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GABA Mediation of the Central Effects of Acute and Chronic Ethanol in Mice

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DAR, M S. AND W. R. WOOLES *GABA mediation of the central effects of acute and chronic ethanol in mice* PHARMACOL BIOCHEM BEHAV **22**(1) 77–84, 1985.—In acute ethanol studies aminooxyacetic acid (AOAA) alone produced marked hypothermia although a test dose of ethanol was able to produce a further drop in body temperature in AOAA treated mice Even though tolerance to ethanol-induced hypothermia was present in ethanol-dependent mice, AOAA administration was able to produce a further decrease in body temperature. Bicuculline potentiated ethanol-induced hypothermia in the acute studies but the tolerance to hypothermia which had developed in ethanol-dependent mice prevented the bicuculline-induced potentiation of ethanol hypothermia AOAA markedly potentiated acute ethanol-induced motor incoordination whereas bicuculline had no effect Although partial tolerance had developed to ethanol-induced motor incoordination in dependent mice, AOAA potentiated, whereas a lower dose of bicuculline antagonized, motor incoordination In the acute studies ethanol had a biphasic effect on AOAA-induced GABA accumulation in the hypothalamus and corpus striatum low doses prevented and a slightly higher dose was without effect on GABA accumulation Ethanol-dependent mice were unable to respond to an AOAA-induced increase in GABA accumulation although basal levels of GABA were unaffected by chronic ethanol ingestion. The results show that brain GABA or GABA-mediated central mechanisms may be involved in the mediation of ethanol-induced motor incoordination but not hypothermia

Ethanol GABA Motor incoordination Hypothermia Tolerance

THE depressant effect of ethanol on the central nervous system may be due to an ethanol-induced alteration in the concentration or the action of various neurotransmitters [5,9] or to an ethanol-induced alteration in the interaction between various central transmitters [13]. Of the various neurotransmitters known to be influenced by ethanol, the inhibitory transmitter gammaaminobutyric acid (GABA) has been reported to be involved in ethanol-induced alteration of motor function [5,10] and behavioral changes [20].

GABAergic agents have been shown to be involved in ethanol-induced impairment of motor function [12] Frye and Breese [9] have recently shown that manipulation of GABA receptor activity by GABAergic agents leads to modulation of ethanol-induced motor impairment Similarly, it is thought that GABA is involved in the regulation of body temperature,although there is no agreement on this point. A GABA-induced increase in body temperature [4], a decrease [24], or both [7], have been reported

Since ethanol is known to produce motor incoordination and hypothermia, the present studies were designed to evaluate the ability of GABAergic agents to modify the motor incoordination and the hypothermic effect of acute and chronic ethanol administration. We have attempted to correlate modifications of ethanol-induced effects in either parameter with aminooxyacetic acid (AOAA) induced changes in GABA concentration in the hypothalamus and the corpus striatum The hypothalamus was chosen because of its prominent role in the regulation of body temperature. The corpus striatum was chosen because the nigrostriatal pathway, a loop in the corpus striatum, involves dopaminergic, cholinergic and GABAergic neurons. The GABAergic striatonigral pathway is involved in the control of posture and voluntary movements [21]. There is also evidence for dopaminergic involvement in various CNS effects of ethanol such as locomotor stimulation [19], impairment of motor performance [1,17] and loss of the righting reflex [8]. It is reasonable to assume that drugs which effect the corpus striatum should effect the action of ethanol. Others have studied the effect of acute ethanol and GABAergic drugs on parts of the brain more directly involved in motor coordination [5,9].

METHOD

Charles River male mice (22–28 g) of the CD strain were used throughout the study. All mice were bred locally by the Animal Resource Center of the Medical School They were maintained at constant temperature (25°C) and a 12 hr light-dark cycle. In the acute studies mice were allowed food and water ad lib and were housed five to a cage.

Mice used in the chronic ethanol studies were fed a Carnation Slender diet fortified with vitamins to which ethanol was added in an amount to provide 33% of total calories [6]. Control mice received the same diet except that an isocaloric amount of sucrose was added in place of ethanol. Mice on chronic studies were pair-fed and housed in individual cages. They were fed the diet for 10 days and consumed an average daily amount of diet comparable to 22 g /kg of ethanol. All mice on the ethanol diet were studied 24 hours after ethanol was last given.

Aminooxyacetic acid (AOAA), an inhibitor of GABA transaminase activity [23], and bicuculline, a post-synaptic GABA antagonist [21] were used to increase the concentration of GABA and to block the neural pathways activated by GABA Bicuculline (Sigma Chemical Co , St Louis, MO) was dissolved in a minimum volume of 0 1 N HCl, diluted with saline to desired volume and brought to pH 6 6 with 1 N NaOH Bicuculline was administered in the amount of 1 5 and 3 0 mg/kg IP 10 min before ethanol and AOAA was given in the amount of 6 25, 12 5 or 25 mg/kg either 3.5 or 4 hr prior to the administration of ethanol, except in one study it was given 0 5 hr prior to ethanol Ethanol was given in the amount of 1 5, 2 0 or 3 5 g/kg IP as a 10% w/v solution In separate groups of mice, blood ethanol levels were determined at 0 5, 1, 1 5 and 2 hr after 1 5 and 2 g/kg from tail blood samples by the method of Bonnichsen [3]

Hypothermia

Body temperature was determined by telethermometry (YSI Model 42, Yellow Spring Inst , OH) with a thermistor probe inserted 2 cm into the rectum In all hypothermia studies body temperatures were recorded at 0 5, 1 0, 1 5 and 2 hr after the administration of a test dose of 2 g/kg of ethanol given IP In the chronic ethanol studies, the same test dose of ethanol was administered to one half the mice on both the ethanol and isocaloric sucrose control group, and the other half received either bicuculline or AOAA prior to ethanol

Motor Coordination

A subsedative dose of 1 5 g/kg IP of ethanol was used in all motor coordination studies and the degree of motor incoordination was determined using a standard mouse rotorod treadmill (UGO Basile, Verese, Italy) under conditions and times as reported previously [6] The rotorod data is expressed as an activity ratio which is defined as the ratio of the time animals were able to remain on the rotorod after drug and/or ethanol administration compared to pre-drug and/or ethanol values In the acute studies ethanol-induced motor incoordination was evaluated after bicuculline, and AOAA treatment at 15 min intervals for one hour after ethanol In the chronic studies mice fed the ethanol and the isocaloric control diet were divided into groups of 15 mice each and received bicuculline or AOAA followed by ethanol or the saline control solution

GABA Levels

The ability of acute ethanol administration to alter the synthesis of GABA was measured indirectly by determining the accumulation of GABA in the hypothalamus and the corpus striatum, 4 hr following the administration of AOAA and 30 min after the administration of each dose of ethanol This time interval was chosen because ethanol-induced motor incoordination and hypothermia were very pronounced 30 min after ethanol and peak blood levels of ethanol were present 30 min after administration The ethanol doses of 1 5 and 2 g/kg were chosen because these were the doses used in the motor coordination and the hypothermia experiments, respectively. In the chronic ethanol studies, the synthesis of GABA in the hypothalamus and corpus striatum was studied in the same manner 24 hours after the ethanol diet was removed In the motor coordination, hypothermia and GABA accumulation studies AOAA was given 3 5 hr prior to ethanol We chose this time because a pilot study had shown

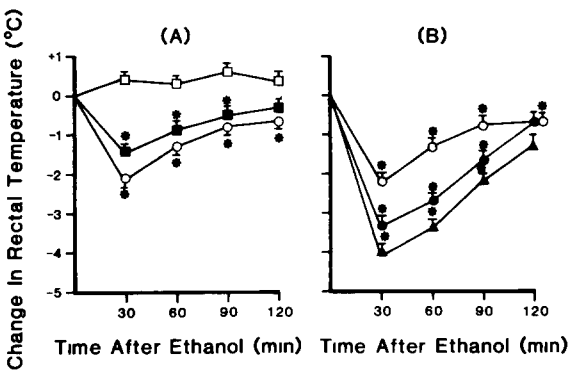

FIG 1 Effect of aminooxyacetic acid (AOAA) side A and bicuculline (Bic) side B on acute ethanol-induced hypothermia (A) ○—○ saline+ethanol, ■—■AOAA (25 mg/kg)+ethanol □—□ AOAA (25 mg/kg)+saline, (B) ○—○ saline+ethanol, ●—● Bic (1 5 mg/kg)+ethanol, ▲—▲ Bic (3 mg/kg)+ethanol Each point represents the mean±S E M of at least 8 mice A test dose of 2 g/kg of ethanol was used *$p<0$ 05

that GABA levels were markedly increased 4 hr following AOAA

In a separate study we evaluated the ability of 1 5, 2 and 3.5 g/kg of ethanol to modify the accumulation of GABA in the hypothalamus and the corpus striatum produced by 25 mg/kg of AOAA In contrast to the other studies ethanol was administered 30 min after AOAA and the concentration of GABA was determined 4 hr after AOAA The concentration of GABA was determined by the enzymatic (Sigma Co , St Louis, MO) method [2] Mice were sacrificed by microwave irradiation (1 33 kw, 2450 MHz and 500 msec) Within 45 seconds after sacrifice brains were removed and dissected on a cold plate The hypothalamus and bilateral corpus striata were removed according to the method of Glowinski and Iverson [11].

Statistical analysis employed either the Students' *t* test or the one way analysis of variance for repeated measures followed by planned comparison among the treatment means at each time period A $p<0$ 05 was accepted as a measure of significance

RESULTS

Hypothermia

In these experiments the test dose of 2 g/kg of ethanol was administered four hours after the administration of 25 mg/kg of AOAA or the saline control solution It must be noted that basal body temperature dropped markedly after AOAA, and at 2 hr after AOAA, body temperature had dropped to 30°C from a control level of 36 8°C and by 4 hr after AOAA, when ethanol was administered, body temperature was 32°C The data in Fig 1A shows that in normothermic animals ethanol lowered body temperature for 120 min after the test dose from a mean basal body temperature of 36 8°C The data also show that ethanol was able to produce a significant decrease in body temperature in AOAA-treated mice even though body temperature was reduced to 33 4°C by AOAA when ethanol was given, $F(12,64)=2$ 25, $p<0$ 01 Body temperature in mice given AOAA alone was nearly stable over the 2 hr recording period (mean 34.2°C) even though it was still significantly reduced

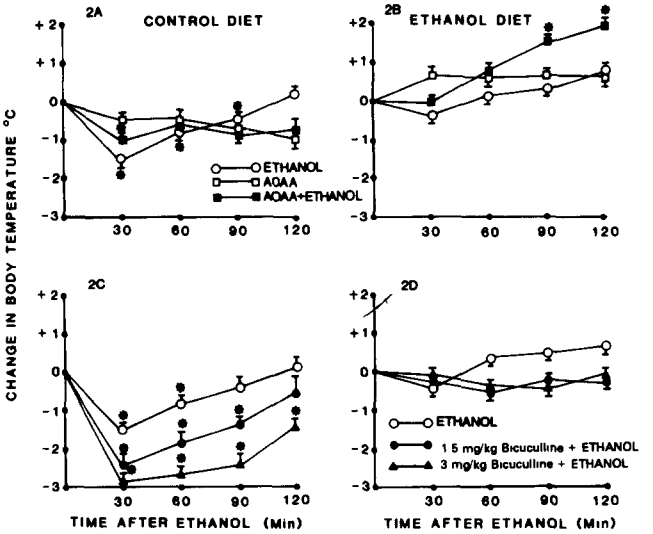

FIG 2 Effect of aminooxyacetic acid (AOAA and bicuculline (Bic) on ethanol (2 g/kg) induced hypothermia in ethanol dependent and pair-fed control mice Each point represents the mean±S.E M. of at least ten mice. *$p<0$ 05

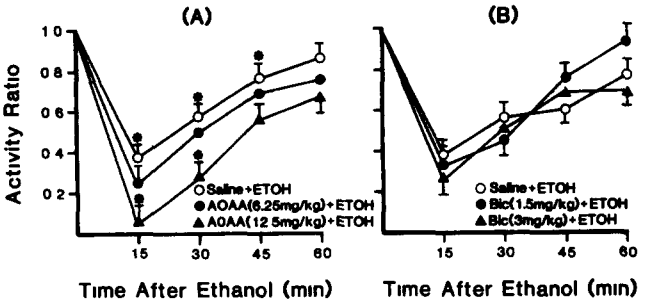

FIG 3 Effect of aminooxyacetic acid (AOAA) and bicuculline (Bic) on acute ethanol (1 5 g/kg) induced motor incoordination in mice Each point represents the mean±S.E.M of 15 mice. *$p<0.05$.

The ethanol-induced hypothermic response to bicuculline is shown in Fig. 1B. The test dose of ethanol produced the expected hypothermic response from 37.4°C after ethanol. However, the ethanol-induced hypothermia was markedly enhanced by the preadministration of 1.5 and 3.0 mg/kg of bicuculline from the mean basal temperature of 36.8°C and 37.1°C, respectively, even though these same doses were without a hypothermic effect when given alone, $F(12,100)=7.65$, $p<0.0001$.

In the chronic ethanol studies (Fig. 2) the test dose of ethanol produced the expected hypothermia in the isocaloric sucrose control groups from a mean basal body temperature of 35 8°C to 34.3°C (Figs. 2A and 2C), which persisted for approximately 90 min. In this experiment, as in the acute experiment, mean body temperature of AOAA treated mice was depressed to 32.4°C from the time of injection until the time ethanol was administered When ethanol was administered to isocaloric control mice treated with AOAA (Fig 2A) there was a further drop in body temperature of 0.8°C, $F(20,284)=4$ 84, $p<0.001$. However, in mice maintained on the ethanol diet, tolerance to the hypothermic effect of ethanol was apparent, since the decrease in body temperature produced by acute ethanol was only 0.42°C (mean basal temperature being 36.4°C) in ethanol dependent mice (Fig. 2B) at 30 min compared to a decrease of 1.75°C in the corresponding control group (Fig. 2A) Also in the ethanol-dependent mice the administration of an ethanol test dose to AOAA-treated mice was not able to produce a further drop in body temperature, although there was a more rapid rate of recovery toward normal body temperature observed in this group from 30 1 to 32 4 over the 2 hr period (Fig 2B) Even though there was a more rapid return toward control body temperature, the mice in this group still had a lowered body temperature.

In mice maintained on the isocaloric sucrose control diet, doses of 1.5 and 3 mg/kg of bicuculline markedly potentiated the hypothermic effect of ethanol from a basal temperature of 36 7°C to 34 4°C and 33.9°C in the 1.5 mg/kg and 3 mg/kg dose, respectively (Fig. 2C). In mice maintained on the ethanol diet (Fig. 2D), tolerance to ethanol hypothermia was observed since the test dose of ethanol produced no change in basal body temperature. Bicuculline, which markedly potentiated ethanol-induced hypothermia in the isocaloric sucrose control group was unable to produce any change in body temperature after a test dose of ethanol in ethanol dependent mice.

Motor Incoordination

In both the acute and chronic studies each mouse served as his own control. Based upon previous observations [6] we chose a subsedative dose of 1 5 g/kg of ethanol that produced severe motor incoordination 15 min after injection which persisted for at least 45 min (Fig. 3). Severe motor incoordination was observed in all mice treated with 25 mg/kg of AOAA. For this reason we did not use this dose of AOAA in any study involving motor incoordination. Doses of 6.25 and 12 5 mg/kg of AOAA produced minor and transient motor incoordination in treated mice. At 4 hr after AOAA administration, the time at which ethanol was administered, no motor impairment was evident and the activity ratio of all AOAA-treated mice was 1 As shown in Fig. 3A, both doses of AOAA potentiated the motor incoordination produced by the acute administration of ethanol in a dose-dependent fashion, $F(6,108)=2.62$, $p<0$ 02. On the other hand, the administration of 1.5 and 3 mg/kg of bicuculline did not alter the degree of motor incoordination produced by the acute administration of ethanol (Fig. 3B). Either dose of bicuculline,when given alone, did not produce any impairment of motor coordination at any time period.

The ability of AOAA to alter motor incoordination in mice maintained on the ethanol diet is shown in Fig. 4. In the isocaloric sucrose control group the administration of both doses of AOAA markedly potentiated the motor incoordination produced by ethanol (Fig. 4). It should be noted that the potentiation of motor incoordination produced by these doses of AOAA was comparable to that observed in the acute study at 15 min after ethanol. However, in the isocaloric sucrose control group (Fig. 4) the severity of motor incoordination was much greater at 30, 45 and 60 min after ethanol (planned comparisons after ANOVA yielded $p<0.05$ at each time period) than at the comparable times in the acute ethanol study

In the chronic ethanol-treated group, a test dose of ethanol produced a significantly less severe fall in motor coordination at 15 min, which returned to pre-ethanol

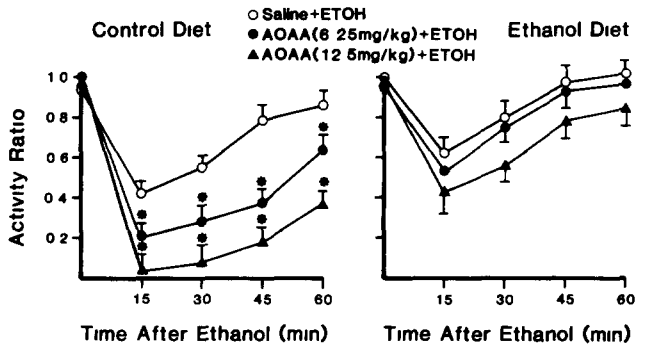

FIG 4 Effect of aminooxyacetic acid (AOAA) on ethanol (1 5 g/kg) induced motor incoordination in ethanol dependent and pair-fed control mice Each point represents the mean±S E M of 15 mice *$p<0$ 05

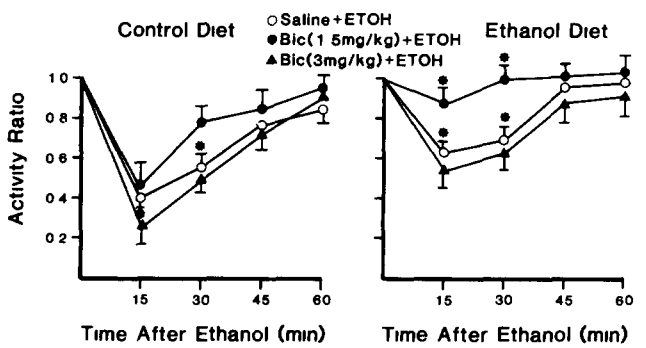

FIG 5 Effect of bicuculline (Bic) on ethanol (1 5 g/kg) induced motor incoordination in ethanol dependent and pair-fed control mice Each point represents the mean±S E M of 15 mice *$p<0$ 05

levels by 45 min (Fig 4), suggesting the development of a partial tolerance to motor incoordination in ethanol dependent mice. The dose of 6 25 mg/kg of AOAA, which markedly potentiated ethanol-induced motor incoordination in the isocaloric sucrose control group, did not alter motor incoordination produced by a test dose of ethanol to ethanol dependent mice. Similarly, the ethanol-induced motor incoordination was only slightly potentiated ($p>0.05$) by a dose of 12 5 mg/kg of AOAA in ethanol dependent mice, whereas in the isocaloric sucrose control group this dose of AOAA markedly potentiated ethanol motor-incoordination (Fig. 4).

In the isocaloric sucrose control group, 1 5 and 3.0 mg/kg of bicuculline did not alter ethanol-induced motor incoordination (Fig 5) which is similar to the results obtained with bicuculline and acute ethanol administration However, in ethanol-dependent mice a dose of 1.5 mg/kg of bicuculline markedly, $F(15,207)=3\ 30$, $p<0\ 001$, antagonized motor incoordination produced by a test dose of ethanol, whereas a dose of 3 mg/kg had no effect on ethanol-induced motor impairment (Fig 5)

GABA Levels

We tried to correlate these effects of ethanol on hypothermia and motor coordination with changes in GABA concentration after inhibition of GABA-transaminase activity by AOAA in the hypothalamus and the corpus striatum In these studies the concentration of GABA was determined 4 hr after the administration of AOAA and 30 min after ethanol These times were chosen because GABA levels after AOAA were markedly elevated at this time and also because blood ethanol levels were maximal 30 min after it was administered

In the acute studies, the concentration of GABA was increased in a dose dependent manner after doses of 6.25 and 12 5 mg/kg of AOAA in both the hypothalamus and the corpus striatum (Fig 6) However, the AOAA-induced rise in GABA levels in both tissues was completely abolished by the administration of 1.5 g/kg of ethanol administered 30 min before sacrifice A dose of ethanol of 2 g/kg was not able to block the dose-dependent rise in GABA levels in the hypothalamus produced by doses of 6.25 and 12 5 mg/kg of AOAA In fact, the rise in hypothalamic GABA levels produced by AOAA after 2 g/kg of ethanol was comparable to the dose-dependent rise in GABA levels observed in the saline control group After the administration of 2 g/kg of

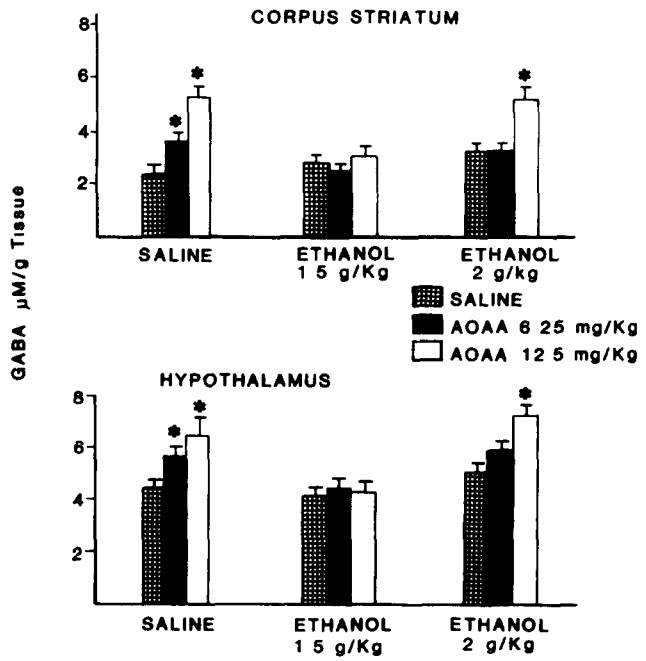

FIG 6 Effect of acute ethanol on the levels of GABA in corpus striatum and hypothalamus of saline and aminooxyacetic acid (AOAA) pretreated mice Each bar represents mean±S E M of at least 10 mice *$p<0$ 05

ethanol only the 12 5 mg/kg dose of AOAA was able to produce an increase in GABA levels in the corpus striatum.

In mice maintained on the ethanol diet, both doses of AOAA were unable to increase the concentration of GABA in the hypothalamus (Fig 7). In the isocaloric sucrose control group the rise in GABA concentration in the hypothalamus produced by 6.25 and 12 5 mg/kg was 22% and 66%, respectively When a test dose of 1 5 g/kg of ethanol was given to ethanol dependent mice pretreated with AOAA only, the dose of 12.5 mg/kg of AOAA was able to increase hypothalamic GABA levels However, the increase was only 30%, whereas in the isocaloric control group the same dose of AOAA produced a 110% rise in GABA levels When the test dose of ethanol was 2 g/kg, only the dose of 12 5 mg/kg of AOAA was able to produce a rise in hypothalamic GABA

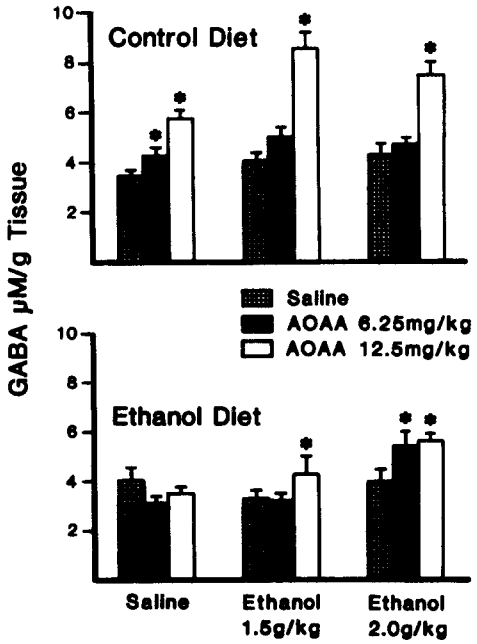

FIG. 7. Effect of ethanol on the levels of GABA in the hypothalamus of ethanol dependent and pair-fed control mice after saline and aminooxyacetic acid (AOAA) pretreatment. Each bar represents mean±S E M. of at least 10 mice *$p<0$ 05.

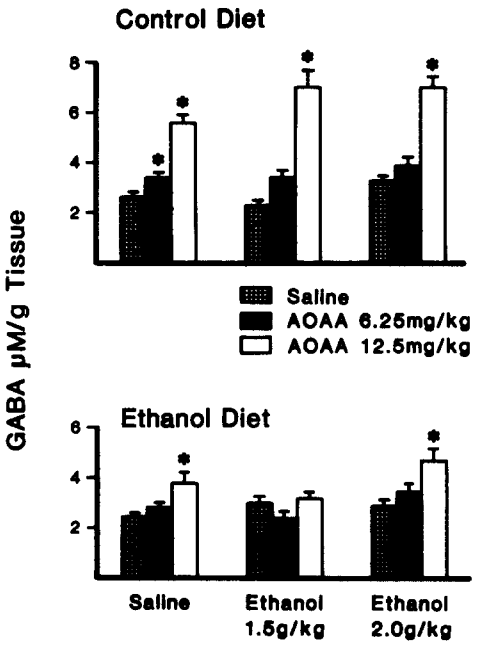

FIG. 8 Effect of ethanol on the levels of GABA in the corpus striatum of ethanol dependent and pair-fed control mice after saline and aminooxyacetic acid (AOAA) pretreatment. Each bar represents mean±S.E.M. of at least 10 mice *$p<0.05$.

levels in the isocaloric control group. The rise produced by 12 5 mg/kg of AOAA in ethanol-dependent mice was only 45% compared to a rise of 76% produced by the same dose in the isocaloric sucrose control group (Fig. 7). On the other hand, a dose of 6 25 mg/kg, which did not increase GABA levels in the control group, produced a 32% increase in dependent mice challenged with 2 g/kg of ethanol.

In the corpus striatum of ethanol-dependent mice, the AOAA-induced effect on GABA levels was only slightly different (Fig. 8) The chronic ethanol diet did not completely abolish the AOAA-induced rise in GABA levels produced by a dose of 12.5 mg/kg (Fig. 8) . However, the increase in GABA levels was only 58% in the ethanol-dependent group compared to a rise of 115% induced by the same dose in the isocaloric control group (Fig 8). When a test dose of 1.5 g/kg of ethanol was given to ethanol dependent mice pretreated with AOAA, the AOAA-induced rise in GABA levels in the corpus striatum was completely prevented when compared to the dose-dependent increase produced in the isocaloric sucrose control group (Fig. 8). When the test dose of ethanol was 2 g/kg, only a dose of 12.5 mg/kg of AOAA was able to increase GABA levels in ethanol dependent mice. However, the increase in these mice was markedly blunted and was only 57% compared to a rise of 113% produced by the same dose in the isocaloric control group.

We were impressed that the GABA response to AOAA could be prevented by chronic ethanol administration in the hypothalamus and the corpus striatum We were also impressed by the fact that AOAA-induced GABA response in ethanol-dependent mice was strikingly different even though the test doses of ethanol used were not markedly different. These studies suggested that perhaps small doses of ethanol inhibited, whereas larger doses had no effect on AOAA-induced changes in GABA. To evaluate this possibility, we added a higher dose of ethanol, 3.5 g/kg, as a test dose and determined the ability of 25 mg/kg of AOAA to increase GABA levels in the corpus striatum and the hypothalamus of normal mice. As shown in Table 1, the lowest dose of ethanol used, 1.5 g/kg, significantly decreased the GABA accumulation after AOAA pretreatment in both tissues, whereas doses of 2.0 and 3.5 g/kg of ethanol did not alter the GABA accumulation in the hypothalamus and corpus striatum. The blood alcohol concentration following 2 g/kg of ethanol was slightly, but significantly, higher than that produced by 1.5 g/kg at 0.5 hr (Fig. 9). Furthermore, the blood alcohol levels remained stable for 90 min after 2 g/kg, whereas after 1.5 g/kg blood alcohol decreased from 30–120 min after administration.

DISCUSSION

The results of the present study are compatible with the suggestion that there may be an interaction between GABAergic drugs and ethanol in relation to some of the CNS effects of ethanol. In the acute experiments, AOAA administration alone produced a marked decrease in body temperature. This AOAA-induced hypothermia was potentiated by the administration of an acute dose of 2 g/kg of ethanol, which suggested the possibility that the pathways and/or mechanism(s) for GABAergic and ethanol produced hypothermia are different. This interpretation is supported by the observations made in the chronic ethanol studies. Animals given the ethanol diet for 10 days were tolerant to the hypothermic effect of acute ethanol. In these tolerant mice AOAA administration was able to produce profound hypothermia. However, administration of a hypothermic test dose of ethanol to AOAA-treated tolerant mice was not able to cause a further drop in body temperature as it did in the

TABLE 1

EFFECT OF ETHANOL ON GABA ACCUMULATION IN HYPOTHALAMUS AND CORPUS STRIATA AFTER INHIBITION OF GABA TRANSAMINASE BY AMINOOXYACETIC ACID (AOAA)*

No	Treatment	GABA (μM/g tissue) Hypothalamus	Corpus Striatum
1	Saline + Saline	3 79 ± 0 40 (10)	3 04 ± 0 33 (10)
2	AOAA + Saline	14 31 ± 1 24 (10)	13 43 ± 0 40 (10)
3	AOAA + Etoh 1 5 g/kg	10 73 ± 1 27* (9)	10 35 ± 0 87† (10)
4	AOAA + Etoh 2 0 g/kg	12 59 ± 0 63 (10)	11 80 ± 0 91 (10)
5	AOAA + Etoh 3 5 g/kg	13 20 ± 1 12 (10)	11 78 ± 1 18 (10)

Data expressed as mean ± S E M Numbers in parentheses indicate number of animals used

*25 mg/kg IP 30 min before ethanol and 4 hr before sacrifice

†Significant compared to AOAA + Saline, $p<0$ 05

acute studies This data clearly establishes that the hypothermia produced by the GABAergic agent, AOAA, and ethanol is mediated by different neuronal pathway(s) and/or by different mechanism(s) It should be pointed out that since AOAA-induced GABA accumulation occurred over a 4 hr period, it is possible that during this time other neurotransmitter mechanism(s) may have been activated, which mediated the effect of ethanol

The results obtained with the GABA antagonist, bicuculline, were not as decisive as those obtained with AOAA Bicuculline-induced blockade of GABA receptors or neural pathways had no effect on body temperature when bicuculline alone was given. However, bicuculline markedly potentiated ethanol-induced hypothermia To be consistent with the results obtained with AOAA, blockade of GABA neural mechanisms would be expected to have no effect on ethanol-induced hypothermia It is also possible that blockade of GABA neural mechanisms by bicuculline allows other neurotransmitter mechanisms to come into play and potentiate the ethanol response as suggested previously

Pretreatment with AOAA produced a significant, dose dependent potentiation of acute ethanol-induced motor incoordination whereas pretreatment with bicuculline did not (Fig. 3) These findings are generally in agreement with those of Hakkinen and Kulonen [12] and with the recent report by Frye and Breese [10] who showed that AOAA increased while bicuculline decreased, the motor impairment produced by ethanol in rats performing a tilted plane test and aerial righting reflex, respectively

In mice maintained on the chronic ethanol diet, tolerance to ethanol-induced motor incoordination was present, as shown by a less severe decrease in the activity ratio and a more rapid recovery to control values (Figs 4 and 5) In tolerant mice, only the highest dose of AOAA used was able

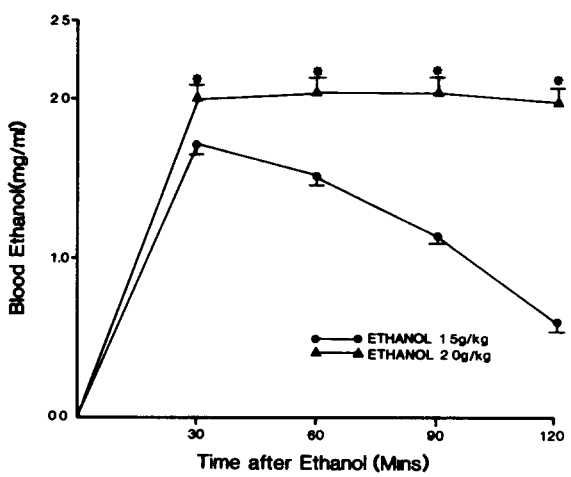

FIG 9 Blood ethanol levels after the administration of 1 5 and 2 0 g/kg IP of ethanol Each point on the curve represents the mean± S E M of 5 mice *At least $p<0$ 05

to potentiate ethanol-induced motor incoordination, and the degree of potentiation produced was much less than that produced by the same dose in non-tolerant mice In the case of bicuculline administration to tolerant mice, there was no potentiation of ethanol-induced motor incoordination and the lowest dose used, 1 5 mg/kg, actually prevented motor incoordination produced by ethanol

It was clear that an acute dose of 1.5 g/kg of ethanol completely prevented an AOAA-induced rise in GABA concentration in the hypothalamus and corpus striatum, whereas

a dose of 2 g/kg only prevented the rise in GABA concentration produced by 6.25 mg/kg of AOAA and did not prevent the increase produced by 12.5 mg/kg It is somewhat difficult to explain these effects caused by doses of ethanol that are not much different. AOAA is known to inhibit the activity of GABA-transaminase, the enzyme responsible for the catabolism of GABA. Since ethanol was given 3.5 hr after AOAA, it is reasonable to assume some accumulation of GABA had occurred in this time period. Since GABA levels 30 min after ethanol were similar to the levels in untreated control mice, it is possible the dose of 1.5 g/kg of ethanol caused the release of accumulated GABA in these tissues. This sudden release of GABA could explain the potentiation of ethanol-induced motor incoordination produced by AOAA. This effect of ethanol is compatible with the results obtained in the bicuculline experiment. Since bicuculline blocks GABA activated neural pathways, the release or lack of release of GABA should have no effect on acute ethanol-induced motor incoordination, and this is what was observed. The observed differences in AOAA-induced GABA accumulation produced by the two doses of ethanol suggests the possibility of a biphasic ethanol response The response produced by 2 g/kg of ethanol was associated with a slightly higher initial blood level which remained stable over the next 90 min. On the other hand, the dose of 1.5 g/kg was associated with a slightly lower initial blood alcohol level and the declining phase of the blood ethanol curve. Although we did not monitor blood ethanol levels in AOAA and bicuculline treated mice, the possibility exists that these agents altered the metabolism of ethanol. We are unable to find any reports in the literature to support this possibility.

It is known that low doses of ethanol have a stimulant effect and that GABAergic agents, including AOAA, will block the stimulant effects of ethanol, [1,5]. This raises the interesting possibility that GABAergic drugs may selectively block the stimulatory effect of ethanol, thereby allowing full expression of the depressant effect of ethanol The results of the present study are compatible with this possibility, particularly the results obtained with AOAA. Since the influence of bicuculline on motor incoordination was observed only in mice made tolerant to ethanol-induced motor impairment by chronic ethanol, it is possible that the effects of bicuculline required alteration of central GABA receptors which is known to occur following chronic ethanol consumption [22,25]

One of the most significant findings in the present study was that mice maintained on a chronic ethanol diet and made tolerant to ethanol-induced hypothermia, and partially tolerant to motor incoordination, were unable to respond to the administration of AOAA by increasing the concentration of GABA in the hypothalamus and the corpus striatum (Figs. 7 and 8), as was observed in the isocaloric sucrose control group. However, it must be noted that the basal levels of GABA in both the hypothalamus and the corpus striatum of ethanol-dependent mice were similar to that measured in the isocaloric sucrose control group showing that chronic ethanol intake did not interfere with the normal steady state concentration of GABA, but was selectively able to prevent the rise in GABA produced by the inhibition of GABA-transaminase by AOAA. A recent report by Wixon and Hunt [26] showed that the rate of AOAA-induced GABA accumulation was decreased in the corpus striatum, cerebellum and cerebral cortex of rats during withdrawal from ethanol. Other studies recently reviewed by Hunt [14] have shown that acute and chronic ethanol may increase or decrease brain GABA

The protocol of the present experiments required that ethanol-dependent mice were studied 24 hr after ethanol was removed which was the first day of the withdrawal syndrome. It is known that during the ethanol withdrawal period there is a central hypogabaergic state [26] which may explain the inability of mice in the ethanol withdrawal state to respond to AOAA stimulation These findings are in agreement with the report of Wixon and Hunt [26] who showed that AOAA-induced GABA accumulation was significantly reduced during the ethanol withdrawal syndrome in the rat. Since the amount of GABA accumulated after AOAA inhibition of GABA-transaminase has been taken as an indication of GABA synthesis [15], the lack of effect in ethanol-dependent mice may be due to inhibition of GABA synthesis However, the levels of GABA in the hypothalamus and corpus striatum of ethanol-dependent mice were similar to the levels in the isocaloric control group, suggesting that the effect of ethanol was not on the basal or steady state GABA levels but only upon AOAA-induced changes in GABA accumulation

Hyperexcitability is a common feature of the ethanol withdrawal state probably caused by a hyperadrenergic state [16] The decreased central GABAergic state in ethanol withdrawal favors hyperexcitability and it is known that drugs which increase central GABA levels decrease ethanol-withdrawal convulsions [18]. A reduction in neuronal GABA activity may also have contributed to the development of tolerance to ethanol-induced motor incoordination by reducing central inhibition, which may compensate for the depressant effect of ethanol [18].

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