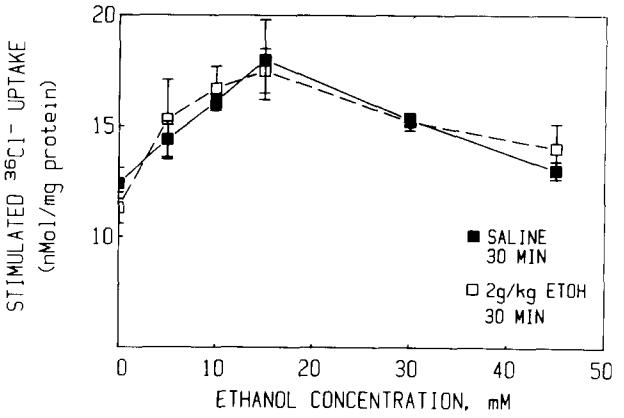

FIG. 5. Effect of in vitro ethanol on muscimol-stimulated $^{36}Cl^-$ uptake 30 min following injection of 2 g/kg ethanol (open squares) or saline (closed squares) in vivo. The ordinate represents $^{36}Cl^-$ influx in nmol/mg protein/3 sec. The concentration of muscimol was 2 μM. The abcissa represents the concentration of ethanol in mM. Each point represents mean±SEM, n=8. A significant effect of ethanol concentration, $F(1,5)=65.7$, $p<0.001$, was observed.

righting reflex) and 24 hr after injection of 4 g/kg ethanol (Fig. 4 lower and upper panel, respectively). Ethanol addition in vitro failed to augment stimulated $^{36}Cl^-$ flux in membranes from animals treated with ethanol for only 5 min. An increase in muscimol-stimulated $^{36}Cl^-$ influx was obtained in membranes prepared 24 hr following in vivo ethanol treatment, with ethanol-treated mice responding similarly to the saline controls.

In contrast to the effects of a high dose of ethanol, following injection of a low dose of ethanol (2 g/kg), cerebellar microsacs responded like control membranes to in vitro ethanol augmentation of muscimol-stimulated $^{36}Cl^-$ influx (Fig. 5). Unlike the 4 g/kg dose of ethanol, the 2 g/kg ethanol dose did not produce righting reflex loss (hypnosis) in these mice and in agreement with other studies [27] some locomotor stimulation was observed.

DISCUSSION

The present findings indicate a role for the $GABA_A$ receptor-operated chloride channel in the development of ethanol tolerance. Brain membrane vesicles (microsacs), prepared from mice chronically treated with ethanol, were resistant to in vitro ethanol potentiation of muscimol $^{36}Cl^-$ flux. This resistance to ethanol augmentation of chloride flux represents a form of functional (cellular) tolerance. Chronic ethanol-treated membranes differed from pair-fed control membranes only in their response to ethanol. Thus, it is unlikely that in vivo ethanol exposure acts directly at the level of the $GABA_A$ receptor to alter GABA-mediated chloride flux. As mentioned in the introduction, ethanol tolerance and dependence are hypothesized to result from a decrease in GABAergic sensitivity. The present findings do not support this contention because the potency and efficacy of muscimol were not altered by ethanol administration. However, our data only measured cerebellar GABA functioning, and it is possible that GABA receptor subsensitivity could develop in other brain regions. This lack of an ethanol tolerance-induced change in GABA receptor functioning is not with precedence. Although in vitro exposure to ethanol inhibits [^{35}S]t-bicyclophosphorothionate binding, no changes in binding were found following chronic ethanol exposure [24,35]. In general, chronic ethanol treatments do not markedly or consistently alter benzodiazepine binding [17, 24, 25, 41]. Recently, however, Tamorska and Marangos [33] found chronic ethanol produced a substantial increase in "peripherial benzodiazepine" binding, but this site is not related to GABA receptor chloride channel complex. Thus, there is no compelling evidence to support a direct effect of chronic ethanol treatment on the known receptor sites on the GABA receptor chloride complex. It is possible that ethanol either alters the coupling of the chloride channel to the complex or affects the channel directly.

The functional tolerance obtained in membranes from mice treated with ethanol developed quite rapidly. In vitro ethanol failed to augment muscimol-stimulated $^{36}Cl^-$ flux in microsacs prepared from mice killed 5 min following a single 4 g/kg dose of ethanol. This is indicative of acute alcohol tolerance. Tolerance that develops within the treatment session was first hypothesized by Mellanby in 1919 [20]. Since then, acute tolerance has been demonstrated to a number of alcohol related effects [14]. Most of the research on acute ethanol tolerance has used behavioral performance as a measure of tolerance development and describes the degree of impairment by ethanol is greater when measurements are made during the ascending portion of the blood alcohol curve compared to the same blood concentration on the descending portion of the curve. However, rapid forms of cellular tolerance to ethanol, not related to peripherial absorption and distribution factors, occur in CA1 hippocampal neurons [6] and in mouse erythrocytes [23]. The rapidly developing tolerance in the present study is different from the acute tolerance described by Mellanby [20] since we detected tolerance during the ascending portion of the blood alcohol curve (within 5 min post-injection). Tolerance also appeared to depend on high concentrations of ethanol since a subhypnotic (2 g/kg) dose failed to affect the ability of in vitro ethanol to increase chloride influx. The tolerance was also reversible because ethanol increased muscimol-induced $^{36}Cl^-$ influx in microsacs prepared from mice treated 24 hr earlier with ethanol.

The acute or chronic in vivo alcohol treatments produced essentially complete tolerance to the effects of in vitro ethanol. This is similar to genetic differences in muscimol-stimulated chloride flux since membranes from Short-Sleep mice were found to be completely resistant to effects of ethanol on chloride flux [2]. The complete resistance is somewhat surprising because chronic alcohol treatment as well as genetic selection results in only about a two-fold shift in the ethanol dose-response curve in vivo [7,13]. One possible explanation for this discrepancy is that augmentation of GABA actions is only one of many ways by which ethanol produces loss of righting relfex. Thus, complete tolerance to this one neurochemical action of ethanol will not result in complete resistance to the behavioral effects of ethanol. Another consideration is that our studies of chloride flux examined only cerebellar membranes. It is possible that complete tolerance to effects of ethanol on chloride flux would not be manifested in other brain regions. This hypothesis is supported by brain regional studies of the Long Sleep and Short Sleep mice [2].

Although ethanol (5–15 mM) reliably agumented muscimol-stimulated chloride flux, the shape of the ethanol concentration-response curve varied across experiments. This variation in no way precludes the interpretation of the findings since control and experimental membranes were al-

ways tested in a paired fashion. However, it does suggest that the effect of ethanol on muscimol-mediated chloride flux is sensitive to day-to-day variation in the procedure.

The DBA inbred mouse strains were chosen for the present studies because they have been fully characterized for ethanol tolerance and dependence development using the liquid diet technique in our laboratory [13]. In general, the advantage of using an inbred strain, such as the DBA/2J mice, is directly related to their genetic uniformity. Individual members of an inbred strain are virtually genetically identical with all other members of the strain. This reduction in heritable sources of variation produced by inbreeding also reduces the ability to generalize the results obtained from them to outbred stocks of mice. Thus, it is possible that these findings are specific to the DBA/2J mouse strain and not characteristic of other lines of mice.

In agreement with present findings, previous work has shown that in vitro ethanol will increase $^{36}Cl^-$ conductance through the $GABA_A$ receptor-operated chloride channel in microsacs [1,2], synaptoneurosomes [28, 30, 31] and cultured spinal neurons [37]. Ethanol facilitation of chloride flux in microsacs, unlike that in synaptoneurosomes and cultured spinal neurons, is dependent on agonist activation of the GABA receptor. Ethanol concentrations of 20 mM and greater in synaptoneurosomes [30] and 50 mM in cultured spinal neurons [37] produce a direct stimulation of $^{36}Cl^-$ influx (without the presence of an agonist). In all the reported studies, however, ethanol was able to augment agonist-stimulated chloride influx. Further, in synaptoneourosomes the direct effect of ethanol on basal chloride flux may be partially due to the presence of endogenous GABA since both picrotoxin and bicuculline inhibited ethanol-induced $^{36}Cl^-$ uptake by at least 50% [31].

In summary, the present findings demonstrate that low concentrations (10–45 mM) of ethanol augmented muscimol-stimulated $^{36}Cl^-$ influx into microsacs and that tolerance developed to this in vitro effect of ethanol following either chronic or acute in vivo treatments with ethanol.

ACKNOWLEDGEMENT

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REFERENCES

1. Allan, A. M. and R. A. Harris. Ethanol and barbiturates enhance GABA-stimulated influx of $^{36}Cl^-$ in isolated brain membranes. *Pharmacologist* **27:** 125, 1985.
2. Allan, A. M. and R. A. Harris. Gamma-aminobutyric acid and alcohol actions: Neurochemical studies. *Life Sci* **39:** 2005–2015, 1986.
3. Carlen, P. L. and W. A. Corrigall. Ethanol tolerance measured electrophysiologically in hippocampal slices and not in neuromuscular junctions from chronically ethanol-fed rats. *Neurosci Lett* **17:** 95–100, 1980.
4. Cooper, B. R., K. Viik, R. M. Ferris and H. L. White. Antagonism of the enhanced suseptibility to audiogenic seizures during alcohol withdrawal in the rat by GABA and GABA-mimetic agents. *J Pharmacol Exp Ther* **209:** 396–403, 1979.
5. Cott, J., A. Carlsson, J. Engel and M. Lindquist. Suppression of ethanol-induced locomotor stimulation by GABA-like drugs. *Naunyn Schmiedebergs Arch Pharmacol* **295:** 203–209, 1976.
6. Durand, D., W. A. Corrigall, P. Kujtan and P. L. Carlen. Effects of low concentrations of ethanol on CA1 hippocampal neurons in vitro. *Can J Physiol Pharmacol* **59:** 979–984, 1981.
7. Erwin, V. G., W. D. Heston, G. E. McClearn and R. A. Deitrich. Effect of hypnotics on mice genetically selected for sensitivity to ethanol. *Pharmacol Biochem Behav* **4:** 679, 1976.
8. Fadda, F., E. Mosca, R. Meloni and G. L. Gessa. Suppression by progabide of ethanol withdrawal syndrome in rats. *Eur J Pharmacol* **109:** 321–325, 1985.
9. Frye, G. D., T. L. McCown and G. R. Breese. Characterization of susceptibility to audiogenic seizures in ethanol-dependent rats after micro-injection of gamma-aminobutyric acid (GABA) agonists into the inferior colliculus, substantia nigra or medial septum. *J Pharmacol Exp Ther* **227:** 663–670, 1982.
10. Frye, G. D., T. J. McCown, G. R. Breese and S. L. Peterson. GABAergic modulation of inferior colliculus excitability: Role in ethanol withdrawal audiogenic seizures. *J Pharmacol Exp Ther* **237:** 478–484, 1986.
11. Goldstein, D. B. Alcohol withdrawal reactions in mice: Effects of drugs that modify neurotransmission. *J Pharmacol Exp Ther* **186:** 1–9, 1973.
12. Harris, R. A. and A. M. Allan. Coupling of GABA receptors to chloride channel in brain membranes. *Science* **228:** 1108–1110, 1985.
13. Harris, R. A., D. M. Baxter, M. A. Mitchell and R. J. Hitzemann. Physical properties and lipid composition of brain membranes from ethanol tolerant-dependent mice. *Mol Pharmacol* **25:** 401–409, 1984.
14. Kalant, H., A. E. LeBlanc and R. J. Gibbins. Tolerance to, and dependence on, some non-opiate psychotropic drugs. *Pharmacol Rev* **23:** 135–191, 1971.
15. Leitch, G. J., D. J. Backes, F. S. Siegman and G. D. Guthrie. Possible role of GABA in the development of tolerance to ethanol. *Experientia* **33:** 496–498, 1977.
16. Liljequist, S., S. Culp and B. Tabakoff. Effect of ethanol on the binding of ^{35}S-t butylbicyclophosphorothionate to mouse brain membranes. *Life Sci* **38:** 1931–1939, 1986.
17. Liljequist, S. and B. Tabakoff. Binding characteristics of ^3H-flunitrazepam and Cl-218,872 in cerebellum and cortex of C57/BL mice made tolerant to and dependent on phenobarbital or ethanol. *Alcohol* **2:** 215–220, 1985.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with folin phenol reagent. *J Biol Chem* **193:** 256–275, 1951.
19. Martz, A., R. A. Deitrich and R. A. Harris. Behavioral evidence for the involvement of gamma-aminobutyric acid in the actions of ethanol. *Eur J Pharmacol* **89:** 53–62, 1983.
20. Mellanby, E. Alcohol: Its absorption into and disappearance from the blood under different conditions. *Med Res Committee Special Rep* **31:** 1–48, 1919.
21. Mereu, G. and G. L. Gessa. Low doses of ethanol inhibit the firing of neurons in the substantia nigra pars reticulata: A GABAergic effect? *Brain Res* **360:** 325–330, 1985.
22. Nordberg, A., G. Wahlstrom and B. Eriksson. Relations between muscimol, quinuclidinyl benzilate and nicotine binding sites in brain after very long treatment with ethanol in rats. *Eur J Pharmacol* **115:** 301–304, 1985.
23. Parsons, L. M., E. J. Gallaher and D. B. Goldstein. Rapidly developing functional tolerance to ethanol is accompanied by increased erythrocyte cholesterol in mice. *J Pharmacol Exp Ther* **223:** 472–476, 1982.
24. Rastogi, S. K., R. Thyagarajan, J. Clothier and M. K. Ticku. Effect of chronic treatment of ethanol on benzodiazepine and picrotoxin sites on the GABA receptor complex in regions of the rat brain. *Neuropharmacology* **25:** 1179–1184, 1986.
25. Rottenberg, H. Alcohol modulation of benzodiazepine receptors. *Alcohol* **2:** 203–207, 1985.
26. Sanchez, C. M., M. C. Toledo and M. P. Gonzalez. The chloride channel opening as an energy dependent process. *Rev Esp Fisiol* **40:** 375–380, 1984.
27. Sanders, B. Sensitivity to low doses of ethanol and pentobarbital in mice selected for sensitivity to hypnotic doses of ethanol. *J Comp Physiol Psychol* **90:** 394–398, 1976.

28. Schwartz, R. D., P. D. Suzdak and S. M. Paul. Gamma-aminobutyric acid (GABA) and barbiturate-mediated $^{36}Cl^-$ uptake in rat brain synaptoneurosomes: Evidence for rapid desensitization of the GABA receptor-coupled chloride ion channel. *Mol Pharmacol* **30:** 419–426, 1986.
29. Simler, S., J. Clement, L. Ciesielski and P. Mandel. Brain gamma-aminobutyric acid turnover rates after spontaneous chronic ethanol intake and withdrawal in discrete brain areas of C57 mice. *J Neurochem* **47:** 1942–1947, 1986.
30. Suzdak, P. D., J. R. Glowa, J. N. Crawley, R. D. Schwartz and S. M. Paul. A selective imidazobenzodiazepine antagonist of ethanol in the rat. *Science* **234:** 1243–1247, 1986.
31. Suzdak, P. D., R. D. Schwartz, P. Skolnick and S. M. Paul. Ethanol stimulates gamma-aminobutyric acid receptor mediated chloride transport in rat brain synaptoneurosomes. *Proc Natl Acad Sci USA* **83:** 4071–4075, 1986.
32. Taberner, P. V. and J. W. Unwin. Behavioral effects of muscimol, amphetamine and chlorpromazine on ethanol tolerant mice. *Br J Pharmacol* **74:** 2761–2762, 1981.
33. Tamborska, E. and P. J. Marangos. Brain benzodiazepine bindings sites in ethanol dependent and withdrawal states. *Life Sci* **38:** 465–472, 1986.
34. Thampy, K. G. and E. M. Barnes. Gamma amino-butyric acid-gated chloride channels in cultured cerebral neurons. *J Biol Chem* **259:** 1753–1757, 1984.
35. Thyagarajan, R. and M. K. Ticku. The effect of in vitro and in vivo ethanol administration on [^{35}S]t-butylbicyclophosphorothionate binding in C57 mice. *Brain Res Bull* **15:** 343–345, 1985.
36. Ticku, M. K. and T. P. Burch. Alterations in gamma-aminobutyric acid receptor sensitivity following acute and chronic ethanol treatments. *J Neurochem* **34:** 417–423, 1980.
37. Ticku, M. K., P. Lowrimore and P. Lehoullier. Ethanol enhances GABA-induced $^{36}Cl^-$ influx in primary spinal cord cultured neurons. *Brain Res Bull* **17:** 123–126, 1986.
38. Ticku, M. K. and R. W. Olsen. Gamma-aminobutyric acid stimulated chloride permeability in crayfish muscle. *Biochim Biophys Acta* **464:** 519–529, 1977.
39. Unwin, J. W. and P. V. Taberner. Sex and strain differences in GABA receptor binding after chronic ethanol drinking in mice. *Neuropharmacology* **19:** 1257–1259, 1980.
40. Volicer, L. and T. M. Biagioni. Effect of ethanol administration and withdrawal on GABA receptor binding in rat cerebral cortex. *Subst Alcohol Actions Misuse* **3:** 31–39, 1982.
41. Volicer, L. and T. M. Biagioni. Effect of ethanol administration and withdrawal on benzodiazepine receptor binding in the rat brain. *Neuropharmacology* **21:** 283–286, 1982.
42. Wong, E. H. F., L. M. F. Leeb-Lundberg. V. I. Teichberg and R. W. Olsen. Gamma-aminobutyric acid activation of $^{36}Cl^-$ flux in rat hippocampal slices and its potentiation by barbiturates. *Brain Res* **303:** 267–275, 1984.