Can energy drinks reduce the depressor effect of ethanol?
An experimental study in mice

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Received 7 October 2003; received in revised form 8 May 2004; accepted 29 June 2004

Abstract

Although the popularization of the combined use of alcoholic beverages and energy drinks (ED) containing caffeine, taurine and other substances has increased, there are no controlled experimental studies on the effects of ED alone or combined with ethanol. This work aimed at evaluating the effects of different doses of ED combined or not with ethanol, on the locomotor activity of Swiss mice. The administration of 3.57, 10.71 or 17.86 ml/kg of ED alone increased the locomotor activity of the animals in relation to a control group. Low doses of ethanol (0.5, 1.0 and 1.5 g/kg) alone or in combination with 10.71 ml/kg of ED did not affect their locomotor activity. However, the reduction of activity observed after 2.5 g/kg of ethanol was antagonized by 10.71 ml/kg of ED. Further studies on the mechanisms of this interaction are still needed.

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Keywords: Ethanol; Energy drink; Taurine; Caffeine; Locomotor activity

1. Introduction

We could observe a fast popularization of the combined use of alcoholic beverages and energy drinks in recent years. Most of the beverages known as “energy drinks” consist of a combination of carbohydrates (about 11 g/dl), taurine (about 400 mg/dl), caffeine (about 32 mg/dl), gluconolactone (about 240 mg/dl) and vitamins of the B complex.

There are few studies on this issue on the effects of these beverages in the literature. Some studies reported enhancement of the mood state, as well as physical and psychomotor performance (time of motor reaction, concentration, work memory and subjective sensation of alertness and vigor), after the ingestion of Red Bull [1,2].

Popular reports suggest that energy drinks could reduce the intensity of the depressant effects of ethanol. In a survey carried out in Brazil with 136 users of energy drinks, the main reason reported by them to use energy drinks combined with alcoholic beverages was the reduction of sleepiness and the increase of the sensation of pleasure after its ingestion [3].

Rieselman et al. [4] suggested that the associated use of alcoholic beverages and energy drinks might have reduced the perception of two youngsters regarding the intensity of their alcohol intoxication, thus causing an automobile accident. Ferreira et al. [5], in a study carried out with volunteers, reported that the ingestion of 3.57 ml/kg of energy drink yielded little effect in antagonizing the symptoms of intoxication caused by the ingestion of 0.6 or 1.0 g/kg of ethanol. The performance in a maximal effort test observed after alcohol combined with energy drink ingestion was similar to that observed after alcohol only. No significant differences were detected on blood alcohol
levels, physiological indicators (VO₂, ventilatory threshold, respiratory exchange rate, heart rate and blood pressure) or biochemical variables (glucose, insulin, cortisol, ACTH, dopamine, noradrenaline and adrenaline) [5]. Only a discrete reduction in the disturbing effects of ethanol in a work memory test performance and in the mood state was observed [6].

From our knowledge, no studies on the combined effect of ethanol and energy drinks were conducted in animals. Based on the knowledge of the actions of caffeine and taurine (the main components of energy drinks) on the nervous system, we could assume that these substances may alter the effects of ethanol, mainly through the stimulant effect of caffeine [7] and/or the influence of taurine in the neurotransmission mediated by GABA [8].

Studies with laboratory animals have shown that the previous or concomitant administration of taurine affects the pharmacological and behavioral effects of ethanol [8–11]. Aragon et al. (1992) [10] showed that the simultaneous administration of taurine reduced the stimulant effect of 1.0 g/kg ethanol on the locomotor activity in mice. On the other hand, under similar circumstances, the administration of taurine reduced the depressant effect of 2.0 g/kg of ethanol on the locomotor activity. These results suggest that the interaction taurine–ethanol might take place in a dose-dependent way, reducing both the stimulant and depressant effects of ethanol. Some studies reported that the administration of taurine reduced the sleeping time induced by ethanol in mice [12–14]. Taurine has also been reported to decrease the aversive effects of high ethanol doses, possibly by restoring ethanol-induced perturbations of cellular calcium homeostasis [15,16]. Besides, taurine may lead to a reduction in the aversive behavioral effects of ethanol, by reducing acetaldehyde concentrations [17].

The co-administration of caffeine can enhance the reinforcing effects of ethanol in a dose-dependent way [18] and increase the locomotor stimulation caused by the administration of ethanol in rodents [19–21]. Based on a set of studies, Kunin et al. [18] considered two possible ways caffeine could produce this effect. First, caffeine (or other stimulant drugs as amphetamine or nicotine) could increase ethanol consumption in rodents by a “self-medication” mechanism, in order to reduce their stimulant effect. The second possible explanation would be the development of sensitization to the reinforcing effects of ethanol, induced by caffeine. There are some data supporting this hypothesis but others are in disagreement with it.

As mentioned above, there are some studies on the ethanol–taurine and ethanol–caffeine interactions but, to our knowledge, there are no controlled studies on the effects of the combined administration of ethanol and both drugs, or ethanol and energy drinks (that contain taurine and caffeine). Considering this paucity of studies on the interaction of energy drink (or its components) and ethanol, as well as the increasing consumption of this combination by young adults, this study aimed at evaluating the toxicity and the behavioral effects of an acute administration of energy drinks combined with ethanol.

2. Materials and methods

2.1. Animals

Albino Swiss male mice, from the colony of the Department of Psychobiology/UNIFESP (35–50 g), aged 75 days at the beginning of the experiments, were used for this experiment. Animals were housed in groups, in plastic cages, with free access to Purina lab chow and water at all times. Lights in the colony room were on between 7:00 a.m. and 7:00 p.m. and temperature was kept at 22±1 °C. Testing was always carried out during the light cycle. In all experiments, each animal was used only once.

2.2. Drugs

The drugs used were: energy drink (Red Bull®) containing by 100 ml: a mixture of sucrose and glucose (11.3 g), taurine (400 mg), caféine (32 mg), glucono-lactone (240 mg), inositol (20 mg), niacin (7.2 mg), pantenol (2.4 mg), B2 (0.64 mg), B6 (0.8 mg), B12 (0.4 mcg), citric acid, caramel coloring, artificial flavoring and sparkling water. We defined one dose of energy drink as the equivalent of a 250-ml can ingested by a 70-kg individual, which corresponds to the administration of 14.3 mg/kg of taurine and 1.14 mg/kg of caféine per dose. The doses used were equivalent to the ingestion of 1 can (3.57 ml/kg), 3 cans (10.71 ml/kg), 5 cans (17.86 ml/kg) and 10 cans (35.70 ml/kg).

Ethanol p.a. (Synth®) was diluted in water or in the energy drink, in concentrations varying from 6% v/v (0.5 g/kg) to 23% v/v (2.5 g/kg), according to the dose.

All the drugs were administered orally (gavage).

2.3. Equipment

- Metal bar cages (15 (width)×25 (length)×20 (height) cm) were used to the observation of the animals’ general behavior.
- In order to record locomotor activity, we used eight identical acrylic activity boxes. Activity boxes [20 (width)×40 (length)×25 (height) cm, Ópto-Varimex Mini, Columbus Instruments, OH] were equipped with 16-photodetector–LED pairs in each dimension (i.e. x, y and two z-planes) spaced 2.5 cm apart and located 2.2 cm above the floor. There was a set of photodetectors located 1.7 cm below the floor and another set located 15.9 cm above it. Activity was measured in number of
photobeam interruptions (counts), using an analyzer system (interface), which sent information (counts) to the computer software.

- Gas Chromatograph Shimadzu 14B with Head-space system, fitted with flame ionization detector and capillary column Megabor (CBP20W 25-100), with hydrogen 4.5 FID as carrier gas, was used to analyze blood ethanol concentration.

2.4. Procedures

2.4.1. Pharmacological screening

In order to evaluate the general effects on behavior, as well as possible acute toxicity, 80 mice (n=20/dose) were administered 3.57, 10.71, 17.86 or 35.70 ml/kg of energy drinks. Immediately after the administration, the animals were individually placed in metal bar cages in order for us to observe, according to a previously described methodology, the occurrence and/or intensity (0, absence; 1, low intensity; 2, middle intensity; 3, high intensity) of the following symptoms: writhing, piloerection, locomotor behavior, salivation, tremors, lachrymation, eyelid ptosis, muscular tonus alterations, convulsions, alterations in posture, urinary frequency, defecation or death [22].

Two trained researchers not blind to the study independently observed the behavior of each animal. After discussing the individual rank attributed to each one, a consensual rank was determined. The observation was made for a total period of 24 h, being continuous in the first 4 h. Eight and 24 h after drug administration, the animals were observed for a period of 10 min.

2.4.2. Study on the effects of the administration of energy drinks on the locomotor activity

Four groups of animals (n=20 mice/group) received, respectively, 3.57, 10.71 or 17.86 ml/kg of energy drink or water (control), being immediately placed in individual cages where their locomotor activity was recorded for 45 min. Each animal was used only once. The experiment was replicated using other 20 mice per group. Due to the lack of differences regarding age, weight and locomotor activity between both experiments, the data were clustered.

2.4.3. Study on the interaction between ethanol and energy drinks on the locomotor activity

In order to evaluate the effects of four different doses of ethanol (0.5, 1.0, 1.5 and 2.5 g/kg) combined or not with 10.71 ml/kg of energy drink, 256 mice were tested on locomotor activity cages. The tests were carried out with four groups (n=16/group): control (water), ethanol, energy drink and ethanol+energy drink, for each of the doses of ethanol mentioned above. Immediately after drug administration, the animals were placed in the activity cages, being their locomotor activity recorded for 45 min. Since the experiments were carried out on different days, in every day there were animals from all groups.

2.4.4. Determination of blood ethanol concentration

In order to evaluate the effects of energy drink on blood ethanol levels, mice received 0.5, 1.5, 2.0 or 3.0 g/kg of ethanol combined or not with 10.71 ml/kg of energy drink. Thirty minutes after the administration, 20 μl of blood from their caudal vein was collected for later analysis of the blood ethanol concentration. The blood samples were placed in
20-ml vials containing 1 ml of propanol (0.02% v/v). The vials were immediately sealed and stored at −20 °C for later analysis by gas chromatography, using propanol as the internal standard [23]. Each animal was used once.

2.4.5. Ethics

The research project was approved by the Committee of Ethics in Research of UNIFESP (563/01). All the procedures were carried out in accordance with the norms of the International Guiding Principles for Biomedical Research Involving Animals (CIOMS) and Principles of Laboratory Animal Care (NIH Publ. N.85-23, revised 1985).

2.4.6. Statistical analysis

The frequency of symptoms evaluated in the pharmacological screening in the groups that received different doses of energy drink was compared with the control group frequency by means of the χ² test. For the interval variables with normal distribution, we used the ANOVA (one- or two-way, depending on the analysis), followed by the Newman–Keuls test. In the comparisons involving only two groups, we used the Student’s t test. The level of significance for all the analysis was 5% (P < .05). The results are presented as mean ± standard deviation. We used the STATISTICA software to run the tests (StatSoft).

3. Results

3.1. Pharmacological screening

In all doses evaluated (3.57, 10.71, 17.86 and 35.70 ml/kg), no significant differences were detected between the groups that received energy drinks and the control group, as regards the symptoms evaluated in the pharmacological screening.

3.2. Effects of the administration of energy drinks on the locomotor activity

Fig. 1 shows that all the doses of energy drink administered (3.57, 10.71 and 17.86 ml/kg) caused an increase in the locomotor activity of the animals in relation to the control group in all the moments analyzed, as well as in relation to the total activity in 45 min. A two-way ANOVA detected group [F(3,154)=13.46; P < .01] and time [A: F(2,120)=105.4, P < .01; B: F(2,120)=113.4, P < .01; C: F(2,120)=137.9, P < .01; D: F(2,120)=66.9, P < .01], as well as their interaction [A: F(6,120)=4.3, P < .01; B: F(6,120)=3.1, P < .01; C: F(6,120)=4.9, P < .01; D: F(6,120)=4.0, P < .01]. One-way ANOVA (D): 15 min: F(3,60)=6.20, P < .01; 30 min: F(3,60)=21.69, P < .01; 45 min: F(3,60)=11.04, P < .01.

Fig. 2. Locomotor activity of mice, evaluated for 45 min, in three 15-min periods, after the administration of 10.71 ml/kg of energy drink alone or in combination with 0.5, 1.0, 1.5 and 2.5 g/kg of ethanol or water (Control). The results are presented by mean ± S.D. (A) *Higher than in the control group at 0–15 min (P < .05), higher than in the other groups at 15–30 min (P < .01) and higher than in the control and ethanol groups at 30–45 min (P < .01). (B) **Higher than in the other groups at 15–30 min (P < .05) and than in the control and ethanol groups at 30–45 min (P < .05). (C) ***Higher than in the other groups in all the periods analyzed (P < .01). (D) #Higher than in the control group at 0–15 min (P < .01), higher than in the other groups at 15–30 (P < .01) and 30–45 min (P < .01). #*Lower than in the group ethanol + energy drink at 0–15 min (P < .05), lower than in the control and ethanol + energy drink groups at 15–30 min (P < .05) and lower than in the control group at 30–45 min (P < .05). The two-way ANOVA detected the following significant factors: group [A: F(3,60)=6.5, P < .01; B: F(3,60)=4.4, P < .01; C: F(3,60)=6.8, P < .01; D: F(3,60)=15.3, P < .01], and time [A: F(2,120)=105.4, P < .01; B: F(2,120)=113.4, P < .01; C: F(2,120)=137.9, P < .01; D: F(2,120)=66.9, P < .01], as well as their interaction [A: F(6,120)=4.3, P < .01; B: F(6,120)=3.1, P < .01; C: F(6,120)=4.9, P < .01; D: F(6,120)=4.0, P < .01].
As we can observe in Fig. 2A, B and C, the groups that received energy drink alone had an increase in their locomotor activity when compared to the other groups. The doses of 0.5, 1.0 and 1.5 g/kg of ethanol, isolated or combined with 10.71 ml/kg of energy drink, did not significantly alter the locomotor activity of the animals in relation to the control group. However, the lower doses of ethanol (0.5 and 1.0 g/kg) significantly reduced the stimulant effect of energy drink at 15–30 min and tended to reduce it at 30–45 min. The dose of 1.5 g/kg of ethanol significantly reduced the energy drink stimulant effect in all periods evaluated.

The dose of 2.5 g/kg of ethanol (Fig. 2D) significantly reduced the locomotor activity in relation to the control group at 30 and 45 min. This depressant effect of ethanol was reduced by the simultaneous administration of energy drink. On the other hand, in the first 15 min, the administration of energy drink combined with ethanol significantly increased the locomotor activity of the animals in relation to the control group. The group that received ethanol with energy drink presented higher levels of locomotor activity than the group that received ethanol alone, in the 45-min period (P < .02). However, none of the groups differed from the control group, treated with water.

3.4. Determination of the blood ethanol concentrations

Regarding the evaluation of the blood ethanol concentrations (Fig. 3), no differences were detected between the administration of 0.5, 1.5, 2.0 and 3.0 g/kg of ethanol alone or combined with 10.71 ml/kg of energy drink, 30 min after the administration.

4. Discussion

The administration of energy drink enhanced the locomotor activity of mice in a dose-dependent way, being more evident in the dose of 10.71 ml/kg, which is equivalent to the ingestion of three cans of 250 ml by a 70-kg individual. In the pharmacological screening test, no acute toxic effects were detected in a 24-h period, considering the range from 1 to 10 doses.

In comparison to the control group, the administration of low doses of ethanol (0.5, 1.0 and 1.5 g/kg) produced no significant stimulant or depressant effects. However, a depressant effect of the locomotor activity of mice was observed 15 min after the administration of 2.5 g/kg.

The administration of energy drinks did not significantly alter the effects of 0.5, 1.0 or 1.5 g/kg ethanol, but reduced the depressant effect of 2.5 g/kg of ethanol, increasing the locomotor activity to levels similar to those observed in the control group. However, it is noticeable that, although not reaching statistical significance, the energy drink group tended to present higher locomotor activity levels than ethanol and control groups in all experiments. In a previous experiment (unpublished data), we observed that there was no interaction between 3.57 ml/kg of energy drink and the same doses of ethanol used in this study, which suggests that this interaction might be dose-dependent.

The discrepancy between the results obtained in a previous study with human volunteers [6] and those ob-
served in this study might be due to differences between species and/or doses used. The data obtained in a survey on the pattern of use of energy drinks indicated that even though most of the users have reported habitual use of one or two doses per occasion, some of them reported up to four doses [3]. Based on the data obtained in this study, one could suggest that the ingestion of a minimum dose of energy drink would be necessary to antagonize the depressant effects of ethanol in mice. If the same is true for human beings still remains to be proved.

Assuming that energy drinks might enhance the stimulant effects of ethanol or antagonize its depressant effects, further studies are necessary to determine which are the ingredients of the compound responsible for those effects. There are indications, however, that those effects could be due to taurine and/or caffeine.

If we consider the absence of differences in the blood ethanol concentrations between the groups that received ethanol alone or combined with energy drinks, at least in the doses tested (0.5–3.0 g/kg), we can suppose that the reversion of the locomotor depression could be due to the modulating actions of taurine on the neurotransmission mediated by the GABA system, and/or to the stimulant effect of caffeine on the nervous system [24–26].

In this study, we used the dose of 42.86 mg/kg of taurine (present in 10.71 ml/kg of energy drink). The data we obtained are compatible with those collected by Aragon et al. [10], who reported that the concomitant treatment with taurine, in doses of 30, 45 and 60 mg/kg, altered the effects of ethanol on the locomotor activity of mice, evaluated in the open-field, in a dose-dependent way. Administered along with ethanol, taurine reduced the stimulant effect caused by 1.0 g/kg and the depressant effect caused by 2.0 g/kg.

As for caffeine, we used a dose equivalent to 3.43 mg/kg (present in 10.71 ml/kg of energy drink). In rodents, the co-administration of caffeine might enhance the reinforcing properties of ethanol in a dose-dependent way [18], also increasing the locomotor stimulation caused by its administration [19–21]. Due to differences regarding individual sensitivity, there is not yet a consensus on the dose required to reduce the depressant effects of ethanol in humans. Liguori and Robinson [7] observed a discreet reduction in the latency time to brake in a car simulator, after the combined ingestion of 0.6 g/kg of ethanol and 400 mg of caffeine, when compared to the ingestion of ethanol alone. However, caffeine did not totally revert the impairment caused by ethanol on the subjects’ capacity to drive.

The mechanisms underlying the action of caffeine on adenosine receptors are not yet clear, but El Yacoubi et al. [27] suggest that, considering that caffeine stimulates the A2A adenosine receptor and for this mechanism could reduce the hypnotic effects of ethanol, caffeine could be useful as a therapeutic agent in the acute ethanol intoxication. However, since high doses of caffeine may induce adverse effects, caution is necessary [28].

Other substances are present in energy drinks, namely, carbohydrates and vitamins of the B complex which, when administered together, could promote the stimulation of the energy metabolism [29], thus contributing to reduce the depressant effects of ethanol. Consequently, we should not discard a possible contribution of the other components of energy drinks to the reversion of the depressant effects of ethanol in the locomotor activity of mice. Although the influence of carbohydrate should not be disregarded, the data on its influence on the alcoholic intoxication are controversial. Masur et al. [30] reported no significant effect of glucose administration in reducing the subjective effects (self-evaluation) of alcohol intoxication or in promoting a significant reduction of blood alcohol levels, in patients who attended an emergency room. However, Zacchia et al. [31] reported that sucrose administration attenuated some subjective effects of alcohol intoxication without influencing blood alcohol levels. The “placebo effect” could not be ignored in these cases.

To sum it up, the data obtained suggest that the dose of 10.71 ml/kg of energy drinks antagonized the depressant effects of ethanol on the locomotor activity of mice. Considering mice metabolism is at least several times faster than that of humans, the administration of about one or two cans (3.57–7.14 ml/kg) to a person could exert similar effects. However, it is important to notice that the alteration of the levels of locomotor activity in mice to levels similar to those observed in the control groups, cannot be interpreted as a total reversion of the symptoms of the acute effects of alcohol.

Because only the “compound” energy drink was tested, it is necessary to investigate the possible contribution of each of its components to the observed effects. Although the mechanisms underlying these effects are not clearly established, the clinical implications of such a finding might be far reaching. Since this combination has quickly become popular, mainly among the young population, we should consider the possibility that this combination of drugs could increase the reinforcing properties of ethanol, and in this way increase its abuse liability. Other experiments are under way in order to explore in depth the pharmacological interaction between ethanol and energy drinks, as well as its components, after repeated administration.

Acknowledgements

This work was supported by Associação Fundo de Incentivo à Psicofarmacologia (AFIP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - 02/04191-0) and PIBIC/UNIFESP.
References


