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Mirtazapine attenuates the expression of nicotine-induced locomotor sensitization in rats

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ABSTRACT

Nicotine is the primary psychoactive component of tobacco. Many addictive nicotinic actions are mediated by an increase in the activity of the serotonin (5-HT) system. Some studies show that the 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3 receptors have a central role in the induction and expression of nicotine-induced locomotor sensitization. Mirtazapine, an antagonist of the α_2-adrenergic receptors, the 5-$HT_{2A/C}$, and the 5-HT_3 receptors, has proven effective in reducing behavioral effects induced by drugs like cocaine and methamphetamines in human and animal. In this study, we evaluated the effect of mirtazapine on the locomotor activity and on the expression of nicotine-induced locomotor sensitization. We used the nicotine locomotor sensitization paradigm to assess the effects of mirtazapine on nicotine-induced locomotor activity and locomotor sensitization. Mirtazapine (30 mg/kg, i.p.) was administered during extinction. Our study found that mirtazapine attenuated the expression of locomotor sensitization induced by different nicotine doses, decreased the duration of locomotor effects and locomotor activity induced by binge administration of nicotine. In addition, our study revealed that treatment with mirtazapine for 60 days produced an enhanced attenuation of nicotine-induced locomotor activity during the expression phase of behavioral sensitization, compared to that obtained when mirtazapine was administered for 30 days. This suggests that use of mirtazapine in controlled clinical trials may be a useful therapy to maintain abstinence for long periods.

1. Introduction

Tobacco use is one of the greatest global threats to public health (World Health Organization, 2012). It causes serious health problems and increases susceptibility to many other adverse conditions. Nevertheless, despite significant public education efforts and availability of behavioral and pharmacological therapies that promote smoking cessation, the number of smokers is increasing worldwide (Benowitz, 2008).

Nicotine, the primary psychoactive component of tobacco, is thought to play a critical role in tobacco dependence (Balfour, 2009). Nicotine seems to produce its rewarding effects by acting on the nicotinic acetylcholine receptors (nAChRs) and increasing neurotransmission in mesolimbic and nigrostriatal dopaminergic pathways—hence inducing drug-seeking and drug use behaviors (D'Souza and Markou, 2011).

The most widely used pharmacotherapy for smoking cessation is nicotine replacement. Some patients, however, prefer a nicotine-free treatment or one that does not cause serious adverse effects, while others seek alternative treatments (Prochaska and Benowitz, 2016; Benowitz, 2009). Nonetheless, their efforts have hitherto been unsuccessful.

Several studies of smokers indicate that: 1) smokers often have major depressive episodes. 2) They frequently use nicotine as a substitute for antidepressants because of its potential antidepressant effects. 3) Cessation of nicotine use leads to depressive symptoms. 4) Some antidepressants may alter neurotransmission systems involved specifically in nicotine addiction (Oliveira et al., 2017; Hughes et al., 2014; Cahill et al., 2013). This evidence suggests that in general, antidepressants may help to stop smoking.

Some studies have investigated the effects of antidepressants on smoking (Hughes et al., 2014; Cahill et al., 2013), some of them reduce the side effects of nicotine withdrawal (fluoxetine and paroxetine); others reduce impulse to smoke (nortriptyline, and bupropion) or

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relieve withdrawal symptoms (imipramine, venlafaxine, and bupropion); and still others act as a nicotine blocker or have no effect on smoking at all (Brown et al., 2014; Covey et al., 2002; Cinciripini et al., 2005).

Mirtazapine (REMERON, Schering-Plough-Organon 3770 USA) is an atypical antidepressant approved for the treatment of moderate to severe depression with comorbid anxiety disorders (Croom et al., 2009; De Boer, 1996). Some human studies have shown that mirtazapine administration stops the abuse of benzodiazepines and reduces cocaine abuse (Zueco-Pérez, 2002). Furthermore, recent clinical trials have shown that mirtazapine significantly improves symptoms of depression, anxiety, insomnia, and dysphoria that appear during benzodiazepine, methamphetamine, and cocaine withdrawal (Kongsakon et al., 2005). Animal studies indicate that the mirtazapine also decreases the rewarding effects of morphine and inhibits acquisition of morphine dependence (Kang et al., 2008), and attenuates methamphetamine-induced locomotor sensitization (Voigt and Napier, 2012).

We recently reported that daily dosing of mirtazapine (30 mg/kg, i.p.) for 30 days during the cocaine-extinction phase significantly attenuates the induction and expression of cocaine-induced locomotor sensitization, decreases the duration of the cocaine-induced locomotor effect (Salazar-Juárez et al., 2016), and reduce the reacquisition of cocaine self-administration (Barbosa-Méndez et al., 2017a). Collectively, all these studies suggest that mirtazapine may be an effective therapeutic option in the treatment of nicotine use disorders (nicotine-withdrawal).

Since there are no effective therapies against the addictive effects of nicotine and the urgent need to develop a better therapy that smoking cessation and that the use of antidepressants medications such as mirtazapine could be a useful therapeutic option. In this study, we evaluated the effect of mirtazapine on the locomotor activity and on the expression of nicotine-induced locomotor sensitization.

Behavioral sensitization has been shown to be a factor important in the development of drug addiction because of its important role in the relapse in drug use (Vezina et al., 2007; Mao and McGehee, 2010). For our study, we hypothesized that nicotine-induced locomotor activity and the locomotor sensitization can be altered by mirtazapine administration. We found that mirtazapine dosed for 30 days attenuated the expression of nicotine-induced locomotor activity.

2. Materials and methods

2.1. Animals

The study used male Wistar rats weighing 250–280 g at the onset of the experiments. They were housed four per cage in standard plastic rodent cages (57 cm × 35 cm × 20 cm) in a colony room maintained at 21 ± 2 °C and at 40–50% humidity, under a 12-h light/dark cycle (lights on at 7:00 a.m.). The animals had free access to water and rodent chow pellets, except during experimental sessions. All the experiments were conducted during the light phase (between 9:00 a.m. and 3:00 p.m.) of the light/dark cycle. The study procedures were approved by Institutional Care and Use of Laboratory Animals and Bioethics Committees, in strict compliance with the Guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health.

2.2. Drugs

We purchased nicotine (nicotine tartrate salt; Sigma-Aldrich) after obtaining the required regulatory permission and used all study drugs in compliance with official guidelines (COFEPRIS-2016. México). Nicotine and mirtazapine (REMERON, Schering-Plough-Sanfer) were dissolved in physiological saline (0.9% NaCl, Sigma Aldrich). Both solutions were freshly prepared before intraperitoneal (i.p.) administration to the animals. All nicotine solutions were adjusted to pH 7.4 using sodium hydroxide (1 M), and the nicotine and mirtazapine solutions were injected in a volume of 1 ml/kg body weight. During the experiment, the solutions were maintained at −20 °C. Saline was used as a control in all experiments. To determine if mirtazapine could prevent the effects of nicotine, mirtazapine was administered 30 **min** before nicotine (or saline) administration. Previous studies allowed determining the optimal mirtazapine dose (30 mg/kg) and time of treatment, which we used in our experiments (Salazar-Juárez et al., 2016; Salazar-Juárez et al., 2017; Timmer et al., 2000).

2.3. Behavioral sensitization procedure

2.3.1. Apparatus

For each animal, locomotor activity was assessed in transparent Plexiglass activity chambers (50 × 50 × 30 cm) linked to a personal computer. Each activity chamber was surrounded by a 16 × 16 photocell beam array located 3 cm from the floor surface to scan locomotor activity (OMNIALVA, Instruments, Mexico). Photobeam interruptions were automatically quantified with OABiomed software (1.1) and analyzed afterward. Locomotor activity was defined as the interruption of consecutive photobeams (OMNIALVA, Mexico).

2.3.2. Procedure

Spontaneous locomotor activity was estimated with a standard protocol (Salazar-Juárez et al., 2016). Animals were habituated to the activity chambers in three 30-min sessions and were randomly assigned to different pharmacological treatment groups. Locomotor activity was recorded for 30 min. The rats were returned to their home cages after each experimental session had been completed.

2.4. Experimental procedures

The study used 200 male Wistar rats in five groups, and each group underwent a different experiment. For experiments 1, 4, and 5, we used 32 animals further divided into four experimental groups (n = 8); for experiment 2, we used 40 animals in five groups (n = 8); and for experiment 3 we used 64 animals, which were divided into eight experimental groups (n = 8). Each experimental group received a different pharmacological treatment.

2.4.1. Experiment 1. Mirtazapine altered the expression of nicotine-induced locomotor sensitization

This experiment was divided into four experimental phases and explores the effect of the chronic dosing with mirtazapine during the extinction phase on the expression of nicotine-induced locomotor sensitization. Phase I, or the nicotine-induction phase, lasted 10 days. Phase II, or the nicotine-extinction phase, lasted 30 days. Phase III, or the nicotine-expression phase, lasted 25 days. Lastly, Phase IV, or the post-expression phase, lasted 15 days (Fig. 1A).

After three-day habituation, the saline (SAL) and the mirtazapine (MIR) groups received saline solution (9% NaCl, i.p.) and mirtazapine (30 mg/kg, i.p.), respectively, during the four phases above. The nicotine group (NIC) received nicotine (0.4 mg/kg, i.p.) during the induction, the expression, and the post-expression phases. During extinction, nicotine was withdrawn and the group received only saline daily.

The nicotine plus mirtazapine group (NIC + MIR) received nicotine daily during the induction phase. In the extinction and the nicotine-expression phases, the rats received mirtazapine 30 **min** before administration of either saline or nicotine (0.4 mg/kg, i.p.). In the post-expression phase, mirtazapine was withdrawn and the group received nicotine only. After each administration, locomotor activity was recorded for each animal for 30 min.