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Opposite effects of alcohol in regulating stress-induced changes in body weight between the two mouse lines with enhanced or low opioid system activity

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ABSTRACT

Considering the involvement of the opioid system in alcoholism, depression and metabolism - known risk factors in human obesity, we studied the effects of chronic mild stress (CMS) and alcohol intake on body weight in two mouse lines selected for high (HA-high analgesia) or low (LA-low analgesia) swim stress-induced analgesia. In comparison to LA mice, HA mice exhibit an upregulation of opioid receptor system function, different depression-like behavior and reduced energy expenditure in stress. LA animals showed enhanced basal and CMS-induced alcohol drinking versus HA. Now we report different effects of alcohol under no stress (control) and CMS conditions on food intake and body weight between the lines. CMS in animals with no access to alcohol increased body weight in both HA and LA mice, with no effect of CMS on food intake in either line and without differences between the lines. In LA mice alcohol reduced body weight under both conditions although significantly more under the control than CMS conditions. In contrast, in HA mice alcohol increased body weight more under the CMS than under control conditions. The results suggest that opioid system may modulate effects of alcohol on stress -induced changes in body weight.

1. Introduction

Numerous studies suggest comorbidity between chronic stress and development of depression, alcoholism, obesity and metabolic diseases [1,14,20]. Meanwhile, the etiology of increased obesity resulting from exposure to stress, alcohol and from interaction between these risk factors is still unclear. Problems arising in studies concerned with the occurrence of depression, alcoholism and obesity are intractably linked to a limited number of animal models allowing investigation of the multifactorial etiology (encompassing stress, alcohol, genetic factors, and interactions between the factors) of all these three disorders [26].

HA (high analgesia) and LA (low analgesia) mouse lines selected for high or low swim stress-induced analgesia (SSIA) differ substantially in nociceptive responses both before and after stress [19]. Compared to LA mice, HA mice exhibit an upregulation of opioid receptor system function, are more sensitive to morphine, and display a higher analgesic response to μ, δ and κ receptors selective agonists [6,7,13,16]. Further studies with the HA/LA mouse model revealed that analgesia induced by the swim stress correlates with changes in whole body metabolism during swimming [9,10]. Finally, recently we reported that chronic mild stress (CMS) increases alcohol intake in LA mice. On the opposite, in HA mice, that display enhanced opioid system activity, we observed only small effects of CMS on alcohol intake (Fig. 1). We concluded that CMS imposed on individuals with genetically determined low opioid activity favors the development of alcohol abuse [25].

Opioid receptor system is believed to be involved in the complex process of integrating physiological and behavioral systems maintaining energy balance and it may play a primary role in feeding behavior and body weight regulation [23]. We decided, therefore, to test the hypothesis that opioid system activity modulates effects of stress and alcohol on changes in food intake and body weight. Here we report the effects of interaction between CMS, alcohol consumption and opioid system on food intake and body weight.

2. Materials and methods

2.1. Subjects

Six weeks old Swiss-Webster male mice from the colony maintained at the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Jastrzebiec were used in the experiment. The animals

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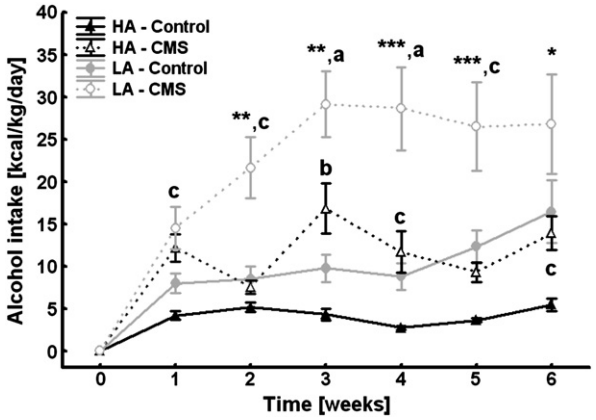

Fig. 1. Alcohol intake [kcal/kg/day] of HA (black lines) and LA (grey lines) mice in a two-bottle free-choice drinking of 8% alcohol and water under no stress conditions (control) and during the chronic mild stress conditions (CMS). Values are mean ± S.E.M.; $N = 10$ per group. $^{*}p<.05$, $^{**}p<0.01$; $^{***}p<0.001$ (*post hoc* test: LA-CMS versus HA-CMS group); $^{c}p<.05$, $^{b}p<.01$, $^{a}p<.001$, (*post hoc* test: CMS versus control group within HA or LA line).

were selectively bred for 70 generations for high (HA line) and low (LA line) SSIA. The selection protocol for the HA and LA lines was described previously [19]. Briefly, outbred mice of either sex were screened for the latency of a hind paw nociceptive reflex on a 56 °C hot plate, 2 min after completion of 3-min swimming in 20 °C water. Those displaying the longest (50 – 60 s) and the shortest (<10 s) post-swim latencies of the hind paw flick or lick response were selected as progenitors of the HA and the LA lines. Similar procedure was repeated in each offspring generation, but only animals displaying the longest and the shortest post-swim hot plate latencies were mated to maintain the lines.

After weaning, mice were kept in groups of 4 – 5 in the polycarbonate shoebox cages with sawdust bedding. They had with free access to tap water and murine chow pellets provided by LABOFEED H, Poland: 22% proteins (with 1,5% of lysine), 5% crude fibre, 4% crude fat, 6.5% crude ash, and 13,4 kcal/g of energy. When entering the experiment, HA mice weighed 37-38 g, and LA mice 34-35 g.

2.1.1. Ethical note

The experimental protocol was approved by the State Ethics Commission, in conformity with Polish law. All the procedures are commonly used and considered ethically acceptable in all European Union countries and North America. They also conform to the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Procedures

Ten days before the procedures, the animals were transferred to individual shoebox cages and remained there throughout the entire six-week experiment. In order to evaluate the effects of chronic mild stressing (CMS), alcohol and genotype, 40 mice of each line were randomly assigned to four equal groups. The CMS group was exposed during 6 weeks to chronic mild stressing (CMS); the Alcohol group was given a choice between tap water and alcohol solution; the CMS+Alcohol group was exposed both to chronic stress and to alcohol drinking; controls were maintained under same environmental conditions as experimental groups. Mice exposed and not exposed to CMS were housed in separate animal rooms at ambient temperature of 22 ± 2 °C and 55 ± 5% relative humidity on a 12-h light/dark cycle (lights on at 07:00).

2.2.1. Chronic mild stress (CMS)

CMS was adapted from the procedures developed by Willner et al. [30] and described previously [25]. Briefly, the animals were subjected for six weeks to various kinds of stress factors changing in 12-h cycles. Each week of stress regime consisted of: two periods of 45^0 cage tilt (12 h), one period of wet sawdust (12 h), two periods of paired housing (1 h) (during this time bottles with alcohol were removed), two periods of low-intensity stroboscopic illumination (8 h), two periods of overnight illumination, one period of removed bedding (12 h), one period of noise emitted by a radio receiver tuned out of the station (white noise combined with cage tilt, 12 h), one period of restrain in a plastic tube 11.5 cm long and 3 cm in diameter (15 min), and two periods of no stress (12 h). The paired-housing stress consisted of exposing a mouse to another stressed mouse of the same line. Each mouse was in successive turns a resident or an intruder, and was paired alternately with two other mice (intruder or resident) throughout the experiment. Stressors were administered in a pseudo-random manner during both light and dark phases. All mice received the same treatment schedule, with treatments occurring in different orders in different weeks.

2.2.2. Alcohol intake and preference testing

Mice could choose freely between 8% alcohol and tap water from two 25-ml glass bottles. To eliminate possible place preference, the position of the alcohol- and the water containing bottle was alternated each day. The bottles were controlled for spillage. The 8% alcohol solutions were prepared by diluting 96% ethanol (Chempur®, Poland) with distilled water. Twenty-four-hour intakes of alcohol and water were assessed by weighing the alcohol and water bottles to the nearest 0.01 g every day. The mice were also weighed at that time. Food was available ad libitum and the amount eaten was assessed three times per week. Daily alcohol intake, in kcal per kg body weight, was calculated after correction of the consumed alcohol solution for the specific gravity of alcohol [29.7 kJ (7. 1 kcal)/g, 23.4 kJ (5.6 kcal)/ml)].

2.3. Data analysis

The general linear models of analysis of variance (ANOVA) were used taking the mouse lines, the condition (CMS vs. control) and the access to alcohol as independent measures. Subsequently, two-, three- and four-way ANOVA model was used to test the effects of conditions and alcohol on food intake and body weight within lines. When a significant effect was revealed by ANOVA, a post hoc analysis for each time point of experiment (weeks) was performed using an all pair-wise Tukey's "honestly significant difference" (HSD) test. The criterion for significance was set at $p<.05$.

3. Results

3.1. Food intake and body weight in control mice

Two-way ANOVA (line and time) showed higher food intake by HA than by LA mice under control conditions ($F(1, 36) = 23.60$; $p<0.001$) (Fig. 2). Time and line x time interaction was nonsignificant.

3.1.1. Body weight

Two-way ANOVA revealed no significant differences in body weight between HA and LA mice. However, body weight changes were observed during the experiment ($F(6, 216) = 2.20$; $p<0.05$ – effect of time, with higher effect of time in LA mice than in HA mice (line x time interaction) ($F(6, 216) = 2.35$; $p<0.05$) – Fig. 3.

3.2. Alcohol intake by unstressed and CMS-exposed mice

Fig. 1 shows alcohol intake (calculated as energy intake - kcal/kg/day) over the course of six weeks of the experiment. Three-way ANOVA showed higher alcohol intake by LA mice ($F(1, 36) = 29.3$; $p<0.001$ - main effect of line). Moreover, LA mice showed enhanced CMS-induced alcohol drinking vs. HA mice ($F(1, 36) = 3.80$; $p<0.05$ - interaction lines x CMS). CMS-exposed LA mice drank almost three times as much as CMS-exposed HA mice, whereas only two-fold difference was seen between unstressed LA and HA mice. The effect of time and line x CMS x time