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Calcium-dependent mechanisms of the reinstatement of nicotine-conditioned place preference by drug priming in rats

G. Biala *, B. Budzynska

Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, 4 Staszica Street, 20-081 Lublin, Poland

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Abstract

Reinstatement of drug-seeking behaviour in animals is relevant to relapse to drug taking in humans. We used the conditioned place preference version of the reinstatement model to investigate the establishment, extinction, reinstatement and cross-reinstatement of nicotine-induced place conditioning in rats. Nicotine produced a place preference to the compartment paired with its injections during conditioning (0.5 mg/kg, i.p., three drug sessions). Once established, nicotine place preference was extinguished by repeated training. Following this extinction phase, nicotine-experienced rats were challenged with nicotine (0.5 mg/kg, i.p.), a cannabinoid receptor agonist WIN55,212-2 (0.5 mg/kg, i.p.), ethanol (0.5 g/kg, i.p.) or D-amphetamine (2 mg/kg, i.p.). The priming injections of nicotine, WIN55,212-2 and ethanol, but not of D-amphetamine renewed a preference for the compartment previously paired with nicotine. Finally, we examined the influence of the calcium channel antagonists, nimodipine (5 and 10 mg/kg, i.p.) and flunarizine (5 and 10 mg/kg, i.p.), on the reinstatement of nicotine place conditioning induced by WIN55,212-2 and ethanol. It was shown that the calcium channel blockers attenuated the reinstatement of nicotine-conditioned response induced by both drugs. As reinstatement of drug-seeking is a factor for the development of dependence, the L-type calcium channel antagonists may be useful in the relapse-prevention phase of addiction treatment, including cannabinoid, ethanol, and/or nicotine dependence.

Keywords: Nicotine; WIN55,212-2; Ethanol; D-amphetamine; Reinstatement; Calcium channel antagonists; Place conditioning; Rats

1. Introduction

Drug addiction is a chronic relapsing brain disorder, characterized by neurobiological changes leading to compulsive drug-seeking and drug taking. The high rate of relapse to drug use after a long period of abstinence is one of the main problems in the treatment of addiction (Fiore, 2000; Shalev et al., 2002). Several factors can elicit drug craving and trigger relapse in humans, including exposure to drug-associated environmental cues, stressors, re-experience of drug, and negative withdrawal symptoms (Brigham et al., 1990; Kassel et al., 2003). High rates of relapse are also characteristic for people trying to quit tobacco smoking during the first 6 months following smoking cessation. The factors mentioned above have been also shown to be associated with smoking relapse, especially exposure to smoking cues, stressors, and aversive symptoms of withdrawal. It has been already documented that nicotine, an alkaloid present in tobacco, is responsible for pharmacological effects of smoking and for its addictive potential, including drug-seeking and relapse.

In animal models, the relapse to drug taking can be investigated in drug self-administration studies and with the place preference paradigm using the reinstatement procedure. The second procedure, conditioned place preference (CPP), is a simple non-invasive method, compatible with classical Pavlovian conditioning (Itzhak and Martin, 2002). In this paradigm, animals are initially trained to associate one distinctive environment with drug injection, and a different environment with vehicle injection. Following training, animals spend more time in the drug-paired environment, when given a choice between the two environments on a drug-free test day. This acquired preference can be extinguished by daily injections of saline (alternatively, the days without any injections) with free access to both drug-paired and saline-paired environments. It was found that after extinction, the non-contingent exposure to the

* Corresponding author. Tel.: +48 81 535 73 71; fax: +48 81 528 89 18.
E-mail address: grazyna.biala@am.lublin.pl (G. Biala).

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drug of abuse acts to renew the significance and attractiveness of the drug-related environmental stimuli (the incentive value of drug-associated cues), drawing the animal to approach and remain in the presence of the drug-related stimuli, resulting in the reinstatement of the CPP (Mueller and Stewart, 2000; Parker and McDonald, 2000). This animal model has been used to measure the appetitive value of different stimuli as well as to evaluate relapse to abuse of drugs, such as cocaine (Mueller and Stewart, 2000), opiates (Parker and McDonald, 2000), alcohol and amphetamine (see review Tzschentke, 1998). Several animal studies have also demonstrated that drugs other than those previously received can reinstate drug-seeking behaviour. This phenomenon, termed cross-reinstatement, has been already described using drugs from different classes. For instance, amphetamine may reinstate responding in heroin-trained animals, whereas morphine infusion into the ventral tegmental area (VTA) or heroin into the nucleus accumbens reinstates cocaine-seeking behaviour (Pierce and Kalivas, 1997; Stewart, 1984).

A large body of evidence indicates the participation of dopaminergic transmission in the reinforcing properties of drugs of abuse, including drug-seeking behaviour (Di Chiara and Imperato, 1988). The mesocorticolimbic dopaminergic system, which projects from the VTA to the ventral striatum, especially to the nucleus accumbens, and the increase in extracellular dopamine concentration in these pathways, is thought to be a major neurobiological substrate of the addictive properties of drugs (Corrigall et al., 1992; Spanagel and Weiss, 1999). For example, amphetamine and cocaine, indirect dopamine agonists, block dopamine transporters, increasing dopamine release in this way (Seiden et al., 1993). Ethanol alters the function of different ionotropic receptors, including the GABA-A/benzodiazepine receptor complex and glutamatergic receptors, but this drug can also modulate the function of neuronal nicotinic cholinergic receptors (AChNRs) (Larsson and Engel, 2004; Davis and de Fiebre, 2006). Concerning cannabinoids, activation of CB1 cannabinoid receptors in the VTA has been shown to enhance mesolimbic dopamine release in the nucleus accumbens indirectly, by disinhibition of GABA-containing interneurons in this area (endocannabinoid retrograde control) (Wilson and Nicoll, 2002). Nicotine is thought to increase dopamine transmission in the nucleus accumbens by stimulating the AChNRs located on the dopaminergic, glutamatergic and GABAergic afferent neurons in this area (Corrigall et al., 1992; Dani et al., 2001). Although the dopaminergic system has been considered as major neural substrate for the motivational and reinforcing properties of nicotine, there is an evidence that other neurotransmitter systems, including the opioidergic and cannabinoid system, might be also involved in its behavioural effects (Biala and Budzynska, 2006; Isola et al., 2002; Valjent et al., 2002).

Tobacco and cannabis are among the most widely consumed drugs of abuse in humans. They share the same biological actions and are used frequently in combination. As already mentioned, nicotine and Δ9-tetrahydrocannabinol (THC), the major psychoactive component of cannabis exert their initial effect at different receptors, highly expressed in the brain, nicotinic acetylcholine ion channel receptors or CB cannabinoid G-protein-coupled receptors, respectively (Pertwee, 1999; Wonnacott, 1997). In animals, they exhibit the main features of addictive drugs. In particular, they have discriminative effects, support self-administration, and facilitate intracranial self-stimulation (Biala, 2003; Biala and Budzynska, 2006; Gonzalez et al., 2005). Concerning polydrug use, it has been confirmed that current smokers and nicotine-dependent subjects had a greater severity of alcohol dependence. Although it has been known that alcoholism and tobacco addiction often co-occur, relatively little is still known about the biological factors that regulate the co-use and abuse of nicotine and alcohol. There is evidence that nicotine modulates the rewarding, aversive, and discriminative stimulus effects of ethanol in rodents (Bienkowski et al., 1998; Korkosz et al., 2006). Given the data already reported, the study of the functional interactions between nicotine and cannabinoid receptor ligands and/or ethanol are of special interest.

Relapse to drug taking remains the most difficult challenge for treatment of addiction, but the neurobiological mechanisms that underlie the persistence of such behaviour remain poorly understood. Although the CPP paradigm has been extensively used to examine the reinstatement of cocaine, amphetamine, morphine or alcohol, relatively few studies have examined reinstatement of nicotine place conditioning. The present experiments were undertaken to further establish a model of nicotine reinstatement and cross-reinstatement in rats. We used the nicotine-conditioned place preference-reinstatement procedure evaluated in our previous studies (Biala, 2003; Biala and Budzynska, 2006). In a set of our experiments, nicotine place preference, once acquired, was extinguished by repeated test trials in a few successive days. Afterwards, in attempt to reinstate nicotine-conditioned place preference, the animals were given a priming dose of nicotine. Based on the finding that similar neural substrates are involved in the rewarding effects of nicotine, cannabinoid receptor agonists, ethanol, or D-amphetamine (Maldonado et al., 2006), we also evaluated cross-reinstatement between nicotine and these addictive drugs. For this purpose, we examined the ability of a priming dose of a synthetic CB1 receptor agonists WIN55,212-2, ethanol and D-amphetamine to reinstate extinguished nicotine place preference. Furthermore, in accordance with previous studies suggesting the participation of calcium ions and calcium channels in several aspects of drug reward and addiction (Biala and Langwinski, 1996), we investigated the influence of calcium channel antagonists on the reinstatement of nicotine-conditioned place preference provoked by a priming dose of compounds mentioned above. In this experiment, two representative L-type voltage-dependent calcium channel (VDCC) antagonists, flunarizine and nimodipine, which are characterized by their high lipophility and central effects, were used. As reinstatement of responding induced by priming injections of drugs is widely accepted as a relevant model to study the mechanisms involved in compulsive drug craving behaviour and relapse, all these experiments were undertaken to further investigate the neurobiological processes underlying relapse to nicotine-taking and polydrug abuse.

2. Materials and methods

2.1. Animals

The experiments were carried out on 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 adapted to the laboratory conditions for at least 1 week. The rats were handled once a day for 5 days preceding the experiments. Each experimental group consisted of 12 animals. The experiments were performed between 9.00 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 the Care and Use of Laboratory Animals (86/609/EEC), and approved by the local ethics committee.

2.2. Drugs

The compounds tested were: (−)-nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA), D-amphetamine sulphate (Sigma, St. Louis, MO, USA), nimodipine (Sigma, St. Louis, MA, USA), flunarizine dihydrochloride (Sigma, St. Louis, MA, USA), ethanol (Polmos, Poland), and WIN55,212-2 (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanonone mesylate; Tocris Cookson, Bristol, UK). The drugs were dissolved in saline (0.9% NaCl). Ethanol was prepared for injections by diluting 95% ethanol to obtain a concentration of 10% (v/v). All agents were administered intraperitoneally (i.p.) in a volume of 5 ml/kg. All the doses are expressed as the salts. Control groups received saline injections in the same volume and by the same route.

2.3. Apparatus

The testing apparatus for the conditioned place preference paradigm was similar to that used by Spyraki et al. (1982). Each of six rectangular boxes (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 were painted black. The central grey area constituted a "neutral" chamber. The testing boxes were kept in a soundproof room with neutral masking noise and dim 40-lx illumination.

2.4. Procedure

The conditioned place preference-reinstatement procedure 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 (Biala, 2003; Biala and Budzynska 2006).

2.4.1. Pre-conditioning

During this phase (day 1), each animal was placed 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 subjects showed a moderate preference for the black compartment.

2.4.2. 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, i.p.) 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 large compartments, as mentioned above. A dose of 0.5 mg/kg nicotine was chosen for conditioning because it is known to produce reliable conditioned place preference in rats, also under our experimental conditions. The control group received vehicle every day. The neutral zone was never used during conditioning and was blocked by guillotine doors.

2.4.3. Post-conditioning (test)

During this phase (day 5), conducted 1 day after the last conditioning trial, animals were placed in the neutral area with the guillotine doors removed and allowed free access to the entire apparatus for 15 min. The amount of time spent by each rat in the two large compartments was recorded. No injections were given on the day of this preference test.

2.4.4. Extinction training

One day after the preference test, the rats were given extinction training daily for 3 days. On each trial, the rat was 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 initial preference test, and on day 8 (Extinction 3), 72 h after this preference test.

2.4.5. Reinstatement

One day after the last extinction trial (day 9), a separate groups of rats received a priming i.p. injection of nicotine (0.5 mg/kg), WIN55,212-2 (0.5 mg/kg), ethanol (0.5 g/kg), D-amphetamine (2 mg/kg), or saline and were immediately tested for reinstatement of conditioned place preference. In other experiments, the groups of rats were treated i.p. with nimodipine (5 and 10 mg/kg) or flunarizine (5 and 10 mg/kg), 15 min prior to administration of ethanol or WIN55,212-2. During this reinstatement test, the rats were allowed free access to the entire apparatus for 15 min, and the time spent in each chamber was measured.

2.5. Statistics

The data are expressed as means ± S.E.M. For CPP paradigm, the statistical analyses of the various treatments were performed

using 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 as statistically significant.

3. Results

3.1. Nicotine-conditioned place preference: expression, extinction and reinstatement

The time spent on the initially less preferred (white) and on 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.

Fig. 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).

Fig. 1 also shows that the time spent in the nicotine-paired chamber gradually diminished over days of three consecutive 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. 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 the time spent in the saline-paired compartment, indicating that nicotine-conditioned place preference had been extinguished by repeated (three) test trials. Interestingly, the priming administration of nicotine (0.5 mg/kg, i.p.) (Fig. 1) completely reinstated the extinguished nicotine-conditioned place preference in rats.

3.2. WIN55,212-2-induced reinstatement

The priming injection of WIN55,212-2 (0.5 mg/kg, i.p.) (Figs. 1 and 2) administered 1 day after the last extinction trial (day 9), completely reinstated the extinguished nicotine-conditioned place preference. Interestingly, both calcium channel blockers also attenuated the priming effect of WIN55,212-2 on nicotine-conditioned place preference [$F(4,26)=12.04$, $p<0.0001$] (Fig. 2). Nimodipine at a dose of 10 mg/kg ($p<0.001$) or flunarizine at the doses of 5 mg/kg ($p<0.05$) and 10 mg/kg ($p<0.001$, Tukey test), injected i.p. 15 min before the priming dose of WIN55,212-2, prevented the reinstatement of previously established nicotine place preference in rats (Fig 2).

3.3. Ethanol-induced reinstatement

The priming administration of ethanol (0.5 g/kg, i.p.) (Figs. 1 and 3) injected 1 day after the last extinction trial (day 9), completely reinstated the extinguished nicotine-conditioned

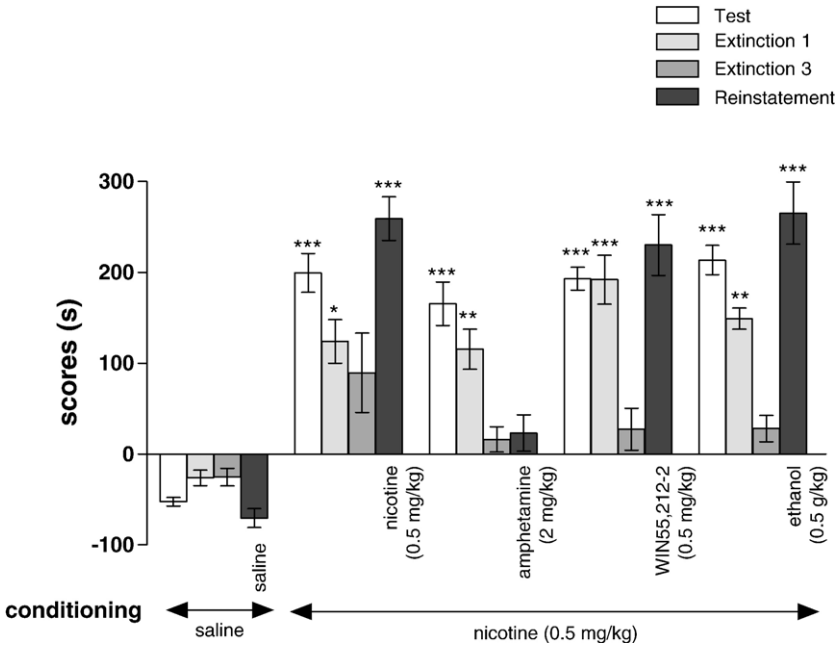

Fig. 1. Reinstatement of nicotine-conditioned place preference in rats caused by a priming dose of nicotine, D-amphetamine, WIN55,212-2 and ethanol. Place preference procedure consisted of pre-conditioning, three conditioning sessions with nicotine (0.5 mg/kg, i.p.), post-conditioning test followed by 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, rats were injected with a priming dose of nicotine (0.5 mg/kg, i.p.), D-amphetamine (2 mg/kg, i.p.), WIN55,212-2 (0.5 mg/kg, i.p.), ethanol (0.5 g/kg, i.p.), or saline. Data represent means ± S.E.M. and are expressed as the difference (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment. $n=12$ rats per group. *$p<0.05$; **$p<0.01$; ***$p<0.001$ vs. saline-conditioned control group receiving saline injection on the reinstatement day (Tukey test).

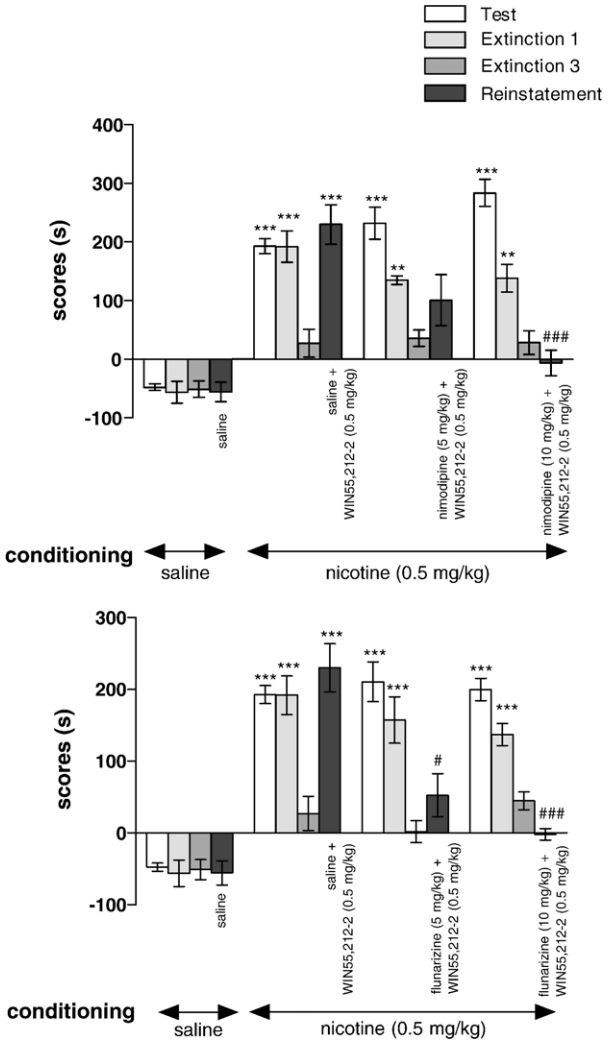

Fig. 2. Effects of nimodipine (5 and 10 mg/kg, i.p.) and flunarizine (5 and 10 mg/kg, i.p.) on the reinstatement of nicotine-conditioned place preference caused by a priming dose of WIN55,212-2. Place preference procedure consisted of pre-conditioning, three conditioning sessions with nicotine (0.5 mg/kg, i.p.), 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 WIN55,212-2 (0.5 mg/kg, i.p.) preceded by an injection of nimodipine, flunarizine or saline. Data represent means±S.E.M. and are expressed as the difference (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment. $n=12$ rats per group. $^{**}p<0.01$; $^{***}p<0.001$ vs. saline-conditioned control group receiving saline injection on the reinstatement day; $\#p<0.05$; $\#\#\#p<0.001$ vs. nicotine-conditioned group given WIN55,212-2 injection on the reinstatement day (Tukey test).

place preference. Pretreatment with calcium channel blockers used, nimodipine and flunarizine, dose-dependently inhibited the priming effect of ethanol in nicotine-treated rats [$F(4,26)=4.07$, $p=0.007$] (Fig. 3). Post-hoc individual comparisons indicated a significant effect of nimodipine at a dose of 10 mg/kg ($p<0.001$) and of flunarizine at a dose of 10 mg/kg ($p<0.001$, Tukey test) (Fig. 3).

Neither of the calcium channel antagonists, at the doses tested, caused significant changes in place preference by themselves. None of the drugs used modified the locomotor activity of rats at the doses tested (data not shown).

3.4. Amphetamine-induced reinstatement

The priming administration of D-amphetamine (2 mg/kg, i.p.) (Fig. 1) injected on the 9th day, after the last extinction trial, did not reinstate the extinguished nicotine-conditioned place preference.

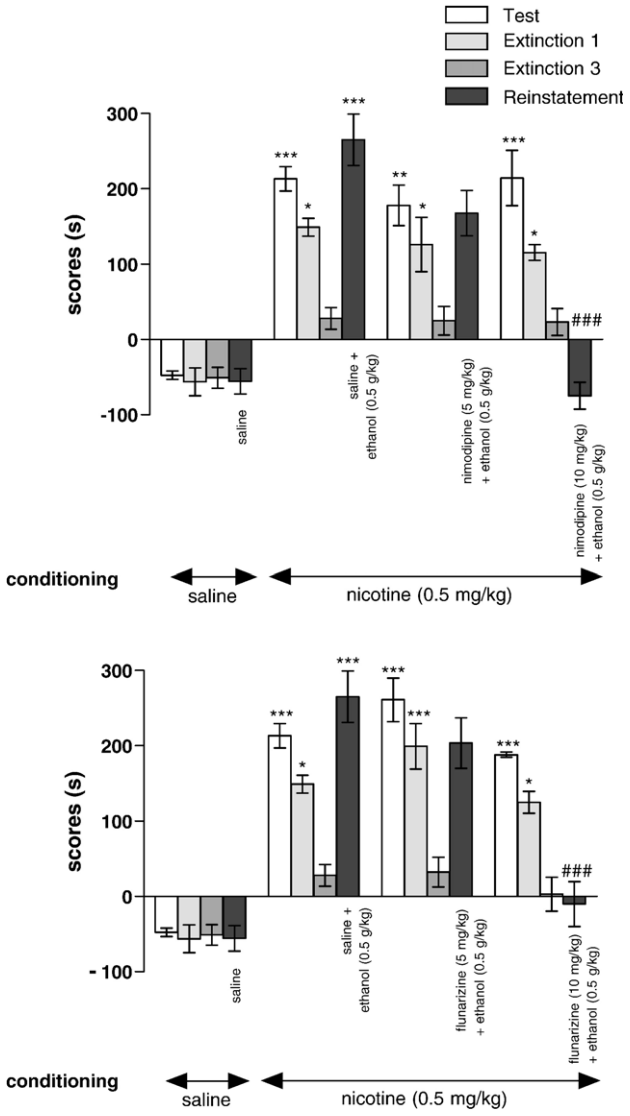

Fig. 3. Effects of nimodipine (5 and 10 and mg/kg, i.p.) and flunarizine (5 and 10 mg/kg, i.p.) on the reinstatement of nicotine-conditioned place preference caused by a priming dose of ethanol. Place preference procedure consisted of pre-conditioning, three conditioning sessions with nicotine (0.5 mg/kg, i.p.), 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 ethanol (0.5 g/kg, i.p.) preceded by an injection of nimodipine, flunarizine or saline. Data represent means±S.E.M. and are expressed as the difference (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment. $n=12$ rats per group. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$ vs. saline-conditioned control group receiving saline injection on the reinstatement day; $\#\#\#p<0.001$ vs. nicotine-conditioned group given ethanol injection on the reinstatement day (Tukey test).

4. Discussion

In the present experiments, we used the conditioned place preference paradigm to further study the extinction and reinstatement of extinguished nicotine place conditioning, a model consistent with drug-seeking behaviour (see review Epstein et al., 2006). In a series of experiments, rats were initially conditioned to associate an environment with nicotine administration. The dose of nicotine was chosen according to the narrow dose range reported to produce CPP in rodents (Biala, 2003; Biala and Budzynska 2006; Calcagnetti and Schechter, 1994). The present study supports and extends our previous findings giving evidence that, in rats subjected to a biased procedure, nicotine reliably induced CPP. Subsequently, once established, nicotine place preference was extinguished by repeated daily testing. Once extinguished, the original place preference can be reinstating by a priming dose of nicotine (Biala and Budzynska, 2006). Our previous results showed that both nicotine and morphine were able to induce reacquisition of this extinguished nicotine-conditioned place preference causing the reappearance of a preference for a compartment previously paired with nicotine (Biala and Budzynska, 2006). In order to further investigate the phenomenon of cross-reinstatement, rats were then given one priming injection of either WIN55,212-2, a CB1 receptor agonist, ethanol or D-amphetamine. The challenge doses of drugs were chosen on the basis of the pilot experiments showing their ability to produce reinstatement in our experimental conditions without affecting locomotor activity of mice (not shown). The results show that WIN55,212-2 and ethanol, but not D-amphetamine are able to induce reacquisition of extinguished nicotine-conditioned place preference. In the second series of experiments, the model of nicotine-conditioned place preference was used to study effects of calcium channel antagonists on drug-primed reinstatement of extinguished place preference. Thus, we investigated the effect of two calcium channel blockers, nimodipine and flunarizine, which act at L-type VDCCs, on the expression of WIN55,212-2 or ethanol-induced reinstatement of nicotine-induced place conditioning. Interestingly, we found that both antagonists, administered prior to the priming injections of drugs used, attenuated, in a dose-dependent manner, their reinstatement effects. These findings emphasize our previous suggestion (Biala and Budzynska, 2006) that calcium ions and calcium channels play an important role in modulating the reacquisition of drug-seeking behaviour following the extinction phase.

In human addicts, high rates of relapse are typical in people trying to abstain from tobacco use, and the relapse is often provoked by acute re-exposure to nicotine after a period of abstinence (Chornock et al., 1992). Using one of the reinstatement models in rats, it has been shown that acute non-contingent administration of nicotine during extinction of nicotine self-administration reinstates responding (i.e. nicotine-taking behaviour) on a lever that previously delivered nicotine (Chiamulera et al., 1996; Shaham et al., 1997). However, the magnitude of the reinstatement of nicotine self-administration was less robust than that for other drugs of abuse, such as cocaine and opioid agonists (LeSage et al., 2004).

Little is known about the possible mechanisms involved in the relapse to nicotine, the most widely used addictive substance. It cannot be excluded that, like other drugs of abuse, environmental cues paired with nicotine also provoke nicotine-seeking, probably by activating the neuronal substrates that mediate relapse after acute re-exposure to the drug. Also in human addicts, drug-associated environmental cues can maintain tobacco use and contribute to relapse even after long periods of abstinence. One could expect that reinstatement of CPP was a consequence of both exposure to nicotine-paired stimuli and nicotine priming. The acute priming injection of the training drug can reinforce the effectiveness and renew the relevance of drug-associated environmental stimuli in promoting drug-seeking behaviours also in our experimental model.

One could propose that the mesolimbic dopamine system, which is involved in the acute reinforcing effect of nicotine (Corrigall et al., 1992) as well as in the reinstatement of opioid and stimulant drugs, is also implicated in the reinstatement effect of nicotine-conditioned place preference. Nicotine shares with other addictive drugs on the property of activating the mesolimbic dopaminergic reward pathway. This drug induces a prolonged increase in firing and burst rates of dopamine neurons in the VTA by interacting with different nicotinic receptors located on these neurons as well as on GABAergic and glutamatergic afferents. This effect promotes a sustained dopamine overflow in the nucleus accumbens (Di Chiara and Imperato, 1988; Pontieri et al., 1996; Wonnacott, 1997). Neuroanatomical studies indicate that the cell body region of the mesocorticolimbic dopamine system in the VTA plays a critical role in drug-induced reinstatement and increased dopamine transmission in the VTA, and likely contributes to drug-seeking behaviour (Di Ciano and Everitt, 2004; Shaham et al., 1997). There is also evidence that several other neurotransmitter systems, e.g. endocannabinoids, opioid system, and serotonin receptors are involved in drug-induced reinstatement, including nicotine effects (Biala and Budzynska, 2006; Castane et al., 2002; Cohen et al., 2005).

A possible role of endocannabinoid system in the motivational effects of nicotine, was also confirmed in our study showing a reinstatement of nicotine-induced place preference in rats by a CB1 cannabinoid receptor agonist. Interestingly, a co-localisation of CB1 receptors and AChNRs has been reported in several brain areas, such as the hippocampus and the amygdala (Picciotto et al., 2000), which supports the possibility of functional interactions between these two systems. Thus, both nicotine and endocannabinoids interfere with GABAergic and glutamatergic control within the VTA over dopamine neurons, acting at the receptors located on afferent terminals, to enhance (AChNRs) or suppress (CB1) local dopamine release, respectively (De Vries and Schoffelmeer, 2005; Tanda and Goldberg, 2003). The possible interactions between cholinergic and endocannabinoid systems have been also confirmed in many behavioural and pharmacological studies. For instance, it has been revealed that non-effective doses of nicotine and THC produced significant CPP in mice when administered together (Valjent et al., 2002). Additionally, the rewarding properties of nicotine, assessed in a place conditioning model, were absent in

knock out mice lacking CB1 receptors (Castane et al., 2002). Rimonabant, a CB1 receptor antagonist, reduced nicotine-induced conditioned preference (Le Foll and Goldberg, 2004) as well as the influence of environmental stimuli on nicotine-seeking behaviours in rats (Cohen et al., 2005). In mice, nicotine facilitated hypothermia, antinociception, hypolocomotion and anxiolytic-like responses induced by THC (Valjent et al., 2002), whereas THC decreased somatic and motivational manifestations of nicotine withdrawal in mice. Moreover, in animals chronically exposed to nicotine, an increase in endocannabinoid levels in the limbic forebrain has been observed (Gonzalez et al., 2002).

Based on evidence for a cannabinoid–dopamine interaction it can be suggested that activation of endogenous cannabinoid system downstream from dopamine synapse may contribute to psychostimulant and nicotine-seeking. In the context of our study showing a cross-reinstatement between nicotine and WIN55,212-2 it has been already revealed that, alike nicotine, systemic administration of this synthetic CB1 receptor agonist in rats increased the activity of dopamine neurons within the VTA. This effect resulted in the increased extracellular dopamine levels within the mesolimbic structures (Tanda and Goldberg, 2003). On the other hand, in vivo microdialysis studies revealed that pre-treatment with rimonabant blocked nicotine-enhanced extracellular dopamine levels in the shell of the nucleus accumbens (Cohen et al., 2002). Endocannabinoid system is also involved in the motivation to seek the drug by dopamine-independent mechanisms, probably by modulation of the impact of reward-related memories. Accordingly, it has been shown that endocannabinoids acting as retrograde messengers mediate synaptic plasticity in several addiction and memory-related brain areas (De Vries and Schoffelmeer, 2005).

It is worth mentioning that the endogenous cannabinoid system plays also a key role in brain mechanisms underlying the motivational effects of drugs of abuse and drug-related stimuli, including drug-seeking. Indeed, high levels of CB1 receptors are present in brain regions that play a pivotal role in relapse-like behaviour in animal, such as the prefrontal cortex, amygdala, nucleus accumbens, striatum and hippocampus (Gardner, 2005). In these regions, CB1 receptor activation causes the release of a variety of neurotransmitters like dopamine, GABA and glutamate, which are implicated in the reinstatement of drug-seeking (De Vries and Schoffelmeer, 2005). Thus, the potent cannabinoid receptor agonist HU210 was able to reinstate cocaine-seeking following long-term extinction of intravenous cocaine self-administration in rats (De Vries et al., 2001). This action was antagonized by the cannabinoid receptor antagonist SR141716A (rimonabant), which suggested a role of CB1 receptors in psychostimulant relapse. Blockade of the CB1 receptors was effective in reducing cue-induced reinstatement of drug-seeking, an animal analogue of cue-induced relapse in human addicts. These relapse-preventing properties were observed with different classes of abused drug, including psychostimulants and opioid agonists (De Vries et al., 2003; De Vries and Schoffelmeer, 2005).

Our experiments also revealed that ethanol could reinstate nicotine-induced CPP in rats. Our results fit well to other previous behavioural experiments in animals showing that alcohol and nicotine produced similar effects in several behavioural models, i.e. sensitization, place preference or self-administration (Biala, 2003; Hoshaw and Lewis, 2001; Risinger and Roger, 1995). In addition, several reports demonstrated cross-tolerance or cross-sensitization between ethanol and nicotine (Al-Rejaie and Dar, 2006b; Biala and Weglinska, 2004; Collins et al., 1996). Other reports revealed behavioural interactions between these drugs in tests of motor activity, alcohol self-administration, learning/memory and anxiety (Clark et al., 2001; Lapin et al., 1995; Nadal and Samson, 1999; Rezvani and Levin, 2002). Results have shown that microinfusion of nicotine significantly and dose-dependently attenuated ethanol-induced ataxia and this effect was blocked by an AChNR antagonist hexamethonium, suggesting participation of nicotinic receptors (Al-Rejaie and Dar, 2006a). Moreover, in mice, ethanol-induced enhancements of locomotor activity and brain dopamine turnover were partially counteracted by mecamylamine, a blood-brain barrier penetrating AChNR antagonist (Blomquist et al., 1992).

It has been well-established that all major pharmacological effects of nicotine are mediated through several different types of AChNRs located within the brain. Ethanol alters the function of different ionotropic receptors, including the GABA-A/benzodiazepine receptor complex, glutamatergic receptors, but this drug can also modulate e.g. enhancing or inhibiting the function of neuronal AChNRs (Davis and de Fiebre, 2006; Larsson and Engel, 2004). Interestingly, ethanol may also exert its effects via direct interaction with AChNRs and/or via enhancement of extracellular acetylcholine levels in the VTA that subsequently stimulate dopamine overflow in the nucleus accumbens (Larsson et al., 2005). An additive or synergistic effect, especially on the release of accumbal dopamine induced by simultaneous administration of ethanol and nicotine, could further contribute to their co-abuse.

Unlike CB1 receptor agonist and ethanol, we have revealed that D-amphetamine did not provoke any reinstatement effect on nicotine-induced place conditioning in rats. Accordingly, other studies have shown no cross-sensitization between the mesoaccumbens dopamine responses to nicotine and other psychostimulants (Henry et al., 1989). It seems unlikely that the mechanisms involved in the nicotine- and psychostimulant-induced responses are identical. However, cross-tolerance between nicotine and cocaine (but not vice-versa) can be demonstrated if several behaviours are observed, while measures of locomotor activity and/or rewarding properties in the CPP paradigm are less sensitive effects (Desai and Philip, 2003).

The present experiments were also designed to further evaluate the possible role of calcium ions and calcium-mediated second messenger systems in cross-reinstatement between nicotine, WIN55,212-2 or ethanol. Our previous results have already shown the inhibition of nicotine- or morphine-induced reinstatement of previously established nicotine place preference in rats by the calcium channel blockers (Biala and Budzynska, 2006). The present data revealed that systemic administration of nimodipine and flunarizine dose-dependently attenuated conditioned nicotine-seeking behaviour in the CPP

model, by blocking the reinstatement effect of WIN55,212-2 and ethanol priming. It is important to note that neither of the calcium channel antagonists, given acutely nor repeatedly at the doses used, had any effects in naive rats and did not provoke any reinforcing effects in the CPP paradigm by themselves. These results are in accordance with our recent data demonstrating that calcium channel blockers inhibit the acquisition and the expression of nicotine-induced sensitization and place preference as well as the expression of behavioural cross-sensitization and cross-reinstatement to locomotor or conditioned rewarding effects between nicotine and morphine (Biala, 2003; Biala and Budzynska, 2006; Biala and Weglinska 2004).

A growing body of evidence indicates that calcium-mediated second messenger systems play an important role in the reinforcing and stimulant effects of psychoactive drugs. For instance, it has been reported that calcium channel antagonists decrease the sensitization response seen after repeated doses of amphetamine (Karler et al., 1991), and amphetamine- or cocaine-induced CPP (Pani et al., 1991; Pucilowski et al., 1993). Taking into account the involvement of calcium channels in drug dependence, it has been shown that administration of some calcium channel blockers decreases the signs of naloxone-precipitated morphine withdrawal syndrome in rats (Antkiewicz-Michaluk et al., 1993). Some studies suggest that calcium-dependent mechanisms are also involved in the behavioural effects of nicotine. Calcium channel antagonists, given intrathecally, reduced significantly the antinociception induced by nicotine (Damaj, 2000). Pretreatment with isradipine, a calcium channel blocker, also produced a significant blockade of nicotine discrimination in rats (Schechter and Meehan, 1992). It is well-established that neuronal nicotinic receptors located at pre- and post-synaptic sites within the central nervous system are highly permeable to calcium ions (Vernino et al., 1992). This high calcium permeability influences intracellular processes and modulates the release of several neurotransmitters, including dopamine, noradrenaline, adrenaline, serotonin, glutamate and acetylcholine itself (Wonnacott, 1997). Given the well-established role of dopamine in many aspects of nicotine addiction, it has been already pointed out that calcium channel antagonists acting at L-type calcium channels are effective blockers of nicotine-evoked dopamine release from rat striatal synaptosomes (Kulak et al., 2001; Prince et al., 1996). This class of compounds, with their antidopaminergic properties, are capable of eliminating the sensitized increase in accumbal and striatal dopamine by blocking the fusion of the synaptic vesicles and the release of neurotransmitters caused by nicotine, impairing the activation of calcium-mediated second messengers. It cannot be excluded that calcium ions may also modulate the salience or incentive value of nicotine-associated stimuli.

It is important to note that calcium channels play also a role in both acute and chronic effects of ethanol. An acute effect of ethanol involves an inhibition of VDCCs, but during ethanol withdrawal an elevation of calcium conductance through the calcium channels was observed (Leslie et al., 1990; Littleton et al., 1990). Indeed, several calcium channel antagonists diminished the signs of ethanol withdrawal, like tremor and seizures in rodents (Pucilowski, 1992). On the other hand, the effects of calcium channel blockers on cannabinoid central action are largely unexplored. Concerning this type of interaction, some reports revealed that cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons (Twitchell et al., 1997). These calcium channels are generally presynaptically located and involved in neurotransmitters release. WIN55,212-2 has been also shown to inhibit calcium channel currents in cell lines and primary neurons expressing rat brain cannabinoid receptors by both CB1 receptor-mediated and direct mechanisms (Shen and Thayer, 1998).

Relapse is a major characteristic of drug addiction and could be used to study the neuronal mechanisms underlying drug craving. Protection of abstinent individuals from relapse is the main goal of drug dependence treatment. The present findings, which reveal the reinstatement of nicotine-conditioned place preference, show analogies with similar phenomenon described in ex-smokers and support the addictive role of nicotine in tobacco smoking. We have described the reinstatement of nicotine-conditioned place preference in rats induced by the CB1 receptor agonist and ethanol, providing circumstantial evidence for ethanol, cannabinoids and nicotine interactions. As concurrent administration of nimodipine and flunarizine completely prevented the reinstatement effects of WIN55,212-2 or ethanol priming, our findings may further indicate a common neuronal pathway and similar calcium-dependent mechanisms involved in the development of reinstatement of nicotine-conditioned place preference provoked by priming injections of both drugs. It is reasonable to conclude that calcium channels may play an important role in the drug-induced neural and behavioural plasticity underlying the development of addiction and relapse to drugs. Since the voltage-dependent calcium channel antagonists can diminish the rewarding and stimulant properties of addictive drugs, this class of compounds could offer an interesting approach to the relapse-prevention pharmacotherapy of addiction including tobacco and polydrug abuse, a quite frequent phenomenon.

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