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BRIEF COMMUNICATION

Effect of Muscimol on Ethanol-Induced Central Nervous System Depression[1]

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MATTUCCI-SCHIAVONE, L. AND A. P. FERKO. *Effect of muscimol on ethanol-induced central nervous system depression.* PHARMACOL BIOCHEM BEHAV 27(4) 745–748, 1987.—In male Sprague-Dawley rats acute ethanol (1.0 and 2.0 g/kg) produced impairment of motor coordination and induced hypnosis (4.0 g/kg). Muscimol (1.25 mg/kg, IP) prior to ethanol administration enhanced motor impairment as measured by the aerial righting reflex. The rate of ethanol disappearance from the blood was unaltered by muscimol. Functional tolerance to the effect of ethanol on sleep time was produced by a 24 hr ethanol inhalation procedure. Animals tested 48 hr after ethanol inhalation exhibited a reduced sleep time from ethanol (4.0 g/kg). Muscimol (1.75 mg/kg) was administered along with ethanol 48 hr following 1 day of ethanol inhalation. Although the animals exhibited tolerance to ethanol-induced hypnosis, they did not manifest tolerance to the effect of muscimol.

Ethanol Muscimol Functional tolerance to ethanol Sleep time

ALTHOUGH ethanol may act to alter various neurotransmitter systems, the GABA (gamma-aminobutyric acid) system is believed to mediate some of the general depressant effects of ethanol [8]. It has been postulated that the interruption of normal GABA neuronal function by ethanol may subserve such CNS depressing effects as motor impairment [4] and hypnosis [11]. Evidence for such GABAergic involvement includes potentiation of acute ethanol-induced motor impairment and hypnosis in rats and mice by pretreatment with GABA agonists and attenuation of these effects by a GABA antagonist [4,11].

Studies on the GABA system following prolonged ethanol administration show that dependence may be associated with alterations in GABAergic function and that the ethanol withdrawal syndrome can be attenuated by GABAergic drugs [5]. Tolerance to ethanol, as well, may be associated with alterations in the GABA system [10] although the development of dependence often accompanies tolerance when a prolonged period of ethanol exposure is involved. The aim of this study is to determine if tolerance to muscimol, a GABA receptor agonist, occurs in animals that are functionally tolerant to ethanol but not physically dependent on ethanol. The use of a 24 hr inhalation procedure [3,14] allows the separation of the phenomena of tolerance to and physical dependence on ethanol and removes physical dependence on ethanol as a variable in the experiments. Previous work indicates that functional tolerance to the effects of ethanol on hypothermia [3] as well as motor impairment and hypnosis [14] can be produced by a short period (24 hr) of ethanol exposure that does not result in ethanol dependence.

This investigation examines the interaction between ethanol and muscimol, a GABA receptor agonist, on two behavioral actions, motor coordination and sleep time in naive rats. In addition, the interaction between ethanol and muscimol on sleep time is studied in rats made tolerant to ethanol by a short period of exposure to ethanol vapor.

METHOD

Male Sprague-Dawley rats (180–220 g) were obtained from Charles River Laboratories, Inc. (Wilmington, MA) and were housed 6 to a cage for 1 week prior to experimentation at 22±1°C with a light cycle from 6:00 a.m. to 6:00 p.m. The animals had free access to Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO) and water; however, they were fasted 18 hr prior to drug or saline administration but water was available ad lib. The bedding used in the cages was ground corn cob, 1/8th in. (Anderson Cob Division, Maumee, OH). Ethanol solutions (10 or 20% w/v) for injection were prepared from 95% ethanol. Muscimol was purchased from Fluka Chemical Corp. (Haupage, NY).

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TABLE 1

ACUTE EFFECT OF ETHANOL (IP) WITH MUSCIMOL (1.25 mg/kg, IP) ON AERIAL RIGHTING REFLEX 60 MINUTES AFTER ETHANOL INJECTION

Control				Muscimol-Treated			
Ethanol Dose (g/kg)	N	Blood Ethanol (mg/ml)	Height of Aerial Righting (cm)	Ethanol Dose (g/kg)	N	Blood Ethanol (mg/ml)	Height of Aerial Righting (cm)
0.0	8	0.00	5.3 ± 0.3*	0.0	6	0.00	6.3 ± 0.8
1.0	7	0.65 ± 0.02	8.7 ± 1.2	1.0	7	0.74 ± 0.03	13.6 ± 1.4‡§
2.0	7	1.67 ± 0.02	17.1 ± 1.0†	2.0	7	1.71 ± 0.02	40.7 ± 5.4‡¶

*Means ± S.E.
†Significantly different from saline-treated animals in Control Group ($p<0.05$).
‡Significantly different from saline-treated animals in Muscimol Group ($p<0.05$).
§Significantly different from Control Group given same dose of ethanol ($p<0.02$).
¶Significantly different from Control Group given same dose of ethanol ($p<0.01$).

TABLE 2

FORTY-EIGHT HOURS AFTER REMOVAL FROM CHAMBERS, THE EFFECT OF MUSCIMOL (MUS, 1.75 mg/kg, IP) ON ETHANOL-INDUCED (ETOH, 4 g/kg, IP) SLEEP TIME

Air-Treated (24 hr)				Ethanol Vapor-Treated (24 hr)			
Drug GIven	N	Blood Ethanol* (mg/ml)	Sleep Time (min)	Drug Given	N	Blood Ethanol (mg/ml)	Sleep Time (min)
ETOH	9	2.90 ± 0.10	193 ± 16†	ETOH	9	3.21 ± 0.14	127 ± 14‡
MUS + ETOH	6	2.77 ± 0.10	267 ± 11§	MUS + ETOH	8	2.88 ± 0.05	191 ± 13¶

*Determined when animals regained righting reflex.
†Means ± S.E.
‡Significantly different from air-treated group given ETOH alone ($p<0.01$).
§Significantly different from air-treated group given ETOH alone ($p<0.01$).
¶Significantly different from ethanol vapor-treated group given ETOH alone ($p<0.01$).

Acute Experiments

A procedure described previously [4] was used to measure the alteration in the aerial righting reflex of rats as an index of motor coordination. Briefly, the rats were held in an inverted position above a foam rubber mat and released. The height was measured by an adjacent meter stick. The minimum height that the animal could successfully right himself on two consecutive releases was used as an index of motor impairment (usually 5 cm for control animals). A successful righting required that all four paws were observed by the rater to touch the mat simultaneously upon landing. To note the effect of ethanol on motor coordination, animals were administered ethanol (1 or 2 g/kg, IP) or saline (0.9% NaCl solution) one hr prior to testing. From each animal immediately after testing, a blood sample (20 μl) was obtained from the orbital sinus to determine blood ethanol concentration according to an enzymatic method [12]. This procedure of using orbital sinus blood is an accurate estimation of brain ethanol content [13].

For the aerial righting reflex test muscimol (1.25 mg/kg, IP) or saline was administered 15 min prior to ethanol (1 or 2 g/kg) injection. Testing occurred 60 min after ethanol treatment, followed immediately by blood sampling to determine blood ethanol content. Also, in separate experiments to study the rate of disappearance of ethanol from blood, muscimol (1.25 mg/kg, IP) was administered prior to ethanol (2 g/kg) injection. Blood ethanol concentrations were determined at 0.5, 2.5, 3.5 and 4.5 hr for each animal. Linear regression analysis was used to determine the rate of disappearance of ethanol from blood.

Function Tolerance Experiments

Animals (group of six) in the chamber were exposed to ethanol vapor (nominal concentration: 28 mg/liter) by inhalation for a period of 24 hr as previously described [3,14]. Briefly, ethanol (95%) was delivered into a warmed flask by an infusion pump at a calibrated rate of 150 mg/min. Air flow of 5.35 liters/min through the flask maintained an ethanol vapor concentration of 28 mg/liter in the chamber. Temperature inside the chamber was 25±1°C. Ethanol vapor concentration was determined twice daily in duplicate at 4 hr into the procedure and at 24 hr. Food and water were available during the period of ethanol vapor exposure. Control animals were exposed to air only in the chamber and food was re-

stricted to match that consumed by the ethanol-treated animals in order to reflect the weights attained by the treated group.

Sleeping time was used as an index of ethanol-induced hypnosis and was measured as the time interval between the loss of the righting reflex after ethanol injection (4 g/kg, IP) and the gain of the righting reflex. The gain of the righting reflex required that the animal be able to re-right himself within one minute, after again being placed on his back. A blood sample (20 μl) was taken from the orbital sinus of each animal after regaining the righting reflex.

These experiments determined the effect of muscimol on ethanol-induced sleep time in tolerant animals. Forty-eight hours after removal of animals from the chamber procedure, both air-treated (controls) and ethanol vapor-treated animals were divided into two groups. The first group of the ethanol vapor- or air-exposed animals received muscimol (1.75 mg/kg, IP) 15 min prior to the 4 g/kg, IP dose of ethanol. The second group of the ethanol vapor- or air-exposed animals received saline injection 15 min prior to the 4 g/kg, IP dose of ethanol. When the animals regained the righting reflex, blood samples (20 μl) were obtained from the orbital sinus to measure ethanol content.

Statistical Analysis

Significant differences were determined by analysis of variance (ANOVA). All multiple comparisons with a control were done by ANOVA followed by Dunnett's test. All data were analyzed using an Apple IIe Computer.

RESULTS

Acute ethanol administration caused an increase in motor impairment as seen by the increased height required for aerial righting and blood ethanol content (Control group, Table 1). When the effect of the GABA receptor agonist muscimol was examined on ethanol-induced motor impairment, there occurred a greater impairment in motor coordination (higher aerial righting scores) when muscimol was combined with ethanol (Muscimol group, Table 1). Blood ethanol content at the time of testing was similar in the control group when these data were compared with the muscimol group. Animals receiving muscimol prior to saline showed no impairment of aerial righting. In another experiment, muscimol (1.75 mg/kg, IP) was administered 15 min prior to saline, and did not induce hypnosis in these animals.

Separate experiments were performed to note if muscimol influenced the rate of ethanol disappearance from the blood. Animals given saline prior to a 2 g/kg dose of ethanol showed an ethanol disappearance rate of 0.32±0.03 mg/ml/hr (N=6). The rate of ethanol disappearance from the blood in animals receiving muscimol (1.25 mg/kg, IP) 15 min prior to ethanol (2 g/kg, IP) was 0.29±0.02 mg/ml/hr (N=6).

After 24 hr of ethanol vapor exposure in the chamber, the ethanol content in the chamber was 26.0±0.8 mg/liter. The blood ethanol content in animals at the end of the inhalation procedure was 4.41±0.06 mg/ml. The rate of disappearance for ethanol from blood was 0.38±0.02 mg/ml/hr (N=6). The changes in body weight that occurred at the end of the 24 hr inhalation were decreases of 13.9±0.06 and 13.4±1.1 percent for the air-treated and ethanol vapor-treated animals, respectively. Also, after removal of the animals from the chambers, no significant signs of physical dependence were exhibited by the ethanol-treated animals when tested every hour for 12 hours as previously described [2,14].

The results for the effect of ethanol (4 g/kg, IP) on sleep time in animals at 48 hr after removal from the inhalation procedure are shown in Table 2. The rate of disappearance from the blood of acute ethanol was unchanged in ethanol vapor-treated animals (0.29±0.01 mg/ml/hr; N=6) and air-treated animals (0.32±0.03 mg/ml/hr; N=6). These data indicate that functional tolerance to ethanol-induced sleep time was produced by the 24 hr ethanol inhalation procedure. Also, the effect of muscimol on ethanol-induced sleep time 48 hr after removal from the chambers is indicated. Muscimol enhanced the sleep time both in ethanol vapor-treated animals (tolerant) and air-treated animals (non-tolerant). The increase in mean sleep time, however, in tolerant and non-tolerant animals was 64 and 74 min, respectively. The data in Table 2 indicate that although animals become functionally tolerant to the effect of ethanol as measured by sleep time (48 hr after 1 day of ethanol inhalation), the animals do no appear to have developed any extensive tolerance to the *in vivo* effect of muscimol.

DISCUSSION

The enhancement of the effect of ethanol on motor coordination and sleep time by muscimol produced in this investigation supports previous results on sleep time in mice [11]. Others also indicate similar enhancement using other GABA agonists, such as aminooxyacetic acid [4,10]. Although it is suggested that GABA mimetic agents may themselves influence behavior [8], our work shows that muscimol has no measurable effect on the aerial righting reflex test or on hypnosis when the drug is given alone. In addition, muscimol does not affect the rate of disappearance of ethanol from the blood, thus eliminating altered ethanol clearance as the reason for the observed effects, a possibility raised by others [1].

Previous neurophysiologic work suggests that acute administration of ethanol augments GABAergic transmission at a postsynaptic site [15]. Recent studies indicate that ethanol enhances GABA receptor-mediated chloride transport [17,18]. Ethanol-stimulated chloride uptake into brain vesicles is attenuated by Ro15-4513, an imidazobenzodiazepine derivative that is a benzodiazepine receptor inverse agonist [18]. Pentobarbital- or muscinol-stimulated chloride uptake into brain vesicles, however, is not attenuated by Ro15-4513. It seems that Ro15-4513 has a unique action on the GABA receptor-mediated chloride ion complex unlike another benzodiazepine receptor inverse agonist [18]. Ro15-4513 also inhibits the acute intoxicating effects of ethanol, e.g., sedation, staggered gait and impaired righting reflex.

It is of interest that although tolerance to the effect of ethanol occurs (Table 2), the effect of muscimol on ethanol-induced sleep time is unchanged after ethanol inhalation. This present finding appears to be supported by some recent *in vitro* evidence in the literature [7,16]. Ethanol does not affect the binding of [^{3}H] muscimol to the GABA receptor or the binding of various radioligands to the benzodiazepine receptor [7]. In addition, *in vitro* work seems to rule out the direct involvement of benzodiazepine and picrotoxin sites on the GABA receptor complex in the phenomenon of tolerance to and withdrawal from ethanol [16]. These investigators suggest that tolerance to, and dependence on ethanol may be due to compensatory changes in other, counter-balancing, neurotransmitter systems. Moreover, ethanol shares a variety of effects with benzodiazepines and barbiturates [17]. Also, behavioral cross-tolerance between ethanol, barbitu-

rates, and benzodiazepines is reported [6,9]. It is not entirely clear, however, that ethanol exerts the same action on GABA receptor activity as do the benzodiazepines or barbiturates [17]. Although evidence in the literature is beginning to suggest a relationship between ethanol, barbiturates, benzodiazepines, and GABA, it appears that further experimentation is required before a definitive explanation can be given.

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REFERENCES

1. Dar, M. S. and W. R. Wooles. GABA mediation of the central effects of acute and chronic ethanol in mice. *Pharmacol Biochem Behav* **22:** 77–82, 1985.
2. Ferko, A. P. and E. Bobyock. Induction of physical dependence in rats by ethanol inhalation without the use of pyrazole. *Toxicol Appl Pharmacol* **40:** 269–276, 1977.
3. Ferko, A. P. and E. Bobyock. Rates of ethanol disappearance from blood and hypothermia following acute and prolonged ethanol inhalation. *Toxicol Appl Pharmacol* **50:** 417–427, 1979.
4. Frye, G. and G. Breese. GABAergic modulation of ethanol-induced motor impairment. *J Pharmacol Exp Ther* **223:** 750–756, 1982.
5. Goldstein, D. Alcohol withdrawal reactions in mice: effects of drugs that modify neurotransmission. *J Pharmacol Exp Ther* **186:** 1–9, 1973.
6. Gougos, A., J. M. Khanna, A. D. Lê and H. Kalant. Tolerance to ethanol and cross-tolerance to pentobarbital and barbital. *Pharmacol Biochem Behav* **24:** 801–807, 1986.
7. Greenberg, D. A., E. C. Cooper, A. Gordon and I. Diamond. Ethanol and the γ-aminobutyric acid-benzodiazepine receptor complex. *J Neurochem* **42:** 1062–1068, 1984.
8. Hunt, W. The effect of ethanol on GABAergic transmission. *Neurosci Biobehav Rev* **7:** 87–95, 1983.
9. Lê, A. D., J. M. Khanna, H. Kalant and F. Grossi. Tolerance to and cross-tolerance among ethanol, pentobarbital and chlordiazepoxide. *Pharmacol Biochem Behav* **24:** 93–98, 1986.
10. Leitch, G., D. Backes, F. Seigman and G. Guthrie. Possible role of GABA in the development of tolerance to alcohol. *Experientia* **33:** 469–498, 1977.
11. Liljequist, S. and J. Engel. Effects of GABAergic agonists and antagonists on various ethanol-induced behavioral changes. *Psychopharmacology (Berlin)* **78:** 71–75, 1982.
12. Lundquist, F. The determination of ethyl alcohol in blood and tissues. *Methods Biochem Anal* **7:** 217–251, 1959.
13. Mattucci-Schiavone, L. and A. P. Ferko. Sampling of orbital sinus blood closely reflects brain ethanol content in rats. *Physiol Behav* **33:** 895–898, 1984.
14. Mattucci-Schiavone, L. and A. P. Ferko. An inhalation procedure to produce tolerance to the behavioral effects of ethanol. *Physiol Behav* **36:** 643–646, 1986.
15. Nestoros, J. N. Ethanol specifically potentiates GABA-mediated neurotransmission in feline cerebral cortex. *Science* **209:** 708–710, 1980.
16. Rastogi, S. K., R. Thyagarajan, J. Clothier and M. K. Ticker. Effect of chronic treatment of ethanol on benzodiazepine and picrotoxin sites on the GABA receptor complex in regions of the brain of the rat. *Neuropharmacology* **25:** 1179–1184, 1986.
17. Suzdak, P. D., R. D. Schwartz, P. Skolnick and S. M. Paul. Ethanol stimulates γ-aminobutyric acid receptor-mediated chloride transport in rat synaptoneurosomes. *Proc Natl Acad Sci USA* **83:** 4071–4075, 1986.
18. Suzdak, P. D., J. R. Glowa, J. N. Crawley, R. D. Schwartz. P. Skolnick and S. M. Paul. A selective imidazobenzodiazepine antagnoist of ethanol in the rat. *Science* **234:** 1243–1247, 1986.