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Fear state induced by ethanol withdrawal may be due to the sensitization of the neural substrates of aversion in the dPAG

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Abstract

The neural substrate underlying the aversive effects induced by ethanol abstinence is still unclear. One candidate for such effects is the dorsal periaqueductal gray (dPAG), a core structure of the brain aversion system. The main aim of this study is to examine the role of the dPAG as a possible locus of the aversive effects following abrupt alcohol withdrawal. To this end, rats were subjected to an oral ethanol self-administration procedure, in which animals were offered 6–8% (v/v) ethanol solution for a period of 21 days followed by an abrupt discontinuation of the treatment on the two subsequent days. Control animals received control dietary fluid for similar periods of time. The effects of ethanol withdrawal were examined in the elevated plus-maze (EPM) (Exp. I), on the prepulse inhibition of startle to loud sounds (Exp. II) and on the freezing and escape responses induced by electrical stimulation of the dPAG (Exp. III). In Experiment III, rats were implanted with an electrode aimed at the dPAG and the number and duration of ultrasonic vocalizations (USVs) were also recorded in the rats that received dPAG stimulation at freezing and escape thresholds. Data obtained showed that ethanol withdrawal elicited significant "anxiety-like" behaviors, as revealed by the decrease in the number of entries into and time spent onto the open arms of the EPM. Startle reflex and prepulse inhibition remained unchanged in withdrawn animals. In addition, discontinuation from the chronic ethanol regimen caused a reduction in the stimulation thresholds for freezing and escape and in the number and duration of USVs. Together, these effects have been interpreted in the frame of a high fear state elicited by activation of the dPAG. These findings are indicative that ethanol withdrawal sensitizes the substrates of fear at the level of this midbrain structure.

Keywords: Ethanol withdrawal; Freezing; Escape; Ultrasonic vocalizations; Startle, dorsal periaqueductal gray

Introduction

It is well established that chronic ethanol administration leads to tolerance and dependence (Koob and Bloom, 1988; Rossetti et al., 1992). Similar to other drugs of abuse, dependence to ethanol has been mainly demonstrated through the expression of a withdrawal syndrome (Schulteis et al., 1995). Abrupt cessation from chronic ethanol administration induces a withdrawal syndrome characterized, among other symptoms, by tremors, agitation, general rigidity, spontaneous seizures, increased sensitivity to audiogenic as well as handling-induced seizures and weight loss (Pohorechy and Brick, 1990). In addition to the peculiar somatic signs of withdrawal, the interruption from persistent intake with most drugs of abuse, including ethanol, can promote disturbances in affective states and emotionality, in particular, high levels of "anxiety" (Markou et al., 1998; Koob and Le Moal, 2006). For instance, an elevation of intracranial self-stimulation threshold – an index of negative emotional state – has been reported following withdrawal from ethanol, nicotine and opiates and psychostimulants such as cocaine and amphetamine (Epping-Jordan et al., 1998; Tyrer, 1988; Pontieri et al., 1995). Moreover, exaggerated emotional reactions have also been observed in abstinent ethanol animals when exposed to a mild stress that did not induce behavioral disturbances in control animals (Valdez et al., 2003). In addition, alcohol-preferring animals are more responsive to both startle-alone and

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potentiated startle stimuli following fear conditioning (McKinzie et al., 2000) and high-alcohol-drinking rats exhibited an excessive fear reaction in standard fear conditioning tasks (Rorick et al., 2003). Finally, exposure to multiple withdrawals from moderate amounts of ethanol induces sensitization of anxiety-like behavior (Overstreet et al., 2002). All these findings support the contention that prior experience with ethanol withdrawal facilitates the occurrence of exaggerated negative emotional responses, mainly fear and "anxiety", to environmental challenges.

A growing amount of evidence has demonstrated that the activation of some mesencephalic structures, particularly the dorsal periaqueductal gray (dPAG), a central structure of the so-called brain aversion system, produces several behavioral and somatic responses characteristic of high fear states that resemble panic attacks (Brandão et al., 1999; Vianna et al., 2001), similar to those observed in animals confronted by predators or by dangerous environmental cues (Graeff, 1990, 2004; Brandão et al., 1999). Similar behavioral and somatic changes have also been reported during alcohol withdrawal (Johnston et al., 1991; Thevos et al., 1991). In this context, drugs that alleviate anxiety in clinical patients or laboratory animals submitted to stress also reduce the anxiety induced by ethanol withdrawal (Saitz and O'Malley, 1997). However, in spite of several studies that report, among other clinical disturbances, high anxiety in ethanol-withdrawn patients as one of the main causes of relapse, relative few studies have examined the neural substrates of the defensive behavior related to the fear/anxiety symptoms following abstinence from chronic ethanol intake.

Freezing, escape, autonomic responses and reduction in the emissions of ultrasonic vocalizations (USVs) are part of the defensive repertoire expressed by animals electrically stimulated at the dPAG. Changes in the sensory gating have also been reported following this stimulation (see Brandão et al., 2005 for a review). The aversive state generated and elaborated in the dPAG is a multifaceted process with sensory, behavioral and autonomic components, and the electrical stimulation of this structure has been used as a model of panic attack (Graeff, 1990, 2004; Brandão et al., 1999, 2005). While the effects of ethanol withdrawal in anxiety have been well investigated (see Kliethermes, 2005 for a review), no study has been conducted on the possibility of activation of the sensory gating of the neural substrates of the defense reaction of the dPAG during this condition. Based on this evidence, the present study was designed to evaluate whether the dPAG could be implicated in the fear state induced by withdrawal from chronic ethanol intake. Thus, in the first part of the present study, general levels of anxiety and sensory processing activity in ethanol-withdrawn animals were assessed by the elevated plus-maze test (EPM) and prepulse inhibition procedure, respectively. In the second part of the study, other groups of rats abstinent from chronic ethanol were evaluated in their reactivity to electrical stimulation of the dPAG at the freezing and escape thresholds and their ability to emit USVs contingent to this dPAG electrical stimulation procedure.

Materials and methods

Animals

Male Wistar rats, weighing 180 g in the average, from the animal house of the Campus of Ribeirão Preto of the University of São Paulo were used. After arrival in the sectorial animal house of the laboratory, these animals were housed in plastic cages under 12:12 dark/light cycle (lights on at 07:00 h) at 20 ± 1°C and given free access to water throughout the experiment. The experiments reported in this article were performed in compliance with the recommendations of SBNeC (Brazilian Society of Neuroscience and Behavior), which are based on the US National Institutes of Health *Guide for Care and Use of Laboratory Animals*.

Chronic ethanol intake

The treatment selected to promote chronic ethanol intake was a forced procedure of fluid dietary intake via drinking an alcohol containing liquid diet. The animals were housed in groups of four by cages (40 × 33 × 18 cm) throughout the experiment. While water was daily available to the animals during the whole period of the experiment, they had free access to food only during the 3 days before the beginning of the treatment. After that, in the two subsequent days, two bottles (120 ml each) containing only a dietary base composed by Sustagen M (chocolate flavor, Mead Johnson) were available to the animals. Sustagen is a ready-to-feed liquid formula providing protein, carbohydrate and fat plus vitamins and mineral salts corresponding to an amount of 1.1 kcal/ml (17.11 g of Sustagen M/60 ml of water by rat/day) and was the only source of food daily available to the animals (Bond and Di Giusto, 1976; Stegink et al., 1983). From this point on, the animals were randomly assigned to two experimental groups: (1) control and (2) ethanol withdrawal. For the control group, the dietary base without alcohol (control regimen) was available for the whole period of the test while the animals of the ethanol withdrawal group were subjected to the ethanol intake procedure for a period of 21 days followed by an abrupt discontinuation of the treatment on the two subsequent days. Then, for this experimental group, the diet was added with different concentrations of ethanol (CH_3CH_2OH). In the first 2 days of treatment, ethanol concentration was 6% (v/v). After that period until the end of the treatment (3rd to 21st day), the ethanol concentration (v/v) was increased to 8%. On days 22 and 23, both groups (control and ethanol withdrawal groups) received the same dietary base without ethanol. The interruption of this chronic schedule of drug intake has been reported to induce clear signs of ethanol withdrawal (Martijena et al., 2001). Control animals received the same dietary base without ethanol. Regardless of the experimental group, the animals consumed the whole diet available every day. Water was available to both experimental groups throughout the experiment. The body weights of the control and ethanol withdrawal groups on the day 21 of the regimen were 246.58 ± 3.12 and 244.33 ± 5.46, respectively. The choice of