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Pharmacology of Ethanol and Glutamate Antagonists on Rodent Sleep: A Comparative Study

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PROSPERO-GARCÍA, O., J. R. CRIADO AND S. J. HENRIKSEN. *Pharmacology of ethanol and glutamate antagonists on rodent sleep: A comparative study.* PHARMACOL BIOCHEM BEHAV **49**(2) 413–416, 1994.—Twenty-five Sprague–Dawley rats were implanted with electrodes for standard sleep–wake cycle recordings. A guide cannula was stereotaxically implanted into the lateral ventricle. Rats were divided into five groups (n = 5) and challenged with an intraventricular administration of 10 μl of a 5 nM solution of either: ethanol (EtOH), MK-801, AP5 (noncompetitive and competitive NMDA receptor antagonists, respectively), CNQX (AMPA receptor antagonist), or saline. Rats were recorded polygraphically for the following 4 h. Results showed that, at comparable doses, all tested drugs reduced REM sleep. No significant changes were detected in slow-wave sleep or wakefulness. This selective effect of glutamatergic antagonists suggests that glutamate may be a selective modulator of REM sleep. These findings also show that EtOH shares similar pharmacological effects on the sleep–wake cycle of the rat. Ultimately, glutamatergic mechanisms could contribute to the EtOH-mediated reduction of REM sleep.

Excitatory amino acids antagonists Ethanol Slow-wave sleep Rapid eye movement (REM) sleep

NUMEROUS investigations have documented that ethanol (EtOH) alters the excitability of neurons in widespread areas of the CNS (3,18) by selectively inhibiting synaptic excitation mediated by *N*-methyl-D-aspartate (NMDA) receptors (13,14, 22). Recently, it has been demonstrated that the systemic administration of EtOH or MK-801 (noncompetitive NMDA antagonist) prevents glutamate release induced by aspartate in the caudate nucleus of freely moving rats (2). In other studies, it has been shown that NMDA antagonists and EtOH share the capacity of impairing memory processes (8,23). Indeed, these drugs effectively prevent the induction of long-term potentiation (LTP is a widely used model of learning) in the hippocampus of the rat (8,15,19). These findings suggest that the syndrome of EtOH intoxication may be due, in part, to the impairement of excitatory amino acid (EAA) processes.

In addition, it is known that acute intoxicating doses of EtOH given to naive or alcoholic humans increases slow-wave sleep (SWS) at the expense of total rapid eye movement (REM) sleep time (24). Such an effect may be reproduced in rats by the inhalation of low doses of EtOH (6).

Local application of glutamate agonists into the pontomedullary region of cats induces REM sleep signs, like atonia and eye movements (12), presumably through an interaction with cholinergic cells (11). In addition, systemic administration of NPC 12626 (an NMDA antagonist) reduces REM sleep in a dose-dependent manner in rats (20). These findings suggest that EAA may be involved in REM sleep modulation.

Due to the existing evidence showing that low doses of EtOH mimic the effect induced by several EAA antagonists in behavioral tests (7,8,23), and that glutamate may play a role in REM sleep modulation, it can be hypothesized that EtOH may mimic some effects induced by EAA antagonists on sleep.

In this study we assessed the effects of EtOH, NMDA, and non-NMDA antagonists on the sleep–wake cycle of rats. We used low doses of these drugs to reveal selective effects on sleep. We decided to determine the similarity of the alterations induced by these drugs to further our understanding of EtOH neuropharmacology and the potential interaction with glutamatergic processes.

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METHOD

Twenty-five male Sprague-Dawley rats (300-350 g at the time of the surgery) were anesthetized with 2.5-3.0% halothane (Halocarbon Laboratories), and surgically implanted for sleep recordings. Two screw electrodes were placed in the parietal bone over the hippocampus (P = 4.0; L = 3.0), to record electroencephalogram (EEG). Two wire electrodes were placed in the external and internal canthus of the orbit to record eye movements (EOG). Postural tone (EMG) was recorded through two wire electrodes inserted into the neck musculature. A stainless steel cannula (23 gauge) was stereotaxically implanted into the lateral ventricle. Rats were individually housed and the light-dark cycle was controlled (12 : 12, lights on at 0630 h).

One week after the surgery, rats were habituated to the recording conditions for at least 2 days. Rats were recorded in a small cage (16 × 10 × 10″) placed inside of an enviromental chamber (35 × 34 × 29″).

Once the habituation period was completed, rats were divided into five groups (n = 5). Each group was challenged with an intraventricular (ICV) administration of 10 μl of a 5 nM solution of either: dizocilpine (MK-801, Merk Sharp and Dohme Research Lab), DL-2-amino-5-phosphono-pentanoic acid (APV, Sigma) (noncompetitive and competitive NMDA antagonists, respectively), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Tocris Neuramin, non-NMDA antagonist) or ethanol (EtOH, J. T. Baker). Control animals received 10 μl of saline. Due to the fact that these drugs have a short half life (about 2 h), rats were continuously recorded for only 4 h after the ICV injection (1000-1400 h). In addition, rats were observed for changes in spontaneous behavior through a one-way window.

In separate experiments the effect of these drugs on body temperature was measured using a similar administration paradigm (data not shown). A lubricated thermistor probe was inserted about 5 cm into the rectum to assess core temperature. A visual inspection of the probe, anus, and fecal matter expelled during this maneuver allowed to determine the absence of bleeding that could indicate intestine rupture. Temperature measurements were taken before and 1 h after the administration of any of the treatments.

Sleep recordings were visually scored and four stages were determined: wakefulness, slow-wave sleep 1 (SWS1), slow-wave sleep 2 (SWS2) and rapid eye movement (REM) sleep. Data were analyzed on the basis of total time in minutes of wakefulness, SWS1, SWS2, and REM sleep, and expressed as a percentage of the total time of recording. Latency to sleep onset as well as latency to the first REM sleep period was evaluated. The frequency and duration of the individual REM sleep episodes were determined. Statistical analysis of the data was carried out using an ANOVA and then a post hoc Sheffé test.

RESULTS

Results summarized in Fig. 1 and Table 1 show that all treatments effectively reduce REM sleep compared to saline, $F(4, 20) = 32.478\ p < 0.0001$. EtOH, as it can be seen in Fig. 1, has a tendency to maximally decrease total REM sleep time. The reduction of REM sleep was a result of a reduction in the frequency of appearance, $F(4, 20) = 11.497, p < 0.0001$, but not in the duration of the individual episodes. However, again EtOH has a tendency to decrease more than the other drugs the frequency of REM sleep. Likewise, EtOH tended to reduce the mean duration of REM sleep episodes. These drugs did not significantly modify the latency for sleep onset; however, EtOH prolonged the latency for the first REM sleep episode, $F(4, 20) = 3.326, p < 0.03$. As for the other stages of the sleep-wake cycle, APV and CNQX had the tendency to increase wakefulness (see Fig. 1). In comparison, EtOH had a stronger tendency to increase this stage, but none of these effects were significantly different from saline. MK-801 was the only drug modifying SWS. It reduced SWS1 and increase SWS2; however, none of these tendencies reached significancy. Regarding core temperature, none of these drugs modified it.

DISCUSSION

Our results show that the ICV administration of saline did not disturb the normal patterns of the sleep-wake cycle, compared to those extensively described in the literature [see (25)]. However, EtOH was unexpectedly potent in its ability to disturb sleep by altering REM sleep parameters. EtOH did not affect the other sleep stages, but it tended to increase wakefulness. In comparison, all three NMDA/non-NMDA antagonists were also capable of reducing REM sleep frequency. None of them modified the other sleep stages, but MK-801 had the tendency to decrease SWS1 and increase SWS2 (Fig. 1).

Others have explored whether low doses of EtOH disturb particular behaviors or the physiology of specific cerebral processes. The systemic administration of intoxicating doses of EtOH induces concentrations of EtOH in the brain of freely moving rats with a range of 200-1200 ng/μl of perfusate obtained with microdialysis probes (4,5). We have observed in pilot studies that similar doses, parenterally administered, dramatically affect sleep by inducing a period of prolonged wakefulness (about 2 h) followed by a period (2 h) of an increase in SWS without REM sleep recovery. This finding suggested that lower doses of ethanol affect sleep more selectively. Indeed, a few studies have proposed that low doses of EtOH induces changes in both behavior and sleep. For example, blood EtOH concentrations as low as about 150 μg/ml induce alterations in fixed ratio and continuous reinforcement schedules (7) and affects the sleep-wake cycle (6) similar to our findings. In addition, low doses of EtOH (200 μg/kg, IV), potentiate cortical GABAergic function in cats (16). These studies demonstrate that very low doses of EtOH affect both behavior and brain physiology.

In this study we have observed that a much lower concentration than those induced by systemic intoxicating doses of EtOH produced more specific effects on the sleep-wake cycle, i.e., EtOH may be able to affect REM sleep before any other stage of the cycle.

Moreover, our results showing that NMDA- as well as non-NMDA-antagonists reduce REM sleep frequency are in concurrence with other studies. For example, regional administration of glutamate agonists into the neocortex (1) or into the pontine area (12) increases sleep as determined by electrophysiological measures. Moreover, microdialysis studies have shown that glutamate reaches its highest concentration in the ventroposterolateral (VPL) thalamic nucleus during SWS (10). Similar studies have suggested that glutamate may affect the dorsal pontine cholinergic systems, those involved in REM sleep generation (11). In addition, REM sleep is blocked by NPC 12626 in a dose-dependent manner (20). These results support an active role of glutamate in REM sleep processes.

There are two brief reports proposing that glutamate antagonists increase both SWS and REM sleep (9,21). However,

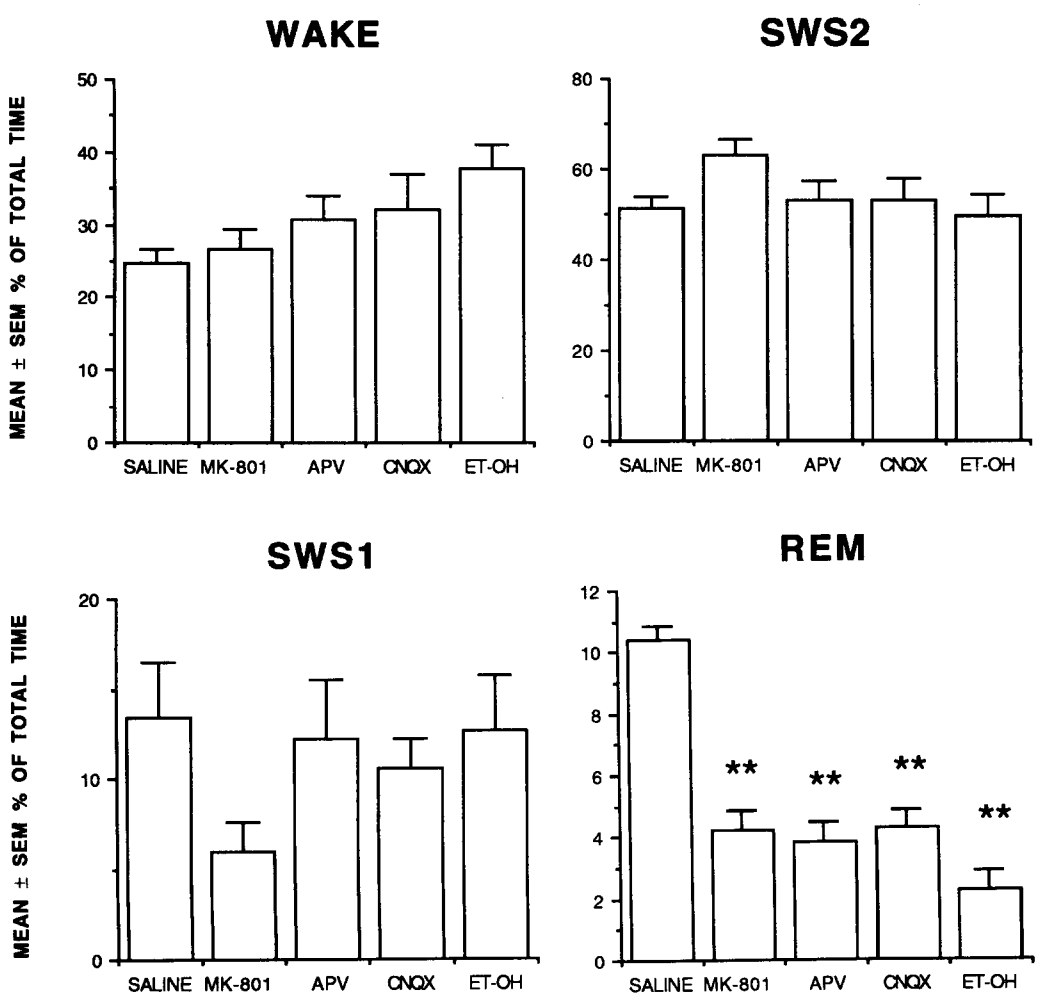

FIG. 1. Illustration of the effect of NMDA (MK-801 AND APV)- and nonNMDA (CNQX) antagonists and ethanol on the sleep-wake cycle of the rat. It can be onserved the prominent reduction of REM sleep elicited by these drugs, $F(4, 20) = 32.478$, $p < 0.0001$.

these effects, as suggested by the authors, may be a result of nonspecific actions of these drugs on the somatosensory systems or through the release of adenosine.

In contrast, our results indicate that low concentrations of glutamate antagonists reduce REM sleep and support the notion that glutamate plays an important role as a putative selective REM sleep modulator (11,12,20).

In summary, we observed that EtOH and the specific EAA

TABLE 1

DRUG-RELATED REM SLEEP PARAMETERS AND SLEEP ONSET LATENCY

	Sleep Lat	REM Lat	REM Freq	REM Dur
Saline	7.8 ± 3.07	22.1 ± 4.12	14.2 ± 1.42	1.85 ± 0.16
MK-801	19.4 ± 4.15	63.44 ± 7.2	7.8 ± 2.68†	1.26 ± 0.06
APV	19.22 ± 4.56	67.9 ± 15.4	6.4 ± 0.92†	1.48 ± 0.15
CNQX	14.16 ± 3.55	67.1 ± 12.13	6.2 ± 0.58†	1.71 ± 0.24
EtOH	15.5 ± 5.23	88.46 ± 20.8*	4.8 ± 1.11†	1.04 ± 0.24

Sleep lat (latency to sleep onset), REM lat (latency to the first REM sleep episode) and REM dur (duration of the individual REM sleep periods) expressed as mean ± SEM in minutes. REM freq (frequency of REM sleep), total number of REM sleep episodes in the total time of recording, $F(4, 20) = 3.326$.

*$p < 0.05$; $F(4, 20) = 11.497$, †$p < 0.0001$.

antagonists used in this study alter the sleep–wake cycle of rats in a comparable fashion. Although EtOH has been shown to have a diversity of actions on a wide variety of neurochemical systems (3,18), EtOH actions on EAA processes in general, and NMDA-related processes in particular, imply that this system may have a unique sensitivity to EtOH's effect. Our results indicate that EAA may play an important role in the selective regulation of REM sleep and that EtOH may primarily disrupt this sleep stage by altering EAA processes. Indeed, EtOH-induced selective effects on REM sleep may come about as a result of an alteration of identified glutamate-sensitive mesopontine neuronal mechanisms known to regulate REM sleep (11,17).

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