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Conditioned reinforcement and locomotor activating effects of caffeine and ethanol combinations in mice

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ABSTRACT

A growing trend among ethanol drinkers, especially young adults, is to combine caffeinated energy drinks with ethanol during a drinking episode. The primary active ingredient of these mixers is caffeine, which may significantly interact with ethanol. We tested the two hypotheses that caffeine would enhance ethanol-conditioned place preference and also enhance ethanol-stimulated locomotor activity. The interactive pharmacology of ethanol and caffeine was examined in C57BL/6 J (B6) mice in a conditioned place preference procedure with 1.75 g/kg ethanol and 3 mg/kg caffeine. Additionally, we used B6 mice to evaluate ethanol/caffeine combinations on locomotor activity using 3 doses of ethanol (1.75, 2.5 and 3.25 g/kg) and 2 two doses of caffeine (3 and 15 mg/kg). Both ethanol and caffeine administered alone increased preference for the drug paired side, although the effect of caffeine was more modest than that of ethanol. The drug combination produced significant place preference itself, but this was not greater than that for ethanol alone. Additionally, the combination of caffeine and ethanol significantly increased locomotion compared to giving either drug alone. The effect was strongest with a stimulatory dose of ethanol (1.75 g/kg) and waned with increasing doses of ethanol. Thus, combinations of caffeine and ethanol had significant conditioned reinforcing and locomotor activating effects in mice.

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1. Introduction

Heavy alcohol consumption continues to be a major health problem in the United States. One estimate indicates that one third of preventable deaths in the United States could be attributed to excessive alcohol (ethanol) consumption (Mokdad et al., 2004). Further, among ethanol drinkers, a growing trend in recent years has been to combine caffeinated energy drinks with ethanol which may facilitate more drinking. Over the past decade, the growth of energy drink sales has been tremendous (Reissig et al., 2009; Seifert et al., 2011). In the United States, epidemiological studies (Arria et al., 2010, 2011) supported by field testing data (Thombs et al., 2009) and frequent reports in the lay press indicate that consumption of ethanol mixed with energy drinks is common, particularly among young people. Unfortunately, consuming these mixtures has been associated with increases in emergency room visits (SAMHSA, 2011), suggesting that consumption of these drinks can be dangerous. In 2010, the Food and Drug Administration banned the manufacture and sale of pre-mixed energy drink/ethanol combinations (e.g. Four-Loko), although drinkers are still free to mix their own. Although mixing caffeinated energy drinks with ethanol appears to be widespread, mixing caffeinated beverages and ethanol is not a new practice nor is it limited to energy drinks. Indeed, other caffeinated drinks like colas are also popular mixers with ethanol (Rossheim and Thombs, 2011; Thombs et al., 2011). Regardless of whether it is an energy drink, cola or coffee, many investigators consider the primary pharmacologically active ingredient of these beverages to be caffeine. Therefore, it is important to examine the interactive pharmacology of these two widely used drugs, caffeine and ethanol, so that the interactive effects can be more clearly understood.

The interactive pharmacological effects of caffeine and ethanol have been studied in humans and to some extent in animal models. For example, research has shown that caffeine can antagonize, although not always completely, cognitive and psychomotor deficits induced by ethanol (e.g. Hasenfratz et al., 1993; Liguori and Robinson, 2001; Mackay et al., 2002). There is also some work using animal models to study the interactive pharmacology of caffeine and ethanol. For example, caffeine injections can increase ethanol drinking in rats (Kunin et al., 2000), and low doses (<10 mg/kg) of caffeine promote significant ethanol-induced taste aversions when combined with a low dose of ethanol (Kunin et al., 2001). Whereas the earlier studies in humans tended to focus on caffeine's ability to antagonize deficits caused by ethanol, the studies in rats indicate that caffeine can actually facilitate ethanol-related behaviors. In fact, recent research suggests that an energy drink combined with ethanol may actually enhance the desire to drink (Marczinski et al., 2013), which could be due to the interaction of the caffeine component of these drinks with ethanol. Therefore, in the context of the current trend to mix caffeinated beverages with ethanol, it is imperative to further develop our understanding of the facilitative effects of caffeine on ethanol-related behaviors. The use of animal models in this endeavor is crucial for establishing a strong

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foundation for additional work which cannot be conducted in humans; for example, examining the interactive effects of ethanol and caffeine in adolescents or at high dose levels.

Our laboratory has previously examined the interactive effects of the psychostimulant methylphenidate with ethanol in C57BL/6 J (B6) mice. In our prior studies, the addition of methylphenidate significantly enhanced ethanol-stimulated locomotor activity, increased signs of ataxia during ethanol intoxication and augmented discrimination of low ethanol doses (Griffin et al., 2010, 2012). Thus, low doses of a widely used psychostimulant co-administered with ethanol facilitated some behavioral effects of ethanol. Therefore, following from this earlier work, the present studies were designed to examine the interactive pharmacological effects of caffeine and ethanol in B6 mice. One common pharmacological target of ethanol and caffeine is the adenosinergic system. In particular, A_1 and A_{2A} adenosinergic receptors have been associated with arousal, locomotion and reinforcement (Hsu et al., 2009; Kuzmin et al., 2006; Lazarus et al., 2011). The adenosinergic system has also been implicated in the reinforcing effects of ethanol (Arolfo et al., 2004; Nam et al., 2013). Although the adenosinergic system is complex, at the most basic level, ethanol administration increases adenosine levels (Dunwiddie and Masino, 2001; Nagy et al., 1990) while caffeine non-selectively antagonizes adenosine receptors (Fredholm et al., 2011), providing a potential mechanistic explanation for the interactive effects of these two drugs noted in humans and laboratory animals. In the present study, we used two well-characterized behaviors, ethanol-induced conditioned place preference and ethanol-induced alterations in locomotor activity to examine ethanol–caffeine interactions. We hypothesized that the addition of caffeine to ethanol intoxication would enhance conditioned place preference and increase locomotor activity compared to either drug alone.

2. Materials and methods

2.1. Subjects

Adult male C57BL/6 J mice were used in these experiments (Jackson Laboratories, Bar Harbor, ME). Mice arrived at 8–9 weeks of age and acclimated at least 1 week before beginning studies. All mice were singly housed under standard conditions (12 h light cycle) in an AAALAC accredited facility with free access to food and water. All procedures were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee.

The mice used in the conditioned place preference experiment (Fig. 1; $n = 48$) were behaviorally naïve at the start of the experiment. The mice used in the locomotor activity experiments were not behaviorally naïve. The mice ($n = 12$) used in the activity experiment shown in Fig. 2 were previously part of a chronic treatment study in which they received vehicle gavage twice daily for 10 days. The mice used in Fig. 3 ($n = 12$) were previously vehicle injected mice and the mice used in Fig. 4 ($n = 12$) were previously ethanol treated mice, both groups from the present conditioned place preference experiment. Before beginning the locomotor activity experiment, the ethanol treated mice used in Fig. 4 had a 3-week washout period after completing the place preference experiment.

2.2. Drugs

Drugs were administered intraperitoneally using 0.9% saline as the vehicle at a volume of 0.02 ml/g of body weight in all experiments. Caffeine was used as the anhydrous base (Fluka, a subsidiary of Sigma-Aldrich, St Louis, MO) and 95% ethanol was obtained from AAPER (Shelbyville, KY). Before use, the ethanol was diluted with 0.9% saline [e.g. 12.2% (v/v) for a 1.75-g/kg dose]. When combined, caffeine and ethanol were administered in the same injection (i.e. simultaneously).

2.3. Conditioned place preference apparatus and procedures

The apparatus and procedures have been recently described (Griffin et al., 2012). We used 6 Med Associates (St. Albans, VT, USA) activity chambers (ENV-510) each equipped with two compartment place preference inserts (ENV-512). The inserts were modified so that the floor was smooth on each side, and the walls and floor were white on one side and black on the other, creating two distinct visual contexts (for complete details, see Griffin et al., 2012). For this experiment, the floor modification consisted of either solid white or black plastic sheets (TAP Plastics, Inc.) cut to fit exactly over the existing floors of the ENV-512 insert.

The dosing groups for this experiment were as follows (all $n = 12$): vehicle; caffeine, 3 mg/kg; ethanol, 1.75 g/kg; and the combination of caffeine and ethanol, 3 mg/kg + 1.75 g/kg. The dose of ethanol was chosen based on previous work (Griffin et al., 2012; Nocjar et al., 1999), indicating that it produces conditioned place preference in B6 mice using well-established procedures in our laboratory. A low dose of caffeine (3 mg/kg) was chosen because the literature indicated it would be borderline in terms of stimulating locomotion (Buckholtz and Middaugh, 1987; Hsu et al., 2009) and as well as producing significant place preference (Bedingfield et al., 1998; Patkina and Zvartau,

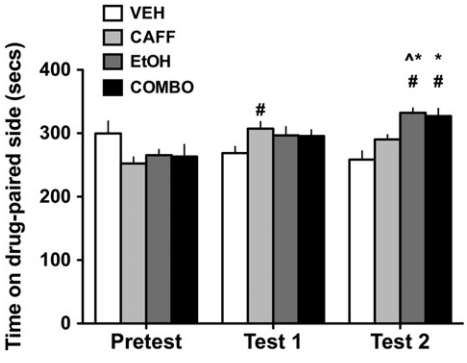

Fig. 1. Conditioned place preference with vehicle (VEH) caffeine (CAFF; 3 mg/kg), ethanol (EtOH; 1.75 g/kg) and the combination of caffeine and ethanol (COMBO) at those same doses. All three active drug groups demonstrated significant place preference, although the effect for caffeine alone was generally weak compared to ethanol and the drug combination. The drug combination did not demonstrate enhanced place preference either in the magnitude of time spent on the drug paired side or in the number of sessions needed to acquire significant place preference. Values are means ± SEM ($^{\#}p < 0.05$ within group compared to pretest; $^{*}p < 0.05$ within test compared to vehicle; $^{\wedge}p < 0.05$ within test compared to caffeine).

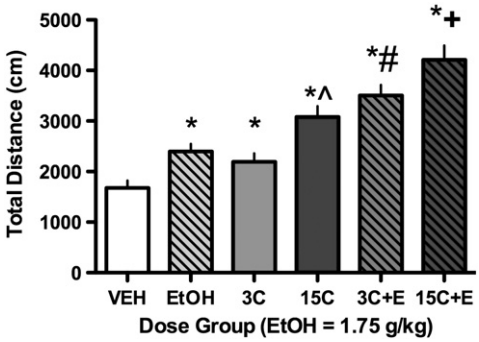

Fig. 2. Locomotor activity after challenge with ethanol 1.75 g/kg, caffeine 3 and 15 mg/kg and the combination of ethanol and caffeine. Ethanol at a known stimulatory dose (1.75 g/kg) increased total distance as did both doses of caffeine when given alone. The combination of caffeine at either dose with ethanol (1.75 g/kg) significantly increased total distance more than either caffeine or ethanol alone. Values are means ± SEM. Key: VEH = vehicle, EtOH = ethanol at the specified dose, 3C = caffeine 3 mg/kg, 15C = caffeine 15 mg/kg, 3C + E = caffeine 3 mg/kg + ethanol at the specified dose, 15C + E = caffeine 15 mg/kg + ethanol at the specified dose ($^{*}p < 0.05$ compared to vehicle; $^{\wedge}p < 0.05$ caffeine 15 mg/kg vs caffeine 3 mg/kg; $^{\#}p < 0.05$ compared to ethanol and caffeine 3 mg/kg alone; $^{+}p < 0.05$ compared to ethanol and caffeine 15 mg/kg alone).