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Expression of behavioral sensitization to ethanol is increased by energy drink administration

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A B S T R A C T

Alcohol abuse and dependence are important medical, social and economical problems, affecting millions of people. A relatively recent habit among young people is mixing alcohol with energy drinks (ED), in spite of the risks involved may be higher than those associated with alcohol consumption alone. The mixture of alcohol and energy drinks, both with stimulant properties, may alter the perception of intoxication and could lead individuals to believe they are less drunk and can drink more or for longer periods of time. In animals, the repeated administration of ethanol can lead to a progressive increase of the locomotor stimulant effect, known as behavioral sensitization, a drug-dependent behavioral plasticity associated with vulnerability to addiction. As well as for addiction, there are clear individual differences in the level of sensitization to ethanol among species and even among individuals from the same strain. The present study assessed how ED affects the expression of ethanol sensitization. Female mice chronically treated with ethanol (2.4 g/kg) were classified as low-sensitized or high-sensitized. Two days later, different groups of mice were submitted to saline + water, ethanol + water or ethanol + ED systemic challenges. As expected, only the high-sensitized group expressed clear sensitization after ethanol administration. However, the administration of ethanol + ED triggered the sensitization expression in the low-sensitized group. These data indicate that the combined use of ED and ethanol can potentiate the stimulant and, consequently, the reward effects of ethanol in previously treated mice. If a similar process occurs in human beings, the use of ED can increase the risk of developing alcohol abuse or dependence.

1. Introduction

Considering that the harmful use of alcohol results in the death of 2.5 million people annually (WHO, Global status report on alcohol and health) many efforts and studies have been conducted in order to determine the factors which facilitate the transition from occasional use to abuse or dependence. Recently, there has been an increase in the combined consumption of alcohol and energy drinks (ED — such as Red Bull®, Flying Horse®, Burn® etc). These beverages contain caffeine and have been marketed as providing increased alertness (Miller, 2008; Reissig et al., 2009; Seifert et al., 2011). Some concerns on the combined use of alcoholic beverages and energy drinks (AED) have been expressed, since recent studies with college students suggest AED consumption increases the probability of binge drinking and dependence development (Marczinski, 2011). There are reports on the use of ED to reduce the depressant effects of ethanol and to extend the duration, or even to increase the intensity, of its stimulant effects (Ferreira et al., 2004a, 2004c). In a previous study, we showed ED significantly reduced the subjective sensations of alcoholic intoxication, although when objectively evaluated they did not reduce the harmful effects of alcohol on visual reaction time, motor coordination and physical performance (Ferreira et al., 2004b). Although some reports did not detect an association between the use of ED and alcohol dependence development (Verster et al., 2012), significant methodological differences must be taken into account. Arria et al. (2011) showed that ED consumption is associated with increased risk of development of alcohol addiction. Recently, other authors (Cheng et al., 2012; Marczinski et al., 2012, 2013) demonstrated that mixing energy drinks with alcohol may increase the motivation to drink and the vulnerability to develop alcohol dependence.

Ethanol reinforcing properties have been associated with the stimulation of the dopaminergic mesocorticolimbic pathway (Wise and Bozarth, 1987). The repeated exposure to drugs of abuse, such as ethanol, progressively increases their psychomotor stimulant effects, a phenomenon known as behavioral sensitization and considered a form of drug-dependent behavioral plasticity associated with addiction vulnerability (Masur and dos Santos, 1988; Masur et al., 1986; Segal and Mandell, 1974; Vanderschuren and Kalivas, 2000). Psychomotor or

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behavioral sensitization to ethanol has been suggested as a behavioral marker for alcohol preference and/or abuse liability in both animals (Grahame et al., 2000; Lessov et al., 2001) and humans (Newlin and Thomson, 1999). This suggests that those individuals whose develop sensitization may be more vulnerable to develop addiction. Besides, there are evidences that behavioral sensitization is associated with relapse in drug addiction (for review see Steketee and Kalivas, 2011).

It is important to note that not all animals from the same species and strain present stimulation after ethanol (Masur and dos Santos, 1988) or develop sensitization. In Swiss mice, it is possible to observe important individual variability in the development and expression of behavioral sensitization to ethanol (Souza-Formigoni et al., 1999). We have recently demonstrated that variations in the development of ethanol sensitization reflect individual differences in addiction vulnerability since ethanol sensitized mice voluntarily drink more ethanol than non-sensitized or saline-treated control mice (Abrahao et al., 2013). Despite the evidence of interaction between the stimulant effects of ethanol and ED, there are few studies on the behavioral effects of AED in animal models of the rewarding properties of drugs of abuse (Ferreira et al., 2004c). Considering that ED can increase the stimulant effect of ethanol, we hypothesized that ED administration could also increase the intensity of behavioral sensitization, as well as the proportion of mice that express it.

2. Methods

2.1. Animals

Albino Swiss female mice, from the Departamento de Psicobiologia-UNIFESP, 35–50 g, aged 75 days at the beginning of the experiment, were housed in plastic cages (44 × 34 × 16 cm, 18–22 animals/cage) with free access to Purina chow and water (lights on 07:00 a.m. and off 07:00 p.m., 22 ± 2 °C). The research project was approved by the Committee of Ethics in Research of UNIFESP (563/01). The procedures were carried out in accordance with international norms of the *Guide for the care and use of laboratory animals* (1996).

2.2. Behavioral sensitization protocol

In order to induce sensitization to the stimulant effects of ethanol, we adopted previously described procedures (Quadros et al., 2005; Souza-Formigoni et al., 1999). For the assessment of their baseline locomotor activity, all the animals were initially evaluated in one 15 min session in a drug free situation, in Opto-Varimex cages (Columbus Instruments, Columbus, Ohio; 47.5 × 25.7 × 20.5 cm), which detect locomotor activity by the interruption of horizontal photoelectric beams. From one day after the baseline test on, seventy six mice were daily treated i.p. with saline (n = 30) or 2.4 g/kg ethanol (n = 46, 15.0% p/v, Synth®) for 21 days and their activity was weekly evaluated for 15 min in locomotor activity cages (Opto-Varimex Mini, Columbus Instruments, Ohio), immediately after the drug administration. Based on their locomotion on day 21, ethanol-treated mice were classified into two groups: the lowest half was considered as low-sensitized and the highest half as high-sensitized. This classification was used to define two profiles of locomotor response after the ethanol chronic treatment, allowing us to evaluate possible factors associated with the individual variability.

2.3. Challenge phase

On day 23, the three subgroups (saline, low-sensitized and high-sensitized) were divided into three challenge groups. The groups were separated taking into account their levels of activity during the development of behavioral sensitization to ethanol, making sure there were no baseline differences among them before the challenges. Different subgroups of mice were challenged with saline i.p. + water p.o; ethanol i.p. + water p.o. or ethanol i.p. + ED p.o (Fig. 1B). The ED Red Bull® (Fuschl/Austria — commercially available) was administered in a dose equivalent to 3 cans (250 ml/can) for a 70 kg human being (10.71 ml/kg). It is important to point out that this dose contains 3.43 mg/kg of caffeine, an important stimulant constituent of Red Bull. After the administration of the drugs, the activity was evaluated for 15 min immediately after drug administrations.

2.4. Data analyses

The locomotor activity counts during the 15 min tests, weekly performed during the treatment, were analyzed by two-way analysis of variance (ANOVA) for repeated measures, being group (saline, low-sensitized and high-sensitized mice) by the independent factor and time (the days of tests) and the repeated measure factor. The data from the challenge phase were also analyzed by a two-way analysis of variance (ANOVA) with group (saline, low-sensitized and high-sensitized mice) and challenge (saline + water, ethanol + water or ethanol + ED) as independent factors. The Newman–Keuls tests for multiple comparisons were used for *post-hoc* analyses.

In order to evaluate whether ED administration would change the proportion of stimulated mice, we computed the number of stimulated mice in each challenge test. We considered "stimulated" those whose locomotor activity levels were above the 95% upper limit of the confidence interval of the high-sensitized group levels on the ethanol + water challenge. In the saline + saline challenge no mice were considered stimulated according to this criterion. The statistical comparison of proportions was made using the test of proportions.

The level of significance adopted was 5% for all analyses. We used the Statistica® v9.0 software for all analyses.

3. Results

Regarding the development of behavioral sensitization phase, the ANOVA, considering the factors group (saline, low-sensitized and high-sensitized) and time of treatment (days 1, 7, 14, 21) detected significant effects of group ($F_{2,73} = 64.28$, $P < 0.001$), time ($F_{3,219} = 106.53$, $P < 0.001$) and their interaction ($F_{6,219} = 41.87$, $P < 0.001$) (Fig. 1A). High-sensitized mice presented higher locomotor activity levels than the other groups on days 14 and 21 ($P < 0.05$) and higher locomotion on day 21 than on days 1 and 7 ($P < 0.05$), demonstrating the development of behavioral sensitization to the stimulant effect of ethanol (Fig. 1A).

The challenge phase of the experiment was performed in order to compare saline, low-sensitized and high-sensitized mice locomotor stimulation after ethanol or the combined administration of ED and ethanol. No differences among groups were found under saline + water challenge ($F_{2,21} = 0.82$). As expected, in the ethanol + water challenge ($F_{2,23} = 6.33$, $P < 0.05$), only the high-sensitized group presented higher activity levels than controls, demonstrating the expression of behavioral sensitization only in those mice that had developed high levels of sensitization to ethanol. However, when the mice received ethanol + ED ($F_{2,23} = 10.90$, $P < 0.05$), higher activity levels were observed both in the low and in the high sensitized groups when compared to saline pre-treated control mice.

Using the criteria of stimulation effect described in Section 2.4, we analyzed the percentage of mice considered stimulated after drug administration. From the high-sensitized group, 87.5% of the mice were considered stimulated after ethanol + water challenge (expression of behavioral sensitization), but after ethanol + ED the percentage of stimulated mice reached the total sample (100%, $P = 0.06$). Considering the low-sensitized mice, there were only 25% stimulated mice in the ethanol + water challenge, but the administration of ethanol + ED induced stimulation in 75% of the low-sensitized mice ($P < 0.01$) (Fig. 1C).

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