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# Neurosteroids Block the Memory-Impairing Effects of Ethanol in Mice

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MELCHIOR, C. L. AND R. F. RITZMANN. *Neurosteroids block the memory-impairing effects of ethanol in mice.* PHARMACOL BIOCHEM BEHAV **53**(1) 51–56, 1996.—Using a win-shift foraging paradigm to assess working memory in C57BL/6 mice, the memory-enhancing effect of low doses of the neurosteroids 5-pregnen-3β-ol-20-one [pregnenolone (PE)], 5-pregnen-3β-ol-20-one sulfate [pregnenolone sulfate (PS)], 5-androsten-3β-ol-17-one [dehydroepiandrosterone (DHEA)], and 5-androsten-3β-ol-17-one sulfate [dehydroepiandrosterone sulfate (DHEAS)] were demonstrated. The neurosteroids 5β-pregnan-3α-ol-20-one [pregnanolone (PA)] and 5β-pregnan-3β-ol-20-one [epipregnanolone (EPI)] disrupted memory in this paradigm. PE, PS, DHEA, DHEAS, and PA were also capable of blocking the memory-impairing effect of 0.5 g/kg ethanol. EPI prevented PA from blocking the effect of ethanol. The influence of these compounds on memory and their interactions on this behavior are consistent with their actions on the $GABA_A$ system.

Memory Ethanol Neurosteroids Pregnenolone Pregnenolone sulfate Pregnanolone Dehydroepiandrosterone GABA

FLOOD and colleagues demonstrated that pregnenolone (PE) and several other steroids that are formed in the brain, including pregnenolone sulfate (PS), dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS), have memory-enhancing effects in mice in avoidance paradigms (5,7,31). The most extensively examined of these neurosteroids, DHEAS, counteracts the amnestic effects of the protein synthesis inhibitor anisomycin and the muscarinic cholinergic receptor blocker scopolamine (7). DHEAS also improves memory in mice with age-induced memory deficits (6).

Several investigators have shown that PS, DHEA, and DHEAS can act as allosteric antagonists in the $GABA_A$-receptor system (4,12,14–16). PE has been suggested to function as an inverse agonist (32). Other steroids in this metabolic pathway that are found in the brain, such as allopregnanolone, act as agonists in the $GABA_A$ system (8,9,24,28,33). Because $GABA_A$ antagonists can improve memory and agonists impair memory (1), probably as a result of the modulation of the cholinergic system by GABAergic neurons, the influence of the neurosteroids on the $GABA_A$ system may account for their influence on memory. This notion is supported by the findings of Mayo et al. (17), who reported that PS improves memory in a Y-maze recognition task, whereas 5α-pregnan-3α-ol-20-one (allopregnanolone) impairs it when the compounds are injected into the nucleus basilis magnocellularis of rats. Because this nucleus, which is a major source of cholinergic neurons and is known to be involved in memory processes, has a GABAergic afferent, these results support the hypothesis that the memory-enhancing effects of the neurosteroids are due to their $GABA_A$-antagonist properties.

The acute administration of ethanol causes an impairment of memory (2,3,19). This effect of ethanol can be enhanced by the $GABA_A$ agonist muscimol and decreased by the $GABA_A$ antagonists picrotoxin and bicuculline (2) and the benzodiazepine receptor partial inverse agonist Ro 15-4513 (3). The purpose of the present experiment was therefore twofold: a) to verify that neurosteroids known to enhance or impair memory in other paradigms can function similarly in a paradigm that tests working memory, and b) to determine whether neurosteroids interact with the amnestic effect of ethanol. We used the win-shift foraging paradigm in this study, because this procedure specifically tests working memory, which is the type of memory particularly disrupted in humans suffering from pathologies associated with dementias such as Alzheimer's disease and stroke (25,29).

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## METHODS

### *Subjects*

All subjects were C57BL/6 male mice obtained from NCI (Frederick, MD). Mice weighed 20–25 g on arrival at the medical center. They were placed in the animal housing facility, which has a 12 L : 12 D cycle and heat- and humidity-controlled rooms. At the completion of the 1-week quarantine, mice were placed in individual cages (18 × 28 × 12 cm, W × L × H) and food restriction was begun. Mice were fed daily at 1200–1300 h. Food was limited until mice were at 80% of free-feeding weight. Each day before feeding, mice were handled and weighed. Behavioral testing was conducted in a laboratory with fluorescent ceiling lighting during the animal's light phase, between 1200 and 1400 h; on these days animals were fed just after testing.

### *Memory Test: Win-Shift Foraging Paradigm*

In the win-shift foraging test of memory (19,25,29) we used a standard T-maze, with the stem and each arm measuring 35 × 5 × 5 cm (L × W × H). Prior to testing, mice were shaped, which consisted of placing the mouse in the maze for 3 min with all doors open and reward (milk) in both goal boxes. This process was repeated until the mouse drank the milk. During memory testing, milk was present in both goal boxes on the first run. Thereafter, the mice were run for 10 trials with a particular intertrial interval with milk present only in the goal box opposite the one entered on the previous trial. The selection of the correct side resulted in the subject's receiving a reward of 0.5 cc of milk, which the subject was allowed to consume. The side of the maze entered was recorded and the percent correct out of the 10 trials was used as an index of memory. During these tests the latency to leave the start box was recorded as an index of motivation. In support of this interpretation, subjects not deprived of food typically do not leave the start box. The time to traverse the maze was recorded as a performance measure.

Prior to drug testing, all animals were tested at progressively greater intertrial intervals to establish their ability to perform the task. Starting at 30 s, the interval was increased in 30-s increments to 180 s. Only one interval was tested on a given day.

### *Procedure*

Mice were injected intraperitoneally (IP) with neurosteroids characterized as $GABA_A$ antagonists DHEA (5-androsten-3β-ol-17-one), DHEAS (5-androsten-3β-ol-17-one sulfate), or PS (5-pregnen-3β-ol-20-one sulfate); the neurosteroid characterized as an inverse agonist, pregnenolone [5-pregnen-3β-ol-20-one (PE)], a neurosteroid characterized as a $GABA_A$ agonist, pregnanolone [5β-pregnan-3α-ol-20-one (PA)], or epipregnanolone [5β-pregnan-3β-ol-20-one (EPI)], a weak $GABA_A$ agonist that can block some of the effects of pregnanolone, 30 min before testing. These compounds, obtained from Sigma Chemical Co. (St. Louis, MO), were suspended in a vehicle of 0.4% Tween 80 in saline and injected in a volume of 0.1 ml/10 g. Controls received the vehicle solution.

Previous studies (19,25,29) and preliminary data with the win-shift foraging paradigm indicated that the easier the task (shorter delays), the less likely for an amnestic agent to disrupt memory, whereas the harder the task (longer delays), the less likely for a drug to be able to improve memory. Therefore DHEA, DHEAS, PE, and PS were tested with the 180-s delay to establish their ability to improve memory; PA and EPI were tested with the 120- or 90-s delay to determine whether they disrupted memory. Only animals capable of performing the task with the 120-s interval at better than chance levels (i.e., >60% correct) were tested with drugs.

To assess the interaction of the neurosteroids with ethanol, mice were injected with a neurosteroid 30 min before testing, then with 0.5 g/kg ethanol, IP, 10 min before testing. Ethanol was prepared with 95% ethanol and saline and delivered in a volume of 0.1 ml/10 g. Controls received an equal volume injection of saline. The 120-s delay was used. The time of testing (i.e., beginning 10 min after injection) was selected to encompass the approximate time of plateau of brain ethanol levels. Previous studies showed that the neurosteroids did not influence ethanol pharmacokinetics (18,20).

After establishing that each animal could perform above chance levels (>60% correct) at a 120-s delay with vehicle/saline, each mouse was tested with vehicle/ethanol, and these data were used as the control data. With at least 2 days between assessments, mice were tested with a series of doses of a neurosteroid, presented in random order. Assessments with vehicle or saline and with vehicle or ethanol were performed at the end of the series of drug testing to assure that all mice could still perform the task at the previous control level and that long-term drug effects or tolerance to the amnestic effect of ethanol had not developed.

### *Statistics*

For statistical analysis percentages were normalized by arcsine transformation, whereas latencies and run times were normalized by reciprocal transformations. Group comparisons were performed using an analysis of variance followed by Dunnett's test to compare each group to the control. To determine whether performance was above chance, a one-tailed *t*-test against a theoretical distribution was performed. Several different groups of animals were employed. A given animal

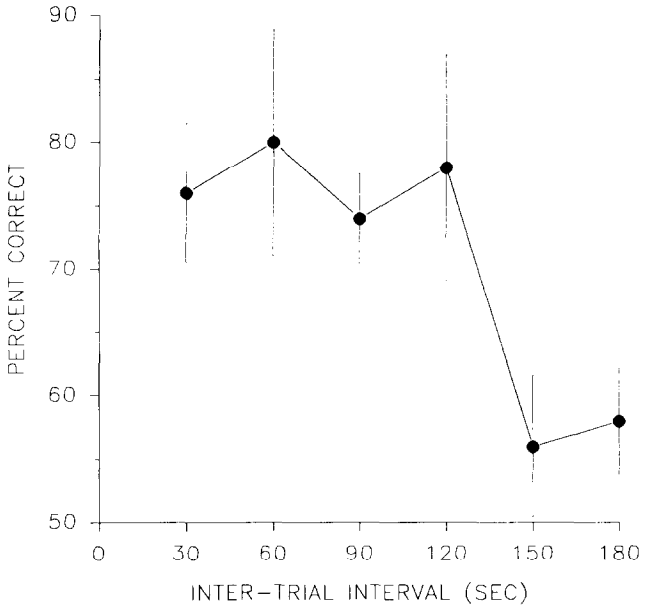


FIG. 1. Performance of C57BL/6 mice in the win-shift foraging paradigm with different intertrial intervals. Percent correct indicates the average percent correct responses out of 10 trials.

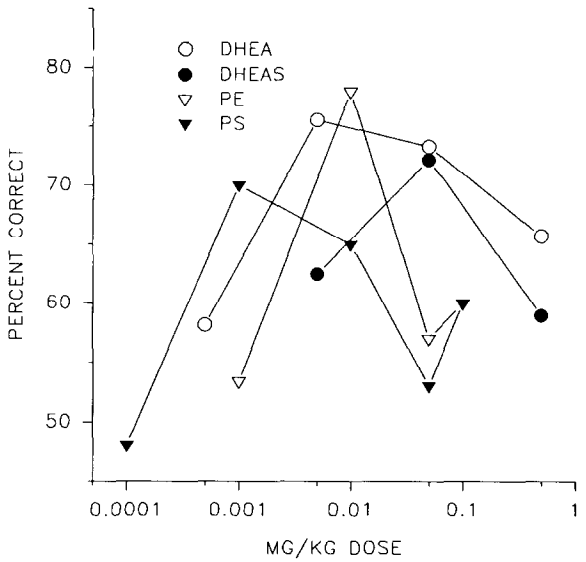


FIG. 2. Percent correct responses in the win-shift paradigm with a delay of 180 s following the administration of various neurosteroids. All values above 70 are significantly different from the control level of 58. $n$ = 6–13 per point.

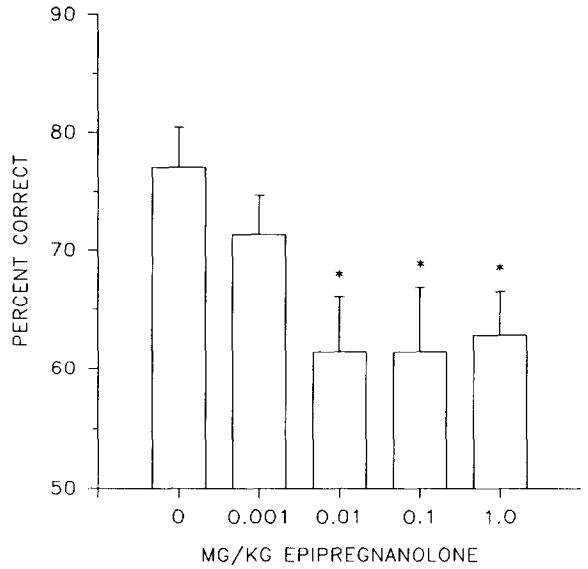


FIG. 3. Percent correct responses in the win-shift paradigm with a delay of 90 s following the administration of various doses of EPI. $n$ = 7. *$p < 0.05$ compared with control.

was used for testing only one neurosteroid, and different animals were used for determining dose-response curves for the neurosteroids from those used for testing ethanol effects.

## RESULTS

### *Baseline*

At short intertrial delays of 30–120 s, mice not injected with drugs entered the opposite side of the maze from that which they had entered on the previous trial significantly more often than chance (>60%). At delays of 150 s or longer, untreated mice performed at chance levels (Fig. 1). As in previous studies (29), it was determined that in this strain of mice, the longest delay at which performance was above chance was 120 s. To observe a disruption in memory, we used a 90- or 120-s delay. However, because of the problem of a ceiling effect, an improvement in memory could not be detected with the use of these short delays. Therefore, to test for an improvement in memory, mice were tested at a delay of 180 s.

### *Neurosteroids*

Administration of a range of doses of DHEA, DHEAS, PE, or PS produced bell-shaped dose-response curves for the enhancement of memory with a 180-s delay (Fig. 2). Neither latency nor run time was altered by these neurosteroids.

As expected, PA disrupted memory compared with controls at a delay of 120 s. From a control level of 77.0 ± 2.6% (mean ± SEM, $n$ = 10), 0.05 mg/kg reduced performance to 61.5 ± 3.8% ($n$ = 10), whereas 0.1 mg/kg reduced performance to 53.0 ± 6.0% ($n$ = 10). Unlike any of the other drugs or drug combinations, mice treated with PA again performed at no better than chance levels 2 days after either dose. Therefore, these mice were not used again and no further doses of PA were tested.

Epipregnanolone also disrupted memory. As shown in Fig. 3, a wide range of doses of epipregnanolone significantly impaired memory on this task compared with controls.

### *Neurosteroids Plus Ethanol*

As previously reported (19), 0.5 g/kg ethanol impaired performance on this memory task (Fig. 4). At 0.05 mg/kg, each of the compounds shown to enhance memory by itself—

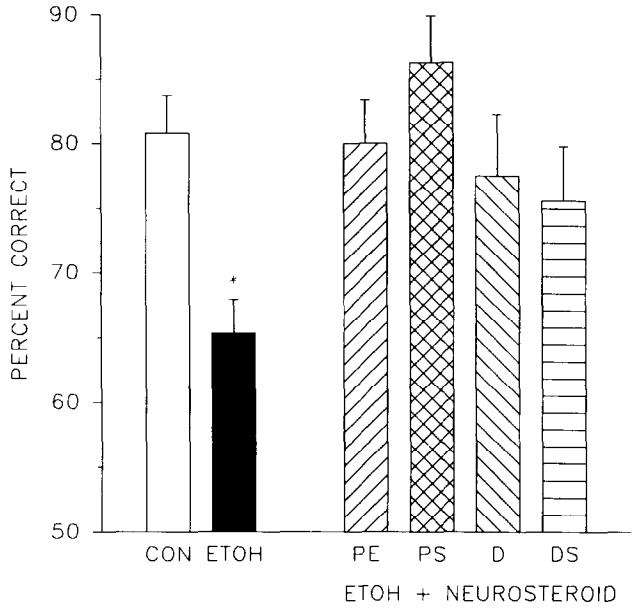


FIG. 4. Percent correct responses in the win-shift paradigm with a delay of 120 s following the administration of 0.05 mg/kg of various steroids followed by 0.5 g/kg ethanol. $n$ = 8–13/group. *$p < 0.05$ compared with all other groups.

DHEA, DHEAS, PE, and PS—blocked the amnestic effect of ethanol (Fig. 4).

This 0.05-mg/kg dose of DHEA in combination with ethanol caused an increase in run time (4.31 ± 1.23 s, mean ± SEM, $n$ = 8) compared with the ethanol (1.17 ± 0.08 s, $n$ = 13) or control (1.44 ± 0.35 s, $n$ = 13) groups. As this group of animals appeared to spend some time sniffing and rearing at the choice point of the maze, the increased run time may be due to a decrease in the stress of food deprivation (anxiolytic effect) reducing motivation rather than an adverse effect on motor abilities (21).

Surprisingly, PA, which disrupted memory by itself, was also effective in a wide range of doses in blocking the amnestic effect of ethanol (Fig. 5). No long-term effect of PA was noted when it was given with ethanol. Both latency and run time increased with doses of PA of 3.0 mg/kg and higher, probably because of an effect on motor activity (18).

In contrast to PA, EPI, which disrupted memory by itself, did not block the amnestic effect of ethanol (Fig. 5).

When EPI was administered at the same time as PA, PA no longer blocked the effect of ethanol (Fig. 6).

## DISCUSSION

Expanding on the findings of Flood et al. (5,7,31) that various steroids improve memory in an avoidance task, this study showed that the neurosteroids affect memory in a paradigm that specifically tests spacial working memory. The compounds that improve memory—DHEA, DHEAS, PS, and PE—have been shown to act as $GABA_A$ antagonists or, for PE, as an inverse agonist (4,12,14-16,32). PA, characterized as a $GABA_A$ agonist, impaired memory. EPI, a very weak $GABA_A$ agonist (26), also impaired memory. These findings are clearly consistent with the actions of the neurosteroids on the $GABA_A$ system and the hypothesis that $GABA_A$ antagonists improve memory, whereas $GABA_A$ agonists impair it (1).

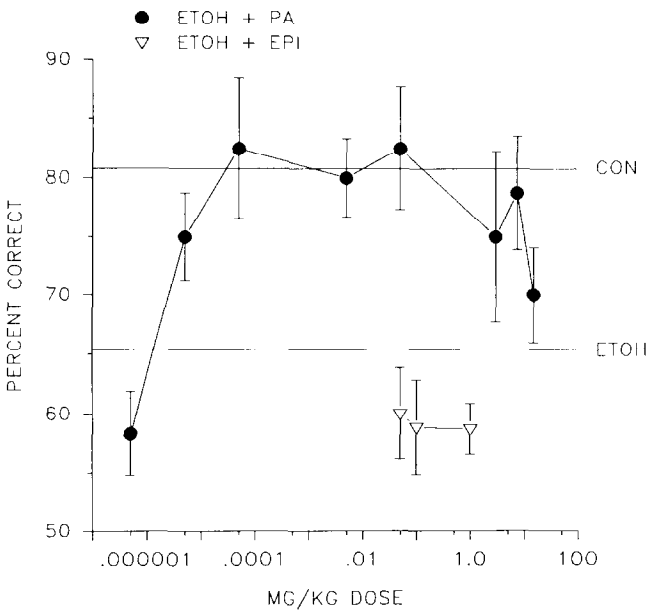


FIG. 5. Percent correct responses in the win-shift paradigm with a delay of 120 s after the administration of various doses of PA or EPI followed by 0.5 g/kg ethanol (ETOH). $n$ = 6-8/group. Horizontal lines indicate the levels of response for mice that received vehicle injections (CON) or those that received only ethanol (ETOH).

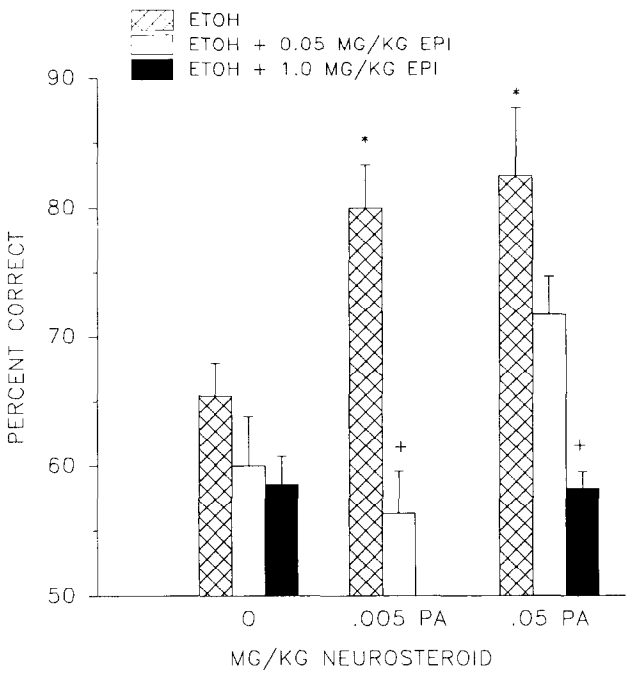


FIG. 6. Percent correct responses in the win-shift paradigm with a delay of 120 s after the administration of various combinations of steroids followed by 0.5 g/kg ethanol (ETOH). $n$ = 6-9/group. *$p < 0.05$ compared with ETOH with no neurosteroid; †$p < 0.05$ compared with ETOH with the same neurosteroid.

All of the compounds that improved memory by themselves—DHEA, DHEAS, PS, and PE—also prevented the memory impairment produced by ethanol. Again, this finding is consistent with other work demonstrating the effects of GABAergic compounds on ethanol-induced memory impairment (2,3).

The surprising finding in this study was that PA, which impaired memory by itself, blocked the memory-impairing effect of ethanol. Allopregnanolone, which has a biochemical profile similar to PA (9), also disrupts memory by itself (17). Majewska (12) demonstrated that the increase in $^3$H-muscimol binding produced by allopregnanolone in rat cortical synaptosomal membranes was significantly reduced in the presence of low levels of ethanol. Thus, whereas both ethanol and PA alone have $GABA_A$-agonist properties, together in low levels they may have a reduced impact on the system.

Other behavioral studies of the influence of PA on the effects of ethanol show that PA enhances ethanol's effects (18). However, these studies used high doses of these compounds to explore the interactions of PA and ethanol on anesthesia, motor activity, or body temperature (18). Different interactions between these two compounds could occur at different doses. Biphasic effects of ethanol are well recognized. In addition, the mechanism of action has been suggested to be different for anesthetic and subanesthetic concentrations of ethanol [i.e., the high-dose effect does not depend on subunit composition of the $GABA_A$-receptor complex, whereas the low-dose effect requires the presence of the $\gamma$-2L subunit (23)]. For PA, differential dose effects have also been noted. In

assessing the electrophysiologic actions of PA, Harrison et al. (9) reported that low levels increased the peak amplitude of GABA responses without effecting baseline membrane current level, whereas higher doses additionally produced a substantial inward current response alone. The existence of high-affinity and low-affinity binding sites for PA in brain membranes has been clearly demonstrated (10), and the existence of multiple steroid binding sites has often been suggested (14,24,27).

EPI, the 3$\beta$ isomer of PA, has been shown to be a specific competitive antagonist of PA at a recognition site for PA at the $GABA_A$ receptor (26,27). EPI's ability to prevent the blockade of ethanol-induced impairment of memory by PA is consistent with the action of EPI as an antagonist of PA at a neurosteroid modulatory site on the $GABA_A$ receptor.

For some of the other neurosteroids, differential interactions with ethanol can be seen by comparing the profile of steroid interactions with ethanol on memory reported here, and previous studies testing anxiety (21,22). In examining the interactions of a range of low doses of several neurosteroids with the anxiolytic effects of a low (1.5 g/kg) dose of ethanol, PE and to some extent DHEAS were effective in blocking the anxiolytic effects of ethanol, but DHEA enhanced the anxiolytic effect of ethanol (21,22). Given that different areas of the brain are involved in the control of different behaviors, these data indicate that, along with different sites of action for various steroids (4,13), the interaction between neurosteroids and ethanol varies across brain areas.

Previous studies have shown that the acute administration of some of the neurosteroids used here does not affect ethanol pharmacokinetics (18,20). Ethanol may, however, affect levels of neurosteroids. Although anesthetic doses of ethanol cause a rise in PE levels (11), moderate levels of ethanol (150 mg%) have been reported not to change the levels of PE or PS in rat brain (30). In contrast, moderate ethanol levels resulted in a dramatic decrease in DHEA and DHEAS in rat brain (30). It is therefore possible that one of the mechanisms for the effect of ethanol on memory is an alteration in levels of neurosteroids.

In summary, the results demonstrate the ability of several neurosteroids to affect working memory in a win-shift paradigm by themselves and to interact with the memory-impairing effect of ethanol. The nature of the action of the neurosteroids in this paradigm is consistent with their effects on the $GABA_A$ system.

ACKNOWLEDGEMENTS

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