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Brief communication

Acamprosate attenuates the handling induced convulsions during alcohol withdrawal in Swiss Webster mice

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ABSTRACT

In the present study, we examined the effects of acamprosate for its ability to reduce handling induced convulsions (HICs) during alcohol withdrawal. Diazepam was used as a positive control. Swiss Webster male mice received three daily IP injections of alcohol (2.5 g/kg) or alcohol (2.5 g/kg)+methylpyrazole (4-MP) (9 mg/kg). (4-MP, being an alcohol dehydrogenase inhibitor slows down the breakdown of alcohol. 4-MP in combination with alcohol exhibits a dramatic increase in blood alcohol level compared to alcohol alone). Ten hours following the last alcohol injection, the mice were picked up by the tail and examined for their seizure susceptibility (HICs). Diazepam, a benzodiazepine known to reduce seizures during alcohol withdrawal, significantly reduced these HICs at doses of 0.25, 0.5 and 1 mg/kg (p's<0.001). Acamprosate, an anti-relapse compound used clinically in newly abstinent alcoholics, also reduced these HICs at doses of 100, 200 and 300 mg/kg (p's<0.05). This study supports the use of acamprosate during periods of alcohol withdrawal as well as during abstinence.

1. Introduction

Withdrawal from chronic alcohol consumption results in a variety of symptoms including hyper-excitability which can manifest as increased tremor, overactivity of the autonomic nervous system, and convulsions which can be potentially lethal [1,2]. Researchers have studied this increased susceptibility to seizures during alcohol withdrawal using rodents, particularly mice. Using a chronic alcohol exposure paradigm, primarily via inhalation chambers, it has been shown that seizure susceptibility increases dramatically with repeated bouts of alcohol exposure and withdrawal [3]. One measure that has been used to assess this withdrawal phenomenon is to assess behavior when the mouse is picked up by the tail. Mice experiencing alcohol withdrawal will often show some seizure activity when picked up in this manner. This handling induced convulsion (HIC) test was first developed by Goldstein and Pal [4] and then used by Crabbe et al. [5,6] and others [7,8]. Using defined criterion, a handling induced convulsion score is easily recorded by an experimenter blind to treatment condition and as stated above, this has been shown to be a sensitive indicator of CNS hyper-excitability during alcohol withdrawal [3]. In the present study, alcohol was used in combination with 4-methylpyrazole (4-MP) to elevate the peak, and prolong the duration of alcohol concentration in the blood. 4-MP is an alcohol dehydrogenase inhibitor and slows down the breakdown of alcohol in blood. Previous studies have shown that the addition of 4-MP in the presence of alcohol increased peak blood alcohol level as well as prolonging exposure by slowing down alcohol metabolism [9] so as to model chronic administration of alcohol.

Benzodiazepines (BZ's) remain one of the standard treatments during alcohol detoxification. One of the key features of the benzodiazepines, such as diazepam, is their ability to reduce the over excitation and increased risk of seizures and delirium tremens associated with alcohol withdrawal [10,11]. The mechanism for this action is primarily due to their modulatory action on the BZ binding site of the gamma-aminobutyric acid-A (GABA-A) receptor [12]. In the present study, diazepam was used as a positive control due to its ability to reduce the HIC susceptibility during alcohol withdrawal using the HIC procedural test [13].

The development of medications for alcohol dependence, more importantly for alcohol withdrawal symptoms has been significantly complicated by the multifaceted actions of alcohol at the neurotransmitter level. Although many medications have been evaluated for the treatment of alcohol withdrawal symptoms including those that interact with dopaminergic, serotonergic, opioid or glutamate and/or GABA systems, however, only naltrexone and acamprosate have

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Table 1
(Modified after Wilson and Little [24])

Score	Symptom
0	No activity on tail lift, or 360° spin
1	Facial grimace or tonic convulsion after 360° spin
2	Tonic/clonic convulsion after 360° spin
3	Tonic/clonic convulsion after 360° spin lasting more than 3 s
4	Tonic/clonic convulsion on tail lift itself

shown some clinical efficacy as a treatment option for individuals with alcohol dependence [14]. Acamprosate (calcium salt of *n*-acetylhomotaurine) has been used in Europe and elsewhere for a number of years and has recently received approval by the FDA in the U.S. to promote abstinence in recently detoxified alcoholics [14,15]. Acamprosate may act on both the positive and negative reinforcing effects of alcohol via its actions on glutamatergic and/or dopaminergic (DA) activity and was shown to reduce alcohol-conditioned anxiety in the elevated plus maze (EPM) [18]. While acamprosate does not possess addictive or reinforcing properties, both clinical and rodent studies have shown that acamprosate can reduce alcohol consumption and relapse [16,17]. In rodents, it has also been reported that acamprosate reduces alcohol-conditioned behaviors, including conditioned cues paired with alcohol [18], and the rewarding effects of alcohol [19]. *In vivo* and *in vitro* studies have also suggested that acamprosate may have neuroprotective effects during alcohol withdrawal [16,20,21].

Preclinical screens based on currently approved therapeutic drug agents could be used to better understand the mechanism for future novel drug targets which may have therapeutic potential in combating alcoholism. Additionally, approved therapeutic agents could be used to increase the predictive validity of such screens. Considering these, we examined the potential anticonvulsant effects of acamprosate on this measure of central nervous system over-excitability during alcohol withdrawal using diazepam as a positive control.

2. Methods

2.1. Animals

Male Swiss Webster mice (Harlan, IN) weighing 26–32 g at the time of the experiments were used. Animals were housed 3/cage in a colony room with a 16/8 light/dark cycle (lights on at 0600 h and off at 2100 h) with *ad libitum* access to mouse chow and water.

2.2. Drugs

Diazepam and 4-methylpyrazole were obtained from Sigma Chemicals, and acamprosate (acetylhomotaurine) was obtained from Merck & Co, Inc, Lyon, France. Diazepam was dissolved in 0.9% saline and was administered IP in doses of 0.25, 0.5 or 2 mg/kg (injection volume 10 ml/kg). Acamprosate was dissolved in 0.9% saline and was administered IP in doses of 100, 200 or 300 mg/kg (injection volume 10 ml/kg). The various doses of acamprosate doses were chosen based on previous literature documenting acamprosate's actions in a variety of alcohol paradigms [22,23]. 4-methylpyrazole (4-MP) (Sigma-Aldrich, USA) blocks alcohol dehydrogenase and thus slows down the breakdown of alcohol (Paez et al., 2004). 4-MP (9 mg/kg) was weighed separately, mixed with alcohol (Aaper Alcohol, Shelbyville, KY: 2.5 g/kg, 20% w/v) and administered intraperitoneally (IP), (injection volume 10 ml/kg) on three consecutive days between 12:30 PM and 02:00 PM. All drug solutions were freshly prepared daily and injected IP, 30 min prior to the behavior assessment.

2.3. Experiment 1

2.3.1. Blood alcohol concentration

Swiss Webster mice were exposed to IP injections of alcohol alone (2.5 g/kg) or alcohol+4-MP (9 mg/kg). Blood samples (40 µl) were taken from a small cut (1–2 mm) at the tip of the tail following the 3rd day of alcohol exposure and analyzed using the Analox AM1 Alcohol Analyzer (Lunenberg, MA). The Analox system measures the rate of oxygen uptake and in the presence of the alcohol oxidase enzyme, it is directly proportional to the alcohol concentration. Samples were taken 30, 60, 120, 240, 480, 600 min and 24 h after injection. 3–4 animals were used for each time point. Two batches of animals were used, with measurements taken at every other time point (30, 120, 480 or 60, 240, 600). Since blood collection constitutes a mild stressor, these animals were not used for the HIC experiments.

2.4. Experiment 2

2.4.1. Handling induced convulsion, HIC

The experimental paradigm for the HIC test used was similar to the one used in our earlier studies [13]. For chronic drug treatment, a total of 8 groups of mice (5/group) were used. 7 groups were given IP administration of alcohol (2.5gm/kg)+4-MP (9 mg/kg) injections for a period of 3 days with injections performed between 1245 and 1400 h. On day 3, after 10 h after they received their last injections, of the 7 groups that received alcohol+4-MP, 6 groups were given either of the three doses of diazepam (0.25, 0.5 and 1 mg/kg), or acamprosate (100, 200 and 300 mg/kg) while the 7th and 8th groups received saline injections. Behavioral scoring was based on a modified scale initially used by Wilson and Little [24]. The procedure began with lifting the mouse from its cage and observing it for any possible convulsions. If no convulsions occurred when picked up, the mouse was gently spun clockwise and counterclockwise 360° using the thumb and forefinger for approximately 5 s. A score of 0 indicated that there was no evidence of any convulsions while a score of 4 indicated severe seizures (Table 1). Scoring was conducted by an experimenter blind to treatment condition. The experimental protocols used in this set of experiments were approved by the University of Kentucky Institutional Animal Care and Use Committee.

2.5. Statistical analysis

The HIC scores were analyzed using ANOVA with treatment as the grouping factor (SPSS Version 12). Significant main effects were broken down with *post hoc* Tukey test. Level of significance was

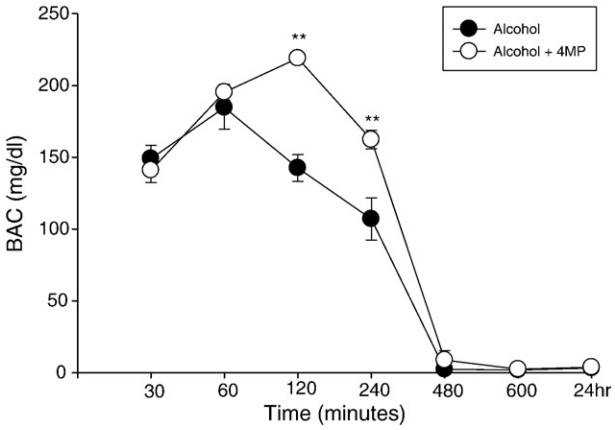

Fig. 1. Blood alcohol concentrations (BAC) after IP administration of alcohol (2.5 g/kg)+4-MP (9 mg/kg) on day 3. Values are expressed as mean±S.E.M. $^{**}p<0.001$ by *post hoc* Tukey HSD against the time points of alcohol group.