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Effects of Ethanol on Passive Avoidance Behavior in the Mouse: Involvement of GABAergic Mechanisms

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CASTELLANO, C. AND F. PAVONE. *Effects of ethanol on passive avoidance behavior in the mouse: Involvement of GABAergic mechanisms.* PHARMACOL BIOCHEM BEHAV **29**(2) 321–324, 1988.—A passive avoidance methodology was used to test the effect of ethanol, and its interference with GABAergic mechanisms, on memory in male CD1 mice. Retention performance was reduced in a dose-related manner, by ethanol and by muscimol, a GABA agonist, while it was increased by the GABA antagonists picrotoxin and bicuculline. These effects were evident when treatments were carried out immediately, but not 120 min, after training, suggesting that they were due to a specific action of the drugs on the time-dependent memory consolidation process. The ethanol-induced reduction of retention performance was enhanced by muscimol and decreased by picrotoxin and bicuculline administrations. Taken together the results confirm the involvement of a GABAergic mechanism in memory consolidation and demonstrate that it underlies the negative effect of ethanol on passive avoidance behavior in the mouse.

Ethanol GABA Memory consolidation Passive avoidance Mice

RECENTLY a number of researches have demonstrated the involvement of the inhibitory neuro-transmitter γ-aminobutyric acid (GABA) in the central actions of ethanol. For instance, it is known that sedation, sleep and anesthesia produced by relatively high doses (2.0 to 5.0 g/kg) of ethanol in rodents are enhanced by GABA agonists whereas they are counteracted by GABA antagonists [10, 13, 15, 17, 20].

Furthermore, it has been shown that acute ethanol treatment increases the brain GABA content in rats and mice [6, 26, 28]. Finally ethanol has been reported to potentiate the inhibition of cortical neurons by GABA in cats [22] and to inhibit, through a GABAergic mechanism, the firing of pars reticulata (PR) neurons in rats [21]. Besides it has been suggested that GABA also plays a role in memory consolidation [11,12]. Indeed the facilitation of maze learning [3] and of brightness discrimination [14] with post-trial injections of the GABA antagonist picrotoxin, and the lack of memory consolidation across sessions in an active avoidance condition following post-trial administration of the GABA mimetic amino-oxyacetic acid (AOAA) [16] have been demonstrated in rats.

Most recently it has been demonstrated that ethanol produces a memory impairment in mice when they are tested in passive avoidance conditions [1, 5, 18]. The aim of the present research was to investigate the involvement of GABAergic mechanisms in the effects of ethanol on memory processes in the mouse.

For this purpose it has been studied whether the GABA receptor agonist muscimol [2], the specific GABA receptor antagonist bicuculline [24], or the blocker of chloride ion channels picrotoxin [29], were able to modify the retention performances of mice tested in the passive avoidance situation.

METHOD

Subjects

Male CD1 mice (River Labs, Como, Italy) weighing about 25 g at the beginning of the experiments were used. Upon their arrival in the laboratory (2 weeks before the experiments), all mice were placed in cages in groups of eight. Food and water were available ad lib, and the mice were kept on a 12-hr light/12-hr dark cycle (7 a.m.–7 p.m.) at a constant temperature (21°C).

In all experiments, the animals were used once. The test used and the experimental procedure adopted have already been described in detail [4]. During training each mouse was placed on a lighted platform, in front of which there was a hole leading to a dark compartment. The floor of this compartment consisted of an electrified grid. When the mouse stepped through the hole (outside the platform) onto the metallic grid it immediately received an inescapable footshock (0.7 mA, 50 Hz) that lasted 1 sec. The mouse was then replaced in its own cage waiting for the experimental test which followed 24 hr later. Test procedures were the same as training except that no shock was administered. The step-through latencies on the test day were assumed an index of memorization by the animals of the previous experience (footshock). The animals underwent a single learning trial before testing. Training and testing were performed between the hours of 2:00 and 5:00 p.m.

TABLE 1

MEAN STEP-THROUGH LATENCIES (±SEM) ON TEST DAY FOR SALINE AND ETHANOL INJECTED MICE

Treatment	g/kg	Latencies (sec)
Saline		98.87 ± 4.9
Ethanol	0.5	101.25 ± 5.7
Ethanol	1.0	50.00 ± 4.6*
Ethanol	2.0	20.62 ± 3.3*

*$p<0.01$ vs. saline.

Groups of eight mice tested in the passive avoidance apparatus 24 hr after training.

The mean step-through latencies on the training day ranged between 3.1 ± 1.2 and 3.6 ± 1.4 sec.

Retention scores of mice treated with ethanol (2.0 g/kg) 120 min after training: saline=96.34 ± 3.9 sec, ethanol=99.50 ± 4.5 sec.

Retention scores of mice that had not received footshock on the training day: saline=3.2 ± 0.6 sec, ethanol (2.0 g/kg)=2.8 ± 0.4 sec.

TABLE 2

MEAN STEP-THROUGH LATENCIES (±SEM) ON TEST DAY FOR SALINE, MUSCIMOL, PICROTOXIN AND BICUCULLINE INJECTED MICE

Treatment	mg/kg	Latencies (sec)
Saline		102.00 ± 4.1
Muscimol	0.5	104.50 ± 3.7
	1.0	58.25 ± 6.2*
	2.0	16.75 ± 2.9*
Picrotoxin	0.25	99.62 ± 5.4
	0.5	152.62 ± 5.2*
	1.0	180.00 ± 0*
Bicuculline vehicle		99.27 ± 5.6
Bicuculline	0.1	105.00 ± 3.8
	0.25	139.62 ± 7.8*
	0.5	193.50 ± 7.2*

*$p<0.01$ vs. saline.

Groups of eight mice tested in the passive avoidance apparatus 24 hr after training.

The mean step-through latencies on the training day ranged between 2.9 ± 0.8 and 3.7 ± 0.9 sec.

Retention scores of mice treated with drugs 120 min after training: saline=96.34 ± 3.9 sec, muscimol (2.0 mg/kg)=94.04 ± 2.9 sec, picrotoxin (1.0 mg/kg)=91.89 ± 3.7 sec, bicuculline (0.5 mg/kg)=101.6 ± 4.6 sec.

Retention scores of mice that had not received footshock on the training day: saline=3.2 ± 0.6 sec, muscimol (2.0 mg/kg)=4.1 ± 0.7 sec, picrotoxin (1.0 mg/kg)=3.9 ± 1.1 sec, bicuculline (0.5 mg/kg)=4.3 ± 1.2 sec.

Procedure

Four different protocols were used.

In a first series of experiments (A) the effects of the post-training administration of ethanol, muscimol, picrotoxin and bicuculline were investigated. Different groups of eight mice each were injected with ethanol (0.5, 1.0, 2.0 g/kg), muscimol (0.5, 1.0, 2.0 mg/kg), picrotoxin (0.25, 0.5, 1.0 mg/kg) or bicuculline (0.1, 0.25, 0.5 mg/kg). An additional group of mice was injected with the bicuculline vehicle only. The performance of these groups was compared to that of saline injected mice. All treatments were carried out immediately after training.

For experiment B four other groups of eight mice each were injected with ethanol (2.0 g/kg), muscimol (2.0 mg/kg), picrotoxin (1.0 mg/kg) or bicuculline (0.5 mg/kg) 120 min after training, and their performance was compared to that of mice injected with saline.

For experiment C four groups of eight mice each did not receive footshock and were injected immediately after training with ethanol (2.0 g/kg), muscimol (2.0 mg/kg), picrotoxin (1.0 mg/kg) or bicuculline (0.5 mg/kg). Their performance was compared to that of saline injected mice.

In experiment D the effect of muscimol (0.5 mg/kg), picrotoxin (0.25 mg/kg) or bicuculline (0.1 mg/kg) against ethanol (1.0 g/kg) on retention performance was investigated. For this purpose different groups of eight animals each were injected with ethanol immediately after training and then, 1 min later, with the GABAergic agents. Their performance was compared to that of a saline plus saline injected group.

All experimental groups were tested in the passive avoidance apparatus 24 hr after the training session. Ethanol concentration was adjusted with saline (0.9% NaCl) so that all animals received 0.1 ml liquid per 10 g of mouse weight [18]. Picrotoxin and muscimol (Sigma Chemical Corp., St. Louis, MO) were dissolved in saline. Bicuculline (Sigma Chemical Corp., St. Louis, MO) was dissolved in a few drops of 0.1 N HCl, after which the final volume was made up with saline. The drug solutions were injected at a volume of 4 ml/kg. Saline (0.9% NaCl; 4 ml/kg) was used for control treatments. All injections were given intraperitoneally (IP).

The results were evaluated by ANOVA (1- and 2-way), in which the mean step-through latencies on the test day were compared [4]. Further analyses for individual between groups comparisons were carried out with post hoc tests (Duncan multiple range test).

RESULTS

Experiment A

Ethanol administration induced a dose-dependent impairment of retention performance (Table 1).

ANOVA (1-way) showed significant difference between groups, $F(3,28)=86.56$, $p<0.001$.

Individual between treatment comparisons showed significant differences between the performance of the control group and those treated with 1.0 and 2.0 but not 0.5 g/kg of ethanol ($p<0.01$).

In a similar fashion the administration of muscimol induced a dose-dependent impairment of retention performance (Table 2).

ANOVA (1-way) showed significant differences between groups, $F(3,28)=83.32$, $p<0.001$.

Individual between treatment comparisons showed significant differences between the performance of the control group and that of the groups treated with 1.0 and 2.0 but not 0.5 mg/kg of muscimol ($p<0.01$).

On the contrary picrotoxin administration induced a dose-dependent improvement of retention performance (Table 2).

ANOVA (1-way) showed significant differences between groups, $F(3,28)=79.41$, $p<0.001$.

Individual between treatment comparisons showed significant differences between the performance of the control

TABLE 3

MEAN STEP-THROUGH LATENCIES (±SEM) ON TEST DAY FOR SALINE, ETHANOL + MUSCIMOL, ETHANOL + PICROTOXIN AND ETHANOL + BICUCULLINE INJECTED MICE

Treatment	g/kg	Treatment	mg/kg	Latencies
Saline		Saline		100.87 ± 4.5
Ethanol	1.0	Saline		62.62 ± 3.8*
Ethanol	1.0	Muscimol	0.5	20.25 ± 4.2*
Ethanol	1.0	Picrotoxin	0.25	93.87 ± 4.8
Ethanol	1.0	Bicuculline	0.1	101.25 ± 5.7

*$p<0.01$ vs. saline.

Groups of eight mice tested in the passive avoidance apparatus 24 hr after training.

The mean step-through latencies on the training day ranged between 3.3 ± 0.6 and 4.2 ± 1.2 sec.

group and that of the picrotoxin (0.5 and 1.0, but not 0.25 mg/kg) injected mice ($p<0.01$).

Moreover the bicuculline administration also induced dose-dependent improvement of retention performance (Table 2).

ANOVA (1-way) showed significant differences between groups, $F(3,28)=23.20$, $p<0.001$.

Individual between treatment comparisons showed significant differences between the performance of the control group and that of the bicuculline (0.25 and 0.5, but not 0.1 mg/kg) injected mice ($p<0.01$). The performance of the mice injected immediately after training with the bicuculline vehicle was not different from that of the saline injected mice.

Experiment B

No difference from the control group was found when animals were treated with ethanol or the other drugs 120 min after training, suggesting that the effects observed were mediated by a specific action of the drugs on the time-dependent memory consolidation process (see Tables 1 and 2).

Experiment C

The mean step-through latencies of the mice that had not received footshock on the training day, but had been injected with saline, ethanol, muscimol, picrotoxin or bicuculline, did not differ significantly from each other (See Tables 1 and 2).

Experiment D

Muscimol (0.5 mg/kg) enhanced while picrotoxin (0.25 mg/kg) and bicuculline (0.1 mg/kg) decreased the effect of ethanol (1.0 g/kg) on retention performance (Table 3).

ANOVA (2-way) showed significant main effects for both ethanol and muscimol, or picrotoxin or bicuculline, treatments, $F(1,28)=212.03$ and 24.85, 34.04 and 16.89, and 12.03 and 12.55, respectively, $p<0.01$.

A significant interaction of ethanol versus muscimol, picrotoxin and bicuculline was also evident, $F(1,28)=33.32$, $p<0.001$; 9.57, $p<0.01$ and 14.39, $p<0.01$, respectively.

Individual between treatment comparisons showed no significant differences: (a) between the performance of the saline and that of the muscimol, or picrotoxin, or bicuculline injected groups, (b) between the performance of the bicuculline, or picrotoxin+ethanol and that of the saline injected mice, and significant differences: (a) between the performance of the ethanol and that of the saline injected groups, (b) between the performance of the muscimol+ethanol, and those of both the ethanol and the saline injected mice ($p<0.01$).

DISCUSSION

From the present research it is clearly evident that ethanol and muscimol exerted dose and time-dependent impairing effects on the retention performance of mice tested in a passive avoidance apparatus. On the other hand, bicuculline and picrotoxin administration improved the retention performance of the animals. In addition, muscimol enhanced, while bicuculline and picrotoxin reduced, the impairment of memory consolidation produced by ethanol.

These results, while confirming our and other results [1, 5, 18] on ethanol-induced memory impairment, indicate that, similarly to ethanol, muscimol exerts an impairing effect in this experimental paradigm, thus supporting that GABA transmission plays a role in memory consolidation, and an excess of GABA might disrupt this process. The GABAergic nature of the phenomenon appears further strengthened by the fact that bicuculline and picrotoxin, two known GABA antagonists, improved the retention performance of mice and antagonized the ethanol effect.

The involvement of GABA in memory consolidation has been suggested by several studies performed in a variety of experimental conditions. It has for instance been demonstrated that post-trial injections of picrotoxin improve memory consolidation in mice tested in a T maze [3] and in rats tested in a brightness discrimination task [14]. In addition, post-trial administrations of amino-oxyacetic acid (AOAA), an inhibitor of GABA catabolism, have been shown to impair memory consolidation in rats tested in active avoidance conditions [16].

There are also evidences that GABA mediates several behavioral effects of ethanol. It has for example been shown that AOAA enhances, while bicuculline reduces, the impairment of motor coordination induced by ethanol in rats in a tilting plane test [15]. Moreover, in the same animal species, the ethanol-induced increase in height of aerial righting is potentiated by muscimol and reduced by bicuculline [13] and, in mice, muscimol potentiates, while bicuculline and picrotoxin reduce, the ethanol-induced sedation [17]. Finally, an enhancement of GABA transmission during the motor incoordination, sedation and sleep produced by ethanol has been observed [10,20]. Thus the results of the present research support the hypothesis that the GABAergic mechanisms are involved in the effects of ethanol, and extend this hypothesis to its action on memory consolidation. Even considering that further neurochemical and neurophysiological studies will be necessary to better clarify the neural bases of this interaction, the present results might have therapeutic implications. It is in fact known that some of the most commonly prescribed anxiolytic drugs, such as benzodiazepines and barbiturates, enhance the GABA-mediated neurotransmission and impair the acquisition of new information [7–9, 19, 23, 25]. In addition, it has been demonstrated that these drugs potentiate the ethanol effects in animals [21] as well as in humans [27]. Since ethanol has been shown to impair memory processes in humans [30], it is pertinent to expect that the amnesia produced by ethanol might be worsened by benzodiazepines and barbiturates thereby adding a further undesirable side effect to these drugs.

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