Pharmacology Biochemistry & Behavior, Vol. 27, pp. 235–238. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Ethanol-Induced Sleep Time: Interaction With Taurine and a Taurine Antagonist[1]

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Received 26 September 1986

FERKO, A. P. *Ethanol-induced sleep time: Interaction with taurine and a taurine antagonist.* PHARMACOL BIOCHEM BEHAV 27(2) 235–238, 1987.—In male Swiss-Webster mice sleep time (hypnosis) was used as an index of ethanol-induced central nervous system depression. Ethanol (4 g/kg, IP) was administered to animals and the onset to sleep time (loss of the righting reflex) and the duration of sleep time were recorded. At the end of the ethanol-induced sleep time, taurine (7.5, 15 or 25 μmol/kg, ICV) was injected. Immediately after the ICV injection of taurine the mice again lost the righting reflex. This effect of taurine occurred in a dose-dependent fashion. In the absence of ethanol, taurine (25 μmol/kg, ICV) did not produce a significant sleep time. In another experiment when TAG, 6-amino-methyl-3-4H-1,3,4-benzothiadiazine-1,1-dioxide HCl, (a taurine antagonist) was given to mice, TAG (0.9 μmol/kg, ICV) significantly reduced the effect of taurine (7.5, 15 and 25 μmol/kg, ICV) to reinstate a sleep time in the presence of ethanol. TAG, however, did not alter ethanol-induced sleep time. These results indicate that taurine (ICV) can enhance the central depressant action of ethanol and that this effect of taurine can be attenuated by TAG. The antagonism of taurine by TAG appears to be noncompetitive in nature.

Ethanol Taurine Taurine antagonist (TAG) Central nervous system depression Sleep time

THE present work studies the effect of taurine on ethanol-induced central nervous system depression. Interest was developed in this project from several earlier reports in the literature. These investigations indicate that taurine decreases the sleep time (hypnosis) of ethanol when both drugs are administered by intraperitoneal injection to mice [2,10]. The maximum effect of taurine to reduce ethanol-induced sleep time (4 g/kg, IP) occurs with 45 mg/kg. Higher doses of taurine produce less of an effect. Another report shows that taurine decreases the sleep time in mice when ethanol and taurine (45 mg/kg) are given simultaneously by IP administration [2]. In addition, taurine fails to antagonize the effect of ethanol on body temperature, seizure susceptibility or brain 5-hydroxyindole acetic acid levels [2]. A later study, however, indicates that taurine does not alter ethanol-induced sleep time in mice when the amino acid is injected IP in a dose similar to that employed in the earlier investigations [16].

Although there appears to be some controversy about the effects of peripheral administration (IP) of taurine with ethanol, the results of various studies may reflect differences in genotype of mice used, their sensitivity to ethanol, age and experimental design. Rather than try to unravel, at the present time, these conflicting results about the intraperitoneal injection of taurine on ethanol-induced sleep time in mice, it was decided to focus on the central effects of taurine following intracerebroventricular (ICV) administration in the presence of ethanol.

Taurine is an amino acid that is present in high concentration in the brain [11]. A recent report indicates that taurine enhances the central depressant properties of ethanol in Sprague-Dawley rats when this amino acid is given by the ICV route [13]. There also is evidence in the literature that taurine does not enter the brain in significant amounts after acute peripheral administration [14]. It, therefore, would be of interest to investigate the effect of taurine on ethanol-induced sleep time in mice following ICV injection to verify the initial report of the taurine-ethanol interaction that occurs in rats [13]. In addition a taurine antagonist, TAG (6-aminomethyl-3-methyl-4H-1,2,4-benzothiadiazine-1,1-dioxide HCl) [20] is administered in the presence of ethanol and taurine. The purpose of these experiments is designed to obtain some information as to the nature of the antagonism of taurine by TAG in the central nervous system. Blood ethanol concentrations are determined in mice when ethanol is administered alone and in combination with taurine and TAG.

METHOD

Male Swiss-Webster mice (24–32 g) were obtained from Charles Rivers Laboratories (Wilmington, MA). The mice were housed 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

[1]This work was supported by BRS Grant #2-SO7-RR05413-24.

TABLE 1

EFFECT OF TAURINE (TAU, μmol/kg, ICV) TO ENHANCE THE CENTRAL DEPRESSANT PROPERTIES OF ETHANOL (ETOH, 4 g/kg, IP) AS MEASURED BY SLEEP TIME

Group	N	Onset to Sleep (sec)	ETOH Sleep Time (min)	TAU Return to Sleep Time (min)†	Blood ETOH mg/ml
ETOH + SALINE (Controls)	10	88 ± 3*	45.9 ± 3.0	2.4 ± 1.3	3.38 ± 0.09
ETOH + TAU (7.5)	7	88 ± 5	56.4 ± 7.4	17.3 ± 3.1‡	3.54 ± 0.04
ETOH + TAU (15.0)	9	93 ± 3	45.2 ± 2.7	28.9 ± 3.2§	3.08 ± 0.06
ETOH + TAU (25.0)	7	100 ± 5	52.0 ± 4.0	49.6 ± 5.0§¶	3.14 ± 0.12

*Values are means ± S.E.M.
†Injections (ICV) were given immediately after regaining the righting reflex following ETOH administration.
‡Significantly different from controls ($p<0.05$).
§Significantly different from controls ($p<0.01$).
¶Significantly different from other ETOH + TAU groups ($p<0.01$).

TABLE 2

THE ANTAGONISM OF TAG (0.9 μmol/kg, ICV) ON THE RETURN TO SLEEP TIME INDUCED BY VARIOUS DOSES OF TAURINE (TAU, μmol/kg, ICV) IN THE PRESENCE OF ETHANOL (ETOH, 4 g/kg, IP)

Taurine Dose	Group	N	Onset to Sleep (sec)	ETOH Sleep Time (min)	TAU-Return to Sleep Time (min)‡	Blood ETOH mg/ml
7.5	Control	6	88 ± 5†	56.4 ± 7.4	19.5 ± 2.6	3.54 ± 0.04
μmol/kg	Treated*	7	92 ± 4	53.5 ± 5.0	8.4 ± 1.2§	3.44 ± 0.10
15.0	Control	8	99 ± 5	48.7 ± 3.6	30.3 ± 3.7	3.33 ± 0.08
μmol/kg	Treated*	6	86 ± 5	49.1 ± 4.8	13.5 ± 2.1§	3.42 ± 0.12
25.0	Control	7	92 ± 4	53.8 ± 4.9	41.9 ± 3.6¶	3.28 ± 0.05
μmol/kg	Treated*	7	93 ± 4	49.7 ± 4.4	16.7 ± 2.0§#	3.37 ± 0.10

*TAG was injected (ICV) 20 min after the loss of the righting following ETOH administration.
†Values are means ± S.E.M.
‡Immediately upon regain of the righting reflex after ethanol administration, TAU (ICV) was given to controls whereas treated animals received a TAU-TAG solution (ICV). In these solutions TAU was 7.5, 15.0 or 25 μmol/kg and TAG was 0.9 μmol/kg.
§Significantly different from corresponding control ($p<0.01$).
¶Significantly different from TAU (7.5 μmol/kg) control group ($p<0.01$).
#Significantly different from TAU (7.5 μmol/kg) treated group ($p<0.05$).

Co., St. Louis, MO) and water; however, they were fasted 18 hr prior to drug or saline administration but water was available ad lib. Ethanol solution (20% w/v) for injection was prepared from 95% ethanol in saline. Taurine was purchased from Sigma Chemical Co. (St. Louis, MO). TAG, 6-aminomethyl - 3 - methyl - 4H - 1,2,4 - benzothiadiazine - 1,1 - dioxide hydrochloride, was a gift from Merck Sharp & Dohme Research Laboratories (Rahway, NJ). Solutions of taurine or TAG were prepared in saline (0.9% NaCl) and adjusted to pH 7.0 with NaOH solution (0.01 N) [13].

Sleep Time Experiments with Ethanol (IP) and Taurine (ICV)

Sleep time (hypnosis) was used as an index of ethanol-induced central nervous system depression 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 3 times within 1 min, after again being placed on his back. In addition the onset of hypnosis (time between ethanol injection and loss of the righting reflex) was recorded.

The purpose of these experiments was to determine if an injection of taurine (ICV) could enhance the degree of central nervous system depression and return the mice to a state of hypnosis (sleep time) when taurine was given at the end of the ethanol-induced sleep time.

Intracerebroventricular injection of drug was administered using a previously described method [17]. The procedure involved cutting the scalp of an anesthetized mouse and injecting (at depth of 3 mm) 2 mm caudal and 2 mm lateral to Bregma using a Hamilton microliter syringe with a 26 gauge needle of $^3/_8$ inch. The correct position of the injection was verified at autopsy by using trypan blue dye.

Animals were administered ethanol (4 g/kg, IP). Twenty minutes after the loss of the righting reflex a 26 gauge needle was used to enter the ventricle of the brain of the anesthetized mouse but no saline or drug was given at this time, since this was the preparatory step for ICV drug administration. Immediately after the animals regained the righting reflex, they received an ICV injection of saline or taurine (7.5, 15 or 25 μmol/kg) in a volume of 5 μl. The duration of the loss of the righting reflex after saline or taurine injection was recorded (Return To Sleep Time). In addition a blood sample (20 μl) was taken from the orbital sinus of each animal when they

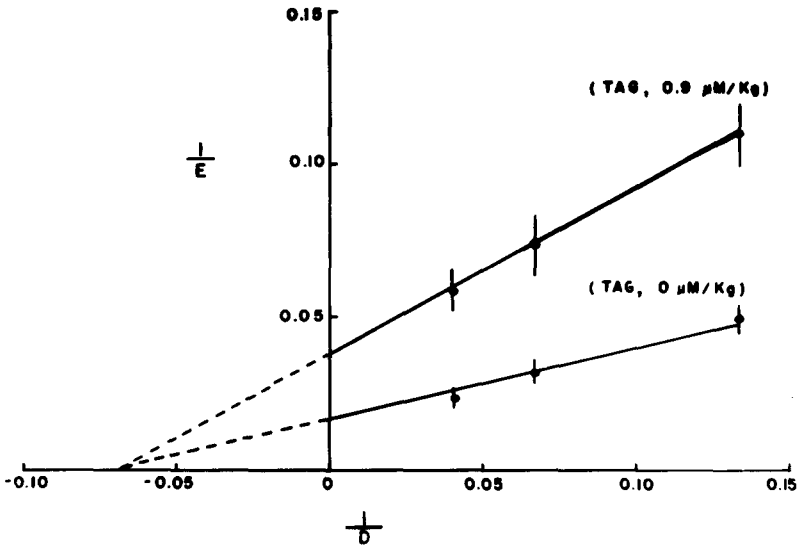

FIG. 1. Double-reciprocal plot of data presented in Table 2 for the taurine-TAG interaction. E is the Taurine-Return to Sleep Time (min) and D is the dose of taurine administered ICV (μmol/kg). Values are the means±S.E.M. of 6 to 8 mice.

regained the righting reflex. Blood ethanol concentrations were determined according to an enzymatic method [12].

To study the effect of taurine alone in mice, the mice were injected (IP) with saline (0.02 ml/g). Twenty min later animals were lightly ether-anesthetized according to a previously described method [17] and injected ICV with saline or taurine (25 μmol/kg) in a volume of 5 μl. Also a similar experiment was performed with TAG administration (ICV) alone in mice.

Experiments with Ethanol (IP), Taurine (ICV) and TAG (ICV)

These experiments were designed to determine if TAG, a taurine antagonist, could alter the effect of taurine in the presence of ethanol. Mice were injected with ethanol (4 g/kg, IP). Twenty min later after the loss of the righting reflex the animals were given ICV injections (5 μl) of saline or TAG (0.15, 0.3 or 0.9 μmol/kg). When the animals regained the righting reflex they were immediately injected intracerebroventricularly (5 μl) with taurine (15 μmol/kg, controls) or a taurine-TAG solution. The taurine-TAG solutions contained a constant amount of taurine (15 μmol/kg) but the TAG concentration was either 0.15, 0.3 or 0.9 μmol/kg. The return to sleep time was recorded. A blood sample (20 μl) was taken from the orbital sinus of each animal when they regained the righting reflex.

The next experiment was repeated in a similar manner to the above experiment, except that the dose of taurine was 7.5, 15.0 or 25 μmol/kg, ICV and the dose of TAG was 0.9 μmol/kg, ICV.

Statistical Analysis

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

RESULTS

The data in this study indicate that the central depressant properties of ethanol are altered by the intracerebroventricular injection of taurine. When taurine was administered ICV to animals immediately after regaining the righting reflex following a previous injection (IP) of ethanol, taurine produced a return to sleep time in these mice in a dose-dependent manner (Table 1). The percentage increase in sleep time for taurine at doses of 15 and 25 μmol/kg was 67 and 186% respectively, when these doses were compared with the lowest dose of taurine administered to the mice. The mice lost the righting reflex at the completion of the ICV injection of taurine, that is, the onset of the second sleep time period was immediate. In addition it should be mentioned that following the ICV injection of taurine in mice previously treated with ethanol (IP), there occurred a respiratory depressant effect that lasted for several minutes when the highest dose of taurine was used. Also two animals died after taurine (25 μmol/kg, ICV) administration.

In another experiment taurine (25 μmol/kg) was administered ICV to lightly ether-anesthetized mice, 20 min after IP injection of saline (0.02 ml/kg). The control animals received 5 μl of saline (ICV) instead of taurine. The sleep times in the controls and taurine-treated mice were 0.9±0.7 and 2.4±0.7 min, respectively. Therefore, taurine did not produce any significant increase in sleep time when the drug was given in the absence of ethanol.

The results of the experiments with taurine and various doses of TAG (a taurine antagonist) in the presence of ethanol indicate that TAG (0.9 μmol/kg, ICV) did not alter ethanol-induced sleep time (Control sleep time = 48.7±3.6 min, N=8 and Treated Group sleep time = 49.1±4.8 min, N=6) but significantly reduced by 55% the effect of taurine to produce a second sleep time period in the presence of ethanol (Control sleep time = 30.3±3.7 min, N=8 and Treated Group sleep time = 13.5±2.1, N=6, $p<0.01$). Lower doses of TAG (0.15 and 0.30 μmol/kg, ICV) were ineffective in antagonizing the taurine reinstated sleep time. TAG administration (ICV) by itself caused no observable alteration in behavior when the mice were compared with saline (ICV) treated animals.

Table 2 shows the effect of TAG to attenuate the return to

sleep time induced by taurine in the presence of ethanol. Three doses of taurine were used in this experiment. In addition, the TAU-Return to Sleep Time data were plotted in the form of double-reciprocal plots (Fig. 1). The effect (E) is the TAU-Return to Sleep Time data that are obtained with the administration of taurine (7.5, 15 or 25 μmol/kg, ICV) in the absence and presence of TAG (0.9 μmol/kg).

DISCUSSION

The results of these experiments show that taurine can prolong the effects of ethanol-induced sleep. It appears that the nature of the interaction between ethanol and taurine is a demonstration of the additive effects of two central nervous system depressants, since taurine has depressant effects on neuronal activity in the spinal cord and brain [5]. In addition high concentrations of taurine are found in the brain, although they are widely but unevenly distributed [11]. Particularly high levels of taurine are found in the olfactory bulb, cerebellum, cerebral cortex, hypothalamus and striatum [3,4]. Reports suggest that taurine may function as a neurotransmitter or play a modulator role in the central nervous system [7, 9, 15].

Recent work [13] with Sprague-Dawley rats shows that ICV injection of taurine after the administration of ethanol (4 g/kg, IP) increases the duration of ethanol-induced sleep time and that TAG, a taurine antagonist [20], reduces this effect of taurine to prolong ethanol-induced sleep time. This present study of the effects of taurine and ethanol in mice confirms the initial investigation from our laboratory in which rats were used. Furthermore, information is contained in the present work (Fig. 1) that suggest that the antagonism of taurine by TAG is noncompetitive in nature [8].

The exact mechanism by which taurine enhances the central depressant effect of ethanol is unknown. There is, however, some evidence to suggest that taurine may be a partial agonist of the GABA receptor complex [15]. In addition, when taurine is applied by microiontophoresis on neurons, it causes an increase in chloride ion permeability, producing hyperpolarization of membranes, and therefore, an inhibitory effect ensues [6]. Recent reports indicate that ethanol has an action on the GABA receptor complex. In rat brain synaptosomes ethanol enhances the postsynaptic GABA receptor-mediated chloride transport and this effect of ethanol does not appear to be related to GABA release [18]. An imidazobenzodiazepine derivative, Ro15-4513, antagonizes ethanol-stimulated chloride uptake into brain vesicles [19]. Ro15-4513 also attenuates the anticonflict activity of low doses of ethanol and the intoxicating effects of higher doses of ethanol. In a study which employed long sleep and short sleep mice, bred for differential sensitivity to the hypnotic effect of ethanol, the results suggest that the effects of ethanol-induced hypnosis in these mice may be related to differences in the sensitivity of the GABA receptor-chloride channel complex to ethanol [1]. Several investigators indicate that the effect of ethanol on the GABA receptor-mediated chloride flux may explain many of the neuropharmacologic properties of ethanol [18,19].

Although some evidence suggest that the interaction between taurine and ethanol in the central nervous sytem may be related to the GABA receptor complex-mediated chloride flux, further experimentation is required before a definitive explanation can be given. In addition, the accumulation of information about the mechanism for the interaction between taurine and ethanol to induce hypnosis may add some insight into the mechanism for the noncompetitive antagonism of taurine by TAG in the central nervous system.

ACKNOWLEDGEMENTS

The author thanks Linda Bush and Colleen Graham for their assistance and typing of the manuscript.

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