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Research report

Alpha1-adrenergic drugs affect the development and expression of ethanol-induced behavioral sensitization

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HIGHLIGHTS

- Mice treated with ethanol (2.2 g/kg) for 2 weeks developed behavioral sensitization.
- Prazosin and Phenylephrine blocked the expression of behavioral sensitization.
- Prazosin + ethanol impaired the expression but not the development of sensitization.
- Phenylephrine+ ethanol did not impair the expression of sensitization.
- Noradrenergic system normal functioning is needed for sensitization expression.

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ABSTRACT

Background: According to the incentive sensitization theory, addiction is caused primarily by drug-induced sensitization in the brain mesocorticolimbic systems. After repeated ethanol administration, some animals develop psychomotor sensitization, a phenomenon which occurs simultaneously with the incentive sensitization. Recent evidence suggests the involvement of norepinephrine (NE) in drug addiction, with a critical role in the ethanol reinforcing properties. In this study we evaluated the influence of an agonist (phenylephrine) and an antagonist (prazosin) of alpha1-adrenergic receptors on the development and expression of behavioral sensitization to ethanol. Male Swiss mice, previously treated with ethanol or saline, were challenged with the combined administration of ethanol (or saline) with alpha1-adrenergic drugs. Prazosin (0.1; 0.5 and 1.0 mg/kg) and phenylephrine (1.0 and 2.0 mg/kg) administration blocked the expression of behavioral sensitization to ethanol. In another set of experiments, mice treated with 0.5 mg/kg of prazosin + ethanol did not present the development of behavioral sensitization. However, when challenged with ethanol alone, they showed the same sensitized levels of locomotor activity of those presented by mice previously treated with ethanol and saline. Phenylephrine (1.0 mg/kg) treatment did not affect the development of behavioral sensitization. Based on this data, we concluded that the alteration of alpha1-adrenergic receptors functioning, by the administration agonists or antagonists, affected the locomotor sensitization to the stimulant effect of ethanol, suggesting that the normal functioning of the noradrenergic system is essential to its development and expression.

1. Introduction

An important question in addiction science is why some individuals consume alcoholic beverages and become addicted while others do not. It is important to identify the features that make some individuals especially susceptible to dependence as well as the underlying neural mechanisms responsible for the transition from occasional psychotropic drug use to addiction [1].

The repeated exposure of animals to alcohol or other psychotropic drugs may produce a sensitized response characterized by increased locomotor activity, known as "behavioral sensitization" or psychomotor sensitization [2–4]. This psychostimulant effect has been associated with the release of dopamine in the nucleus accumbens (NAc), leading to a "sensitized" neural reward circuitry [4]. According to the incentive-sensitization theory of addiction proposed by Robinson and Berridge [5–8] and supported by other authors, the repeated exposure to potentially addictive drugs increases the attribution of the incentive salience

Abbreviations: ANOVA, analyses of variance; ETOH, ethanol; LC, locus coeruleus; NAc, nucleus accumbens; NE, norepinephrine; PFC, prefrontal cortex; Phen, phenylephrine; Praz, prazosin; Sal, saline; S.E.M., standard error of the mean; VTA, ventral tegmental area.

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to stimuli and modifies the neurocircuitry involved in motivation, mainly the dopaminergic mesocorticolimbic pathways [9]. Although incentive sensitization is the critical issue in their incentive sensitization theory of addiction, the locomotor sensitization (or psychomotor sensitization), which is simultaneously observed, can be used as indirect evidence of hypersensitivity in relevant motivation circuitry. Besides, they share common pathways, involving the striatum. The psychomotor activation reflects the engagement of brain incentive systems, among them the mesotelencephalic dopamine systems [10]. The incentive sensitization theory of addiction is also supported by other findings. The repeated drug exposure enhances the capacity to evoke subsequent conditioned place preference, demonstrated as either the need of lower drug doses, or fewer conditioning trials, to establish conditioned place preference [11–18]. Drug self-administration is also enhanced by repeated drug pretreatment, indicated by either its faster acquisition or acquisition at lower doses [19–26]. Besides, pretreatment with other stimulants, such as amphetamine, also accelerated the escalation of cocaine self-administration, when animals were allowed long daily access to the drug [27].

Recent studies have suggested that alpha1-adrenergic receptors modulate the mesolimbic and mesocortical dopaminergic pathways (for a more detailed review see [28]). For instance, alpha1b-adrenergic receptor knockout mice presented a reduced preference for cocaine when compared to wild type mice [29]. Some studies indicated that other manipulations of the NE system affect some behavioral effects of cocaine, amphetamine and ethanol. Additionally, mice whose prefrontal cortical NE pathways were depleted did not develop conditioned place preference to amphetamine [30], cocaine [31] or ethanol [32]. Disulfiram, a dopamine β-hydroxylase inhibitor which reduced norepinephrine production, attenuated the drug-primed reinstatement of cocaine seeking rats [33].

In studies on behavioral sensitization to cocaine [34,35], prazosin, an antagonist of alpha1-adrenergic receptors, blocked the acute locomotor response and the development of behavioral sensitization. Prazosin also blocked the acute stimulant effects of morphine, but not the development of behavioral sensitization to this drug [36]. However, there is no study on the influence of alpha1-adrenergic receptors on the ethanol-induced behavioral sensitization.

We hypothesized that alpha1-adrenergic manipulation, through the administration of an agonist or an antagonist could affect the development or expression of behavioral sensitization to ethanol. According to this hypothesis, mice sensitized to ethanol, if challenged with ethanol + prazosin would not express or develop ethanol-induced behavioral sensitization, or would express it in a lower level than the control group treated with ethanol + saline. On the other hand, the co-administration of ethanol with phenylephrine, an agonist of the alpha1-adrenergic receptor, would increase their locomotor activity, facilitating the development and expression of sensitization.

2. Materials and Methods

2.1. Animals

Male albino Swiss Webster mice 3 months old, weighing 30–50 g, from the Departamento de Psicobiologia and CEDEME – Centro de Desenvolvimento de Modelos Experimentais from Universidade Federal de Sao Paulo, housed under standard laboratory conditions of 12 h light/dark cycle (lights on from 7:00 h to 19:00 h), were used in all experiments. They were provided food and water *ad libitum*. All mice were housed in groups of 15–20 animals per cage. All efforts were made to minimize animals' suffering and to keep the number of animals used to a minimum, according to the NIH guidelines for care and use of laboratory animals. This project was approved by the Committee of Ethics in Research of Universidade Federal de Sao Paulo (CEP 0766/08).

2.2. Drugs

Ethanol (Synth®, Diadema, Brazil) was diluted in saline (15% w/v in 0.9% NaCl). The alpha-1 agonist, phenylephrine hydrochloride (Sigma-Aldrich®, Saint Louis, MO), and the alpha-1 antagonist prazosin hydrochloride (Sigma-Aldrich®, Saint Louis, MO) were dissolved in saline solution (NaCl 0.9%). Sonication was employed to dissolve Prazosin.

2.3. Apparatus

All locomotor activity tests were carried out in Opto-Varimex activity cages [model Opto-M3, with acrylic boxes (47.5 (L) × 25.7 (W) × 20.5 (H) cm); Columbus Instruments, Columbus, OH], which detect locomotion by the interruption of photoelectric beams (16 pairs/cage).

2.4. Experimental procedures

2.4.1. Behavioral sensitization protocol

Initially, all mice were placed in activity cages, for 15 min, to measure their baseline activity, in a drug-free situation. This habituation test, performed 72 h prior to the first day of treatment/test also intended to habituate the mice to the activity cages, reducing an increased activity which could be triggered by the new environment. Seventy-two hours after this test, they were allocated to one out of two experimental groups (equated on basis of their locomotor activity in the habituation session). On day 1 (Monday), mice received ethanol (2.2 g/kg; $n = 132$) or saline ($n = 45$) administration, being immediately tested for 15 min in the locomotor activity cages. Mice received the same treatment on Mondays, Wednesdays and Fridays for two weeks, and were tested on activity cages on days 5, 10 and 15. On days 3, 8 and 12, immediately after ethanol or saline administration, mice were returned to their home-cage. This procedure aimed at preventing strong associations between the treatment given and the contextual cues present in the activity cages

2.4.2. Experiments 1, 2 and 3 – challenges with different doses of phenylephrine and prazosin

This phase of the experiment started five days later (washout period) the treatment phase that lasted two weeks. Mice were allocated to the challenge groups equated on basis of their locomotor activity in the habituation session.

In all experiments mice were submitted to saline and ethanol challenges before the noradrenergic drugs + ethanol challenges. Initially, in the "*Saline challenge*" (day 20) mice received two administrations of saline. In the second challenge, called "*Ethanol challenge*" (day 22), all mice received saline before 2.2 g/kg of ethanol. On days 26 (in experiments 1, 2 and 3) and 28 (in experiment 3) mice received phenylephrine or prazosin at the doses showed in Table 1, which summarizes the experimental procedures adopted in the challenge phase of experiments 1, 2 and 3. Different groups of mice were used in each experiment.

2.4.3. Experiments 4, 5 and 6 – repeated treatment with phenylephrine or prazosin and/or ethanol

The experimental procedures adopted in experiments 4, 5 and 6 were similar to those employed in the previous experiments, except that in the two-week treatment phase mice received phenylephrine