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Effects of the cannabinoid CB1 receptor antagonist AM 251 on the reinstatement of nicotine-conditioned place preference by drug priming in rats

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Abstract:
Tobacco and cannabis are among the most widely abused drugs in humans, and recently, the functional interaction between nicotine and cannabinoids has been reported. The aim of the present studies is to evaluate the role of CB1 cannabinoid receptors in the reinstatement of nicotine-induced conditioned place preference. Nicotine-induced conditioned place preference was established (three-day nicotine sessions, 0.5 mg/kg), extinguished and reinstated by a priming dose of nicotine. It was shown that the CB1 receptor antagonist AM 251 (0.25 and 0.5 mg/kg) in a dose-dependent manner attenuates the reinstatement of nicotine place conditioning. These studies suggest a role for CB1 cannabinoids receptors in preventing the reinstatement of nicotine addiction.

Key words:
nicotine, CB1 cannabinoid receptors, AM 251, reinstatement, place conditioning, rats

Introduction

Drug addiction is a complex dysfunction of the central nervous system (CNS) that manifests in obsessive, sometimes uncontrollable drug craving (defined as an intense desire for a specific object or experience), drug-seeking and drug-taking, despite obvious and serious health and life hazards. For many addicted people, drug dependence is a chronic illness with a high rate of relapses even after long periods of abstinence [13].

Animal models of relapse may be helpful in developing better methods to achieve long-term drug abstinence. In laboratory animals, the reinstatement of extinguished drug-seeking behaviors has been studied with three paradigms using the reinstatement procedure: self-administration, conditioned place preference (CPP) and runway paradigm [14, 17, 23].

Several laboratories have developed a reinstatement procedure based on CPP, which is a simple non-invasive method, compatible with classic Pavlovian conditioning [21]. In these studies, animals are initially trained to associate one environment with a drug injection and a different environment with a vehicle injection. During the test day animals typically spend more time in the drug-paired environment. This acquired preference can be extinguished

by paring injections of saline with both compartments, or by allowing animals to explore these compartments in the absence of the drug. After the extinction, a priming dose of the drug of abuse, or the exposure to a non-drug stimuli reinstates the extinguished CPP. This animal model is used to measure the appetitive value of natural and synthetic substances as well as to evaluate the relapse to the abuse of drugs such cocaine, opiates, nicotine, alcohol and amphetamine [1, 2, 29].

Current concepts of addiction and relapse postulate the participation of mesocorticolimbic transmission in reinforcing effects of the drug of abuse [26]. In particular, midbrain dopaminergic neurons that originate in the ventral tegmental area (VTA) and project to structures associated with the limbic system. As such, the shell region of the nucleus accumbens (NAC) and prefrontal cortex (PFC) play an important role in drug addiction [26]. The dopaminergic neurotransmission in these areas is increased by natural rewards such as food, water, sex and also by a variety of drugs of abuse like opiates, psychostimulants or ethanol [26].

Moreover, enhanced dopamine transmission in the NAC, a critical brain region for drug reward, plays a role in nicotine- and cannabinoid-driven motivation and reinforcement. Nicotine increases the activity of dopamine containing neurons in the NAC through the activation of cholinergic nicotinic receptors (nAChRs) localized directly on dopaminergic neurons [11]. However, this type of receptors is known to desensitize rapidly, whereas experimental evidence suggests that a single injection of nicotine increases the level of dopamine in the NAC for two hours [11]. A long-lasting, high level of dopamine may result from the synaptic release of glutamate by activated nAChRs located presynaptically on glutamatergic neurons [19].

Several lines of research have concluded that the dopaminergic neurons of the mesocorticolimbic system are also under the control of the endocannabinoid system [20, 28]. Cannabinoids are a class of psychoactive agents showing motivational and reinforcing effects [10]. They produce their physiological effects by influencing the activity of cannabinoid (CB) receptors. Two types of CB receptors, CB1 and CB2, have been characterized [16]. CB1 receptors are found in the CNS, whereas CB2 receptors are present at particularly high levels in the immune system [16]. The modulation of dopaminergic neurons by cannabinoids is associated with the activation of CB1 receptors present in the NAC. But, contrary to nAChRs, CB1 receptors are not located directly on dopamine neurons. These receptors are expressed presynaptically on GABAergic and glutamatergic neurons. Thus, they indirectly modulate the dopamine release in the reward system [20, 28].

Nicotine addiction is a complex disorder, and no effective medications are currently available for treatment despite extensive efforts. In agreement with behavioral studies conducted in animals, and in clinical trials (rimonabant is effective in phase III of clinical trials for the treatment of nicotine addiction), the blockade of CB1 receptors may be useful in preventing relapse to drug taking behaviors. The present study was undertaken to examine the ability of the CB1 receptor antagonist – AM 251 to inhibit the reinstatement of extinguished nicotine induced CPP. These experiments may contribute to a better understanding of the neurobiological mechanisms underlying relapse in nicotine addiction and allow the development of more effective pharmacotherapies in the treatment of nicotinism.

Materials and Methods

Animals

Experiments were carried out in naive male Wistar rats weighing 250–300 g (Farm of Laboratory Animals, Warszawa, Poland) at the beginning of the experiments. The animals were kept under standard laboratory conditions (12/12-h light/dark cycle) with free access to tap water and lab chow (Bacutil, Motycz, Poland), and allowed to acclimate to laboratory conditions for at least one week. The rats were handled once a day for 5 days preceding the experiments. Each experimental group consisted of 7–10 animals. The experiments were performed between 8.00 a.m. and 5.00 p.m.

All experiments were carried out according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and the European Community Council Directive of 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the local ethics committee.

Drugs

The compounds tested were: (–)-nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA) and AM 251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, Tocris, MO, USA). Nicotine was dissolved in saline (0.9% NaCl), and the solution pH was adjusted to 7.0 (± 0.2) with dilute NaOH. AM 251 was dissolved in one drop of Tween and diluted in 0.9% NaCl. All agents were administered intraperitoneally (*ip*) in a volume of 5 ml/kg. Control groups received saline injections at the same volume and by the same route.

Apparatus

The testing apparatus for the conditioned place preference paradigm was similar to that used by Spyraki et al. [27]. Each six rectangular box (60 × 35 × 30 cm) was divided into three compartments: two large compartments (20 × 35 cm) were separated by removable guillotine doors from a small central area (10 × 10 cm). One of them had its walls and floor painted white while the walls of the other chambers were painted black. The central grey area constituted a "neutral" chamber, which serves as a connection between the two conditioning chambers and as the start compartment. The testing boxes were kept in a soundproof room with neutral masking noise and dim 40-lx illumination.

Procedure

The CPP-reinstatement paradigm took place on 9 consecutive days and consisted of the following phases: pre-conditioning (pre-test), conditioning, post-conditioning (test), extinction and reinstatement. This method (biased design) was similar to that used in previous experiments [1, 2].

Pre-conditioning

On the first day each animal was placed separately in the neutral area with the guillotine doors removed to allow access to the entire apparatus for 15 min. The amount of time that the rats spent in each of the two large compartments was measured (a baseline preference). All animals showed a moderate preference for the black compartment.

Conditioning

One day after pre-conditioning, the rats were randomized and subsequently conditioned with saline paired with the preferred (black) compartment (the morning sessions) and nicotine (0.5 mg/kg, *ip*) with the other (white) compartment (the afternoon sessions) for 30 min. Sessions were conducted twice each day with an interval of 6–8 h for 3 consecutive days (day 2–4). Injections were administered immediately before confinement in one of the two larger compartments, as mentioned above. A dose of 0.5 mg/kg nicotine was chosen for conditioning because this dosage is known to produce reliable conditioned place preference in rats in our experimental conditions [1, 2, 5]. The control group received an injection of vehicle every day. The neutral zone was never used during conditioning and was blocked by guillotine doors.

Post-conditioning (test)

On day 5, which was one day after the last conditioning trial, the animals were placed in the neutral area with the guillotine doors removed and allowed free access to all compartments of the apparatus for 15 min. The time spent in the drug-paired compartment was recorded for each animal. No injections were given on the day of this preference test.

Extinction training

One day after the preference test, the rats were given extinction training daily for 3 days. For each trial, the rats were placed in the neutral area and allowed to explore both chambers for 15 min. No injections were given during this extinction period. The amount of time that rats spent in each chamber was measured on day 6 (Extinction 1), 24 h after the initial preference test, and on day 8 (Extinction 3), 72 after this preference test.

Reinstatement

One day after the last extinction trial (day 9), separate groups of rats received vehicle or AM 251 (0.25 or 0.5 mg/kg) 15 min before a priming injection of nicotine (0.5 mg/kg) or vehicle, and were immediately tested for reinstatement of conditioned place preference. During this reinstatement test, the rats were al-

lowed free access to the entire apparatus for 15 min, and the time spent in each chamber was measured.

Statistics

The data are expressed as the means ± SEM. For CPP paradigm, the statistical analyses were performed using a one way-analyses of variance (ANOVA) with score (i.e. the differences between post-conditioning and pre-conditioning time spent in the drug-associated compartment) as the dependent factor. *Post-hoc* comparison of means was carried out with the Tukey test for multiple comparisons when appropriate. The confidence limit of $p < 0.05$ was considered statistically significant.

Results

The time spent in the initially less preferred (white) versus the initially more preferred (black) side did not significantly differ between groups on the pre-conditioning day. This side preference was not significantly changed when saline was paired with both compartments during the conditioning sessions (data not shown).

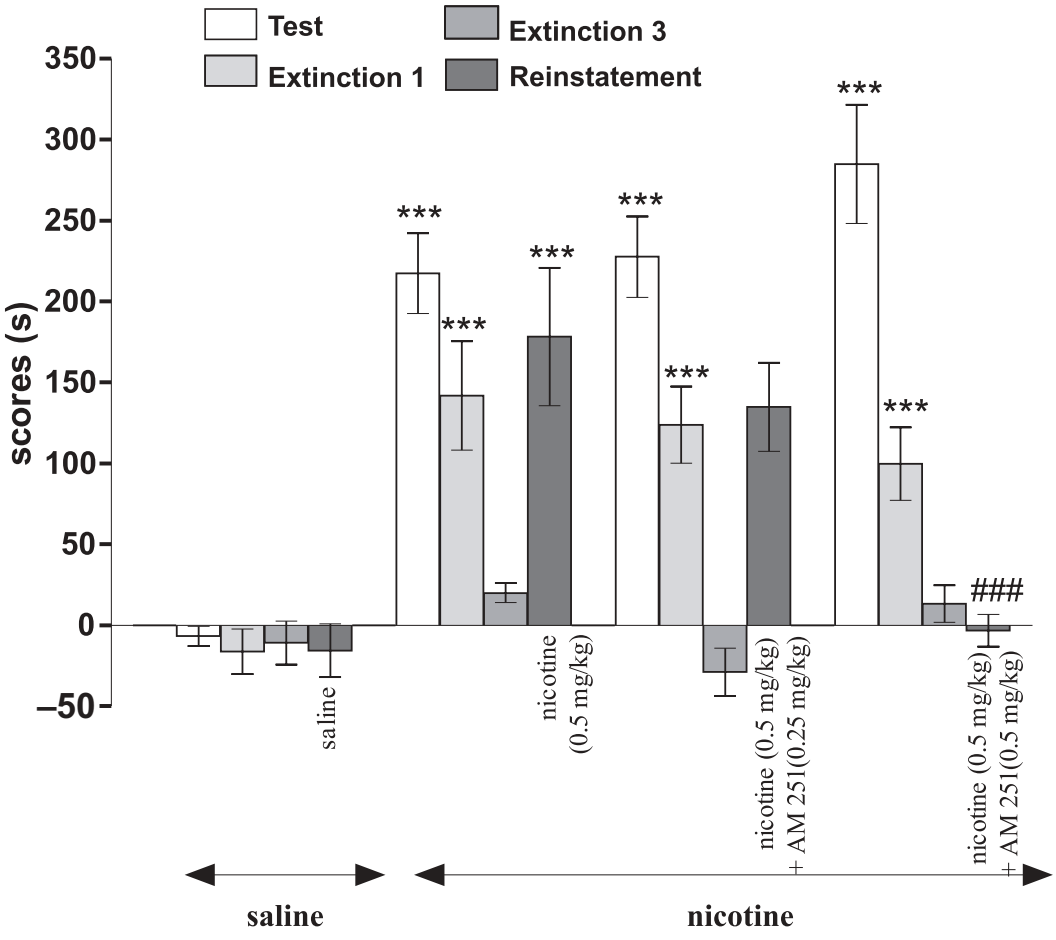

Fig. 1. Effects of AM 251 (0.5 and 0.25 mg/kg, *ip*) on the reinstatement of nicotine-conditioned place preference caused by a priming dose of nicotine. The place preference procedure consisted of pre-conditioning, three conditioning sessions with nicotine (0.5 mg/kg, *ip*), post-conditioning test followed by extinction period, i.e. repeated test trials, 24 h (Extinction 1) and 72 h (Extinction 3) after the preference test. One day after the last extinction trial, extinguished nicotine conditioned place preference was reinstated with a priming dose of nicotine (0.5 mg/kg, *ip*) preceded by an injection of AM 251 or saline. Data represent the means ± SEM and are expressed as scores, i.e. the difference (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment. n = 7–10 rats per group. *** $p < 0.001$ *vs.* saline-conditioned control group receiving saline injection on the reinstatement day; ### $p < 0.001$ *vs.* nicotine-conditioned group given nicotine injection on the reinstatement day (Tukey test)

Figure 1 shows that after three conditioning sessions (days 2–4), nicotine (0.5 mg/kg) induced a clear place preference in animals that had previously received nicotine injections, indicated by a significant increase in time spent in the drug-associated compartment during the post-conditioning test phase (day 5) ($p < 0.001$).

Figure 1 also shows that the time spent in the nicotine-paired chamber gradually diminished over days of repeated test training. On day 6 (first test for extinction, Extinction 1), conducted 24 h after the preference test, animals still spent more time in the nicotine-paired compartment than in the saline-paired one ($p < 0.001$). On day 8 (second test for extinction, Extinction 3), 72 h after the initial preference test, the time spent in the nicotine-paired compartment did not significantly differ from time spent in the saline-paired compartment, indicating that nicotine-conditioned place preference had been extinguished by repeated test trials. The priming injection of nicotine (0.5 mg/kg, *ip*), injected one day after the last extinction trial (day 9), completely reinstated the extinguished nicotine conditioned place preference ($p < 0.001$).

Interestingly, the CB1 receptor antagonist AM 251 dose dependently attenuated the priming effect of nicotine on nicotine-conditioned place preference [$F(15,72) = 110.7$, $p < 0.0001$] (Fig. 1). At a dose of 0.5 mg/kg ($p < 0.001$), but not at a dose of 0.25 mg/kg, AM 251 prevented the reinstatement of previously established nicotine place preference. AM 251, at the doses tested, did not cause significant changes in place preference by itself (data not shown).

Discussion

In the present study, we used the CPP paradigm to study the extinction and reinstatement of extinguished nicotine place preference, a model consistent with drug seeking behavior. As it was previously shown, nicotine in our experiments, nicotine in our experimental conditions induces place preference [1, 2]. The dose of nicotine was chosen according to the narrow dose ranges reported to produce CPP in rats [1, 2, 5]. The present study supports and extends our previous findings supporting the idea that nicotine reliably induces CPP in rats subjected to a biased procedure. Subsequently, once established, nicotine place preference was extinguished by repeated daily testing. As demonstrated previously, a priming dose of nicotine effectively reinstates nicotine-seeking behavior in the CPP-reinstatement paradigm [1, 2].

According to the latest epidemiological data, relapses to drug taking behavior are very important clinical problems. The definition of this phenomenon, in the context of the drug of abuse, refers to the restitution of previously drug-reinforced behaviors by non-contingent exposure to drug or non-drug stimuli after extinction. Several factors were shown to be associated with relapse, including re-exposure to the drug (a priming dose of the drug), presentation of environmental cues associated with drug taking (e.g. location where the drug was consumed or administered) [14, 22, 23], or the exposure to certain stressors [25]. The fact that the same factors provoke relapse in humans and reinstate drug-seeking behavior in animal models may be exploited to develop more effective methods to achieve long-term abstinence. As previously reported, a priming dose of nicotine produces a significant reinstatement effect [1, 2]. However, cues associated with nicotine administration play an important role in the observed effects [3]. The combination of the two stimuli results in an additive effect producing robust reinstatement of nicotine seeking behavior. We can suppose that similar neural circuits mediate both forms of reinstatement. Nevertheless, little is known about possible mechanisms involved in nicotine relapse, but several studies suggest the role of dopaminergic, glutamatergic, GABAergic, endocannabinoid and opioidergic systems in the reinstatement of nicotine seeking behavior [2, 6, 11].

A possible role of the endocannabinoid system in motivational effects of nicotine was also confirmed in our study. The major finding of the present experiments was that the CB1 receptor antagonist AM 251 reduced the reinstatement of nicotine seeking behavior in the CPP-reinstatement paradigm. The observed effect was dose-dependent and significant following a 0.5 mg/kg dose of AM 251. In contrast, a smaller dose, 0.25 mg/kg, did not produce a reduction in the response. On the basis of this research we tentatively conclude that AM 251 reduced the motivational effects of nicotine and the proposed mechanism is mediated *via* CB1 cannabinoid receptors [24].

Moreover, many behavioral and pharmacological studies confirm the interaction between cholinergic and endocannabinoid systems. The recent study showed that nicotine and Δ9-tetrahydrocannabinol

(THC) administered together at ineffective doses produces significant CPP in mice [30]. Other reports revealed that acute nicotine administration potentiates hypothermia, antinociception, hypolocomotion and anxiolytic-like responses induced by an acute administration of THC [30]. Additionally, nicotine does not produce rewarding effects in CB1 knock-out mice, although this effect was observed in wild type animals measured in the CPP paradigm [6]. As mentioned above, the cues associated with nicotine administration are very important for sustaining smoking in humans as well as for reinstating drug-seeking behaviors in laboratory animals. The studies also revealed that the CB1 receptor antagonist rimonabant reduces responses maintained by nicotine-associated cues in the absence of nicotine in a self-administration paradigm [8]. Moreover, rimonabant reduced the expression of nicotine induced CPP [18]. These data may suggest that CB1 antagonists reduce not only the motivational and rewarding effects of nicotine, but also the relapse to drug-seeking behaviors induced by environmental cues. In the context of our study, it is worth mentioning that pre-treatment with AM 251 dose-dependently attenuated the reinstatement of nicotine-seeking behavior produced by nicotine and environmental cues [24].

Recent evidence suggests that the endocannabinoid system plays a prominent role in motivational effects not only of nicotine but also other drugs of abuse. For example, the CB1 antagonist HU210 reinstates self-administration of cocaine after long-term extinction, whereas the CB1 antagonist – rimonabant reverses this effect [10]. Moreover, it has been reported that AM 251 inhibits cocaine-induced reinstatement of previously extinguished cocaine self-administration [31]. These effects may further suggest a role of CB1 receptors in psychostimulant relapse. Additionally, several reports demonstrated that CB1 receptor antagonists inhibit self-administration of ethanol [15] or opioids [4, 12], and prevent acquisition or expression of the CPP induced by morphine or cocaine [7].

The findings in the present study show that AM 251, a CB1 cannabinoid receptor antagonist, is able to attenuate relapse in nicotine seeking behavior observed in the CPP paradigm. As mentioned above, this action may result from indirect or direct modulation of dopamine release by cannabinoids or nicotine, most notably in the NAC. In the context of the present experiments, studies using brain microdialyses in rats [9] showed that dopamine levels increase in the shell of the NAC and the bed nucleus of stria terminals after administration of nicotine, and this effect is antagonized by rimonabant.

In conclusion, the present studies demonstrate that the selective CB1 receptor antagonist AM 251 dose-dependently prevents the reinstatement of nicotine induced place preference caused by a priming dose of nicotine. However, further studies are needed to examine the role of endocannabinoids on different aspects of nicotine dependence. These studies may contribute to the development of more effective pharmacotherapies for tobacco addiction.

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