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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 1mg/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 300mg/kg (p 's < 0.05). This study supports the use of acamprosate during periods of alcohol withdrawal as well as during abstinence.

Keywords

Acamprosate; alcohol; withdrawal; mice; convulsion; Swiss Webster

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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-methyl pyrazole (4-MP) to elevate the peak, and prolong the duration of alcohol concentration in the blood. 4MP 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 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 overexcitability during alcohol withdrawal using diazepam as a positive control.

2. Methods

2.1. Animals

Male Swiss Webster mice (Harlan, IN) weighing 26–32 grams 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 0600h and off at 2100h) 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 10ml/kg). Acamprosate was dissolved in 0.9% saline and was administered IP in doses of 100, 200 or 300mg/kg (injection volume 10ml/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 (4MP) (Sigma-Aldrich, USA) blocks alcohol dehydrogenase and thus slows down the breakdown of alcohol (Paez *et al.*, 2004). 4MP (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 minutes 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 μ l) 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 (Lunenburg, 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 minutes and 24 hr 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) + 4MP (9mg/kg) injections for a period of 3 days with injections performed between 1245 and 1400 hours. On day 3, after 10 hours after they received their last injections, of the 7 groups that received alcohol + 4MP, 6 groups were given either of the three doses of diazepam (0.25, 0.5 and 1mg/kg), or acamprosate (100, 200 and 300mg/kg) while the 7th and 8th group 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 secs. 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 $P < 0.05$. Level of statistical significance between treatment groups was set at $p < 0.05$.

3. Results

3.1. Experiment 1

3.1.1. Effects of 4MP on blood alcohol levels—The first experiment was designed to examine blood alcohol levels following exposure to alcohol alone or alcohol + 4 MP using our exposure regimen. As shown in Figure 1, the group that received alcohol + 4MP displayed significantly higher blood alcohol levels at both the 120 and 240 min post alcohol time point, $F(13, 113) = 113.225$, $p < 0.001$; (** $p < 0.001$ by post-hoc tukey against the appropriate time point between alcohol versus alcohol + 4MP). Blood alcohol levels in both groups were reduced to minimal levels by 480 min post injection.

3.2. Experiment 2

3.2.1. Effects of diazepam and acamprosate on 4MP + alcohol withdrawal induced HIC's—As shown in Figure 2, alcohol + 4 MP resulted in increased CNS excitability during alcohol withdrawal relative to controls. Diazepam and acamprosate significantly reduced seizure susceptibility during withdrawal in the groups that received alcohol + 4MP. For diazepam, ANOVA revealed a main effect of treatment and subsequent *post-hoc* Tukey tests revealed that all three doses of diazepam (0.25, 0.5 and 1mg/kg) significantly reduced HIC scores relative to the control (alcohol + 4MP) group $F(5, 27) = 13.551$, $p < 0.001$. For acamprosate, ANOVA revealed a significant main effect of treatment with subsequent *post hoc* comparisons showing that acamprosate was effective at reducing HIC scores at the 100, 200 and 300mg/kg dose relative to the control (alcohol + 4MP) group, $F(5, 26) = 2.930$, $p < 0.05$.

4. Discussion

In this series of experiments, a relatively simple and rapid procedure to screen compounds for their ability to reduce HIC seizure susceptibility during alcohol withdrawal was described. Three daily injections of a moderate dose of alcohol (2.5 g/kg) in combination with 4-methylpyrazole produced an increase in seizure susceptibility during withdrawal in male Swiss Webster mice relative to either alcohol exposure alone or controls. This was most likely due to the increased and prolonged blood alcohol concentration that occurred as a consequence of exposure to 4MP in combination with alcohol [13]. The findings from this study also showed that the positive control, diazepam, and the anti-relapse agent acamprosate reduced this increase in seizure susceptibility during alcohol withdrawal. Our findings demonstrate that there wasn't a dose-dependent reduction in convulsions for both diazepam and acamprosate. However, the reduced convulsions between doses for either drug were not significantly different from each other. Previous studies in our laboratory suggested that 4MP in the absence of alcohol had no effect on seizure susceptibility and appeared to have little if any behavioral effects when administered in the absence of alcohol [13]. Behavioral variables such as grooming, sniffing and rearing were monitored across the three days of the administration procedure as an indicator of the physical condition of the mice. 4MP, nor alcohol + 4MP

resulted in any obvious adverse effects. This lack of adverse effects replicates some of the earliest work with 4MP and alcohol [25].

It should be noted that the severity of seizure scores following 3 days of 4MP injections in combination with alcohol were not as severe as those typically observed following alcohol exposure via inhalation chambers [26]. Still, the increased seizure susceptibility that was observed was consistent, reliable, and replicable and may be valuable as a model for more moderate, chronic alcohol exposure/use and withdrawal. Recently, this paradigm has been routinely used in our laboratory as a behavioral screening tool for novel therapeutic agents [13].

It was anticipated that diazepam would reduce alcohol withdrawal-induced seizures and this was the case. It is of particular interest that acamprosate had similar anticonvulsant actions with that of diazepam. The underlying mechanism or mechanisms for acamprosate's action are still not well understood. Early on, it was believed that the actions of acamprosate were via its effects on the inhibitory neurotransmitter GABA but this was based primarily on structural analysis with little empirical evidence to support this. Some of the strongest recent evidence supports the actions of acamprosate on glutamate function and/or receptors as the putative mechanism [27].

Acutely, alcohol decreases glutamate and N-methyl-D-aspartic acid (NMDA) receptor activity [28,29] while chronic alcohol exposure produces a compensatory increased sensitivity to glutamate receptor and NMDA receptor activity resulting in neuronal overexcitation and neurotoxicity during alcohol withdrawal [30,31]. Acamprosate reduces NMDA receptor mediated activity (and potentially reduces excitotoxicity) in cortical slices [32] although early data suggested a potentiation of NMDAR response in hippocampal pyramidal CA1 neurons [33]. Prior studies also indicate that acamprosate likely works by blocking the modulatory polyamine site of the NMDAR reducing the ability of polyamines to potentiate NMDAR activity [21,34]. This would have direct relevance for reducing alcohol-withdrawal induced neurotoxicity and potentially reducing the risk for seizures during alcohol withdrawal [35]. Activity at metabotropic GLURs has received considerable interest lately as a means by which relapse to drug use (i.e. alcohol, heroin, cocaine, amphetamine) can occur [36,37,38,39] and this may be a mechanism by which acamprosate reduces relapse to drinking as well as reduce a variety of conditioned responses to alcohol [40].

As a treatment agent, acamprosate has no overt effects on behavior (i.e., no sedation, stimulation or anxiolytic actions). Currently, it is clinically prescribed for newly abstinent alcoholics but typically is not prescribed until after the individual is detoxified and has undergone withdrawal. From the present studies, it is evident that high doses of acamprosate are required to reduce these seizures in mice, despite the relatively mild nature of the HICs in this paradigm. This low potency may explain the lack of previous reports, and reflects similar low potency in all the reported actions of acamprosate.

4.1. Conclusion

The current study suggests that this agent might be useful in conjunction with more traditional treatments (BZ) in detoxification itself and this would protect patients from relapse at an earlier stage. The major value of this study is that it describes a rapid and simple screen which can be used to evaluate novel drugs against alcohol withdrawal. That acamprosate is effective in this screen suggests that this screen may also be used to evaluate anti-relapse agents with an acamprosate-like mechanism. The predictive validity of the screen can, of course, only be assessed when large numbers of compounds have been evaluated this way, and thereafter to determine clinical efficacy for its usage.

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Figure 1.

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

Figure 2.

Effects of IP administration of Diazepam (0.25, 0.5 and 1mg/kg) and acamprosate (100, 200 and 300mg/kg) in male Swiss Webster mice 10 hours after the last 'alcohol + 4MP' injection on day 3 in HIC test. Drug solutions or saline (for 'control' or 'alcohol + 4MP' treated animals) were administered 30 minutes prior to the test. Values are expressed as $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.001$ by *post hoc* Tukey HSD against the alcohol + 4MP group.

Table 1

Modified after Wilson and Little, 1998

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 seconds
4	Tonic / clonic convulsion on tail lift itself