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Reinstatement of nicotine-conditioned place preference by drug priming: Effects of calcium channel antagonists

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Abstract

Reinstatement of drug-seeking behaviour in animals is relevant to drug relapse in humans. In the present study, we used the conditioned place preference paradigm to investigate the establishment, extinction, reinstatement and cross-reinstatement of nicotine-induced place conditioning in rats. Nicotine produced a place preference to the initially less-preferred 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, the reinstatement of place conditioning was investigated. For this purpose, nicotine-experienced rats were challenged with nicotine (0.5 mg/kg, i.p.) or morphine (10 mg/kg, i.p.). These priming injections of both drugs renewed a marked preference for the compartment previously paired with nicotine. In the second step, we examined the influence of the calcium channel antagonists, nimodipine (10 and 20 mg/kg, i.p.) and flunarizine (5 and 10 mg/kg, i.p.), on the reinstatement of nicotine-conditioned place preference induced by priming doses of nicotine and morphine. It was shown that the calcium channel blockers dose dependently attenuated the reinstatement of nicotine place preference induced by both drugs. These findings support the hypothesis that similar neural calcium-dependent mechanisms are involved in nicotine- and morphine-induced reinstatement. Finally, the conditioned place preference paradigm appears to be a useful tool for studies of the relapse of drug-seeking behaviour in laboratory animals.

Keywords: Nicotine; Morphine; Reinstatement; Calcium channel antagonist; Place conditioning; (Rat)

1. Introduction

Relapse to drug use after a long period of abstinence is a common problem of drug users (Fiore, 2000). Several factors seem to be associated with relapse, including exposure to drug-associated cues, stress, negative affect and withdrawal symptoms (Brigham et al., 1990; Kassel et al., 2003). Many detoxified drug abusers report that they relapse due to craving induced by the environment that was previously associated with drug taking. Others report that relapse is followed by exposure to the previously abused drug (Shelton et al., 2004). High rates of relapse are also characteristic for people trying to quit tobacco smoking. It is estimated that 85–100% of smokers that ever experience a smoking lapse will eventually relapse to regular smoking (Kenford et al., 1994). It has been already documented that nicotine, an alkaloid present in tobacco, is responsible for pharmacological actions of smoking and for its addictive effects, 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. In the first model, laboratory animals are initially trained to self-administer drugs by pressing a lever for drug infusion in operant conditioning chambers. Then, the drug-reinforced behaviour is extinguished by substituting the drug solution with saline or by disconnection of the infusion pump. After extinction of the drug-reinforced behaviour, an acute exposure to drug (non-contingent injection, priming dose) or non-drug stimuli (previously associated with the self-administered drug) reinstates drug-seeking behaviour (Stretch et al., 1971). This effect has been described for a number of drugs of abuse, such as alcohol (Le et al., 1998),

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cocaine (de Wit and Stewart, 1981), heroin (De Vries et al., 1998; de Wit and Stewart, 1983) and nicotine (Shaham et al., 1997). The second procedure, conditioned place preference, 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, injection of a drug of abuse (priming dose) reinstated the extinguished place preference, increasing the attractiveness of the environment previously paired with this drug (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 of 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 or heroin into the nucleus accumbens reinstates cocaine-seeking behaviour (Stewart, 1984; Pierce and Kalivas, 1997).

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 ventral tegmental area 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). The opioid receptor agonists enhance dopamine release in terminal regions by inhibiting GABAergic neurons in the ventral tegmental area, which provide tonic inhibition of dopamine neurons (Leone et al., 1991). Nicotine is thought to increase dopamine transmission in the nucleus accumbens by stimulating the nicotinic cholinergic receptors located on the dopaminergic neurons in this area (Corrigall et al., 1992; Dani et al., 2001; Shim et al., 2001). Although the dopaminergic system has been considered as a major neural substrate for the motivational and reinforcing properties of nicotine, there is evidence that other neurotransmitter systems, including the opioidergic system, might be also involved in its behavioural effects (Isola et al., 2002).

Although the conditioned place preference 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 establish a model of nicotine reinstatement in rats. We used the nicotine-conditioned place preference procedure evaluated in our previous studies (Biala, 2003). In a set of our experiments, nicotine place preference, once acquired, was extinguished by repeated test trials in a few successive days. Afterwards, in an 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 and morphine (Biala and Weglinska, 2004; Di Chiara, 2000), we also evaluated cross-reinstatement between nicotine and morphine. For this purpose, we examined the ability of a priming dose of morphine 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 nicotine or morphine. In this experiment, two representative L-type voltage dependent calcium channel antagonists, flunarizine and nimodipine, which are characterized by their high lipophility and central effects, were used. All these experiments were undertaken to study the neurobiological mechanisms underlying relapse to nicotine taking.

2. Materials and methods

2.1. Animals

The experiments were carried out on naïve 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 9–14 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 for Care and Use of Laboratory Animals and were approved by the local ethics committee (The Medical University of Lublin Committee on the Use and Care of Animals).

2.2. Drugs

The compounds tested were: (−)-nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA), morphine hydrochloride (Polfa Kutno, Poland), nimodipine (RBI, Natick, MA, USA) and flunarizine dihydrochloride (Sigma, St. Louis, MO, USA). The drugs were dissolved in saline (0.9% NaCl). 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.

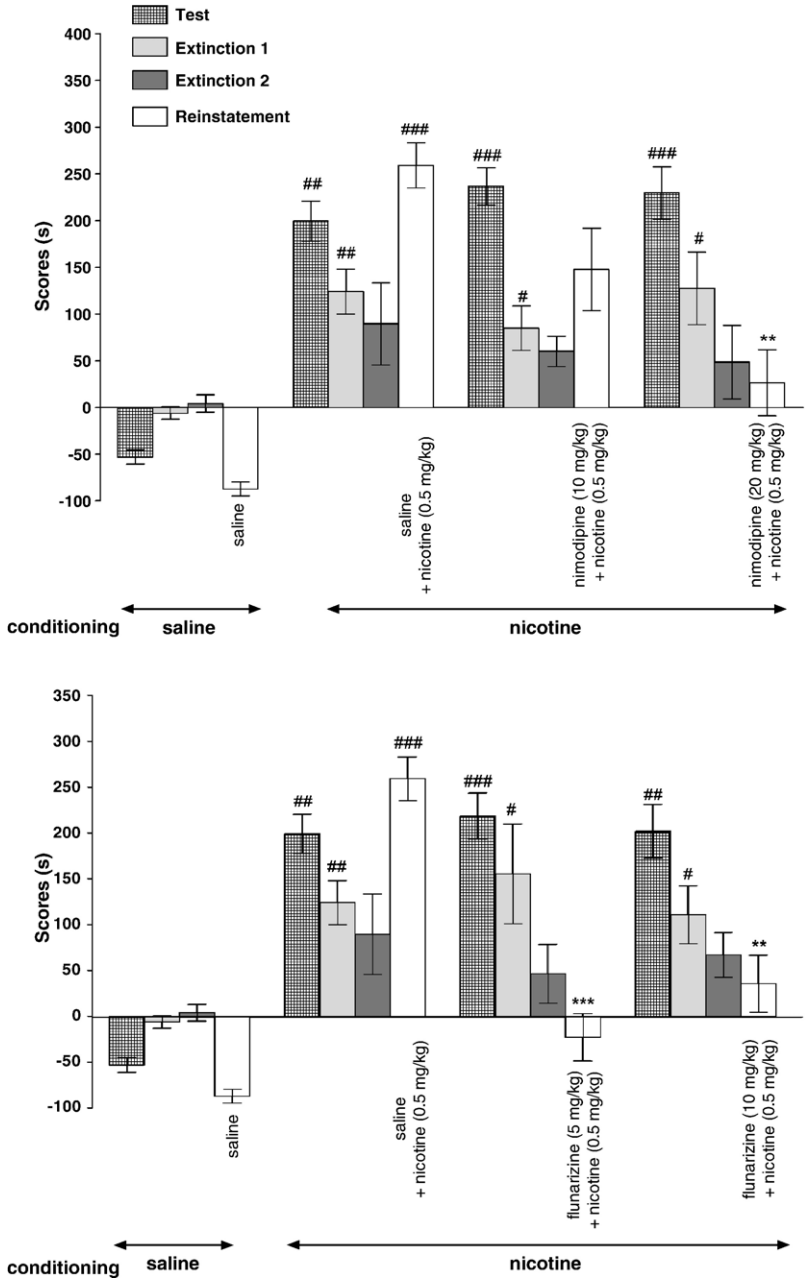

Fig. 1. Effects of nimodipine (10 and 20 mg/kg, i.p.) and flunarizine (5 and 10 mg/kg, i.p.) on the reinstatement of nicotine-conditioned place preference caused by a priming dose of nicotine. Place preference procedure consisted of pre-conditioning, three conditioning sessions with nicotine (0.5 mg/kg, i.p.), post-conditioning test followed by extinction period, i.e., repeated test trials, 24 h (Extinction 1) and 72 h (Extinction 2) 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, i.p.) preceded by an injection of nimodipine, flunarizine or saline. Data represent means ± S.E.M. and are expressed as the difference (in seconds) between post-conditioning and pre-conditioning time spent in the drug-associated compartment. $N = 10–14$ rats per group. $^{\#}P<0.05$; $^{\#\#}P<0.01$; $^{\#\#\#}P<0.001$ vs. saline-conditioned control group receiving saline injection on the reinstatement day; $^{**}P<0.01$; $^{***}P<0.001$ vs. nicotine-conditioned group given nicotine injection on the reinstatement day (Tukey test).

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.

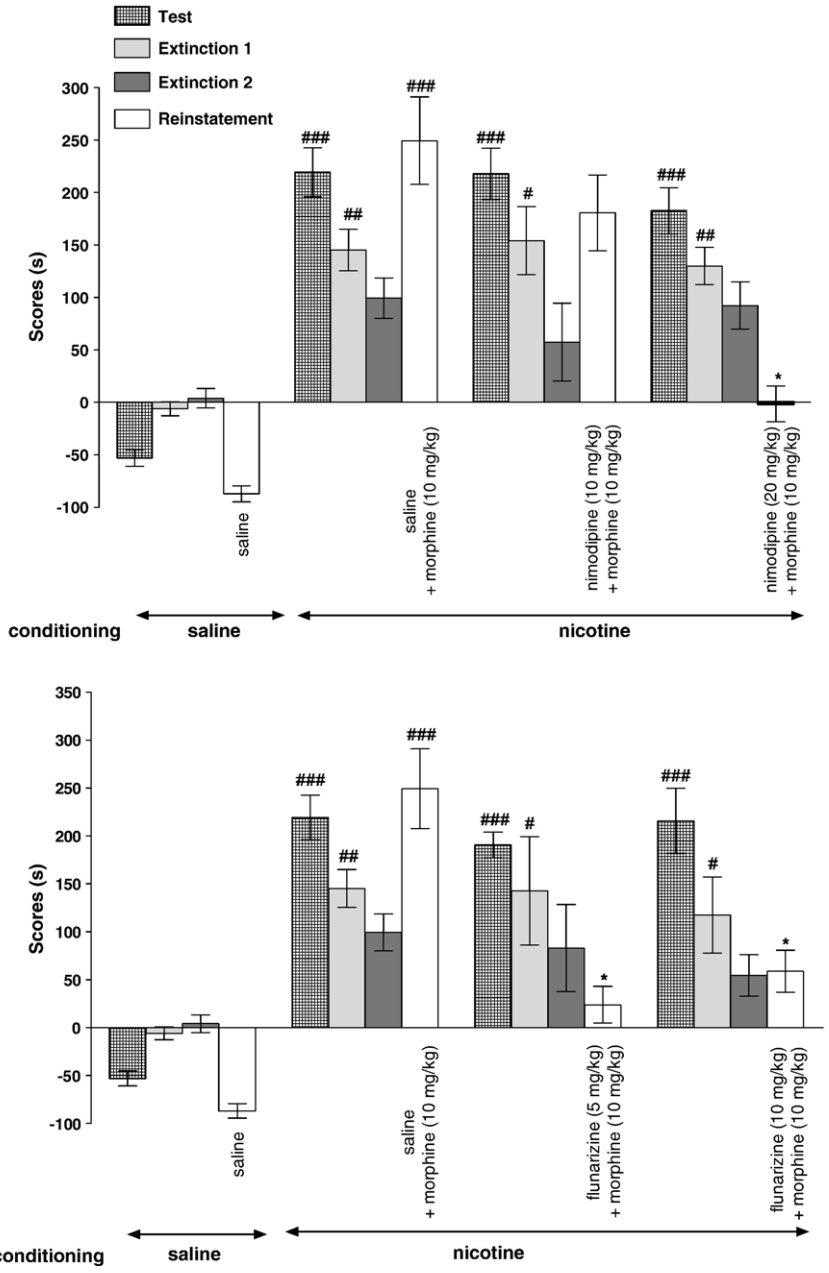

Fig. 2. Effects of nimodipine (10 and 20 mg/kg, i.p.) and flunarizine (5 and 10 mg/kg, i.p.) on the reinstatement of nicotine-conditioned place preference caused by a priming dose of morphine. Place preference procedure consisted of pre-conditioning, three conditioning sessions with nicotine (0.5 mg/kg, i.p.), post-conditioning test followed by extinction period, i.e., repeated test trials, 24 h (Extinction 1) and 72 h (Extinction 2) after the preference test. One day after the last extinction trial, extinguished nicotine-conditioned place preference was reinstated with a priming dose of morphine (10 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 seconds) between post-conditioning and pre-conditioning time spent in the drug-associated compartment. $N=9$–14 rats per group. $^{\#}P<0.05$; $^{\#\#}P<0.01$; $^{\#\#\#}P<0.001$ vs. saline-conditioned control group receiving saline injection on the reinstatement day; $^{*}P<0.05$ vs. nicotine-conditioned group given morphine injection on the reinstatement day (Tukey test).

This method (biased design) was similar to that used in previous experiments (Biala, 2003). The biased design is commonly used by other authors as an alternative to the unbiased design (Bespalov et al., 1994; Tzschentke, 1998).

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 (days 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 everyday. 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 2), 72 after this preference test.

2.4.5. Reinstatement

One day after the last extinction trial (day 9), separate groups of rats received a priming i.p. injection of saline, 0.5 mg/kg nicotine or 10 mg/kg morphine and were immediately tested for reinstatement of conditioned place preference. In other experiments, the groups of rats were treated i.p. with nimodipine (10 and 20 mg/kg) or flunarizine (5 and 10 mg/kg), 15 min prior to administration of nicotine, morphine or saline, respectively. 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. The statistical analyses 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 and extinction

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.

Figs. 1 and 2 show 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).

Figs. 1 and 2 also show 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. On day 8 (second test for extinction, Extinction 2), 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 test trials.

3.2. Nicotine- and morphine-induced reinstatement

The priming injection of both nicotine (0.5 mg/kg, i.p.) (Fig. 1) and morphine (10 mg/kg, i.p.) (Fig. 2) completely reinstated the extinguished nicotine-conditioned place preference. Pretreatment with nimodipine and flunarizine dose dependently inhibited the priming effect of nicotine in nicotine-treated rats ($F_{4,44}=3.23$, $P=0.021$) (Fig. 1). Post hoc individual comparisons indicated a significant effect of nimodipine at a dose of 20 mg/kg ($P<0.01$) and of flunarizine at both doses used: 5 mg/kg ($P<0.001$) and 10 mg/kg ($P<0.01$) (Tukey test). Interestingly, both calcium channel blockers also attenuated the priming effect of morphine on nicotine-conditioned place preference ($F_{4,38}=2.95$, $P=0.032$) (Fig. 2). Nimodipine at a dose of 20 mg/kg ($P<0.05$) or flunarizine at the doses of 5 mg/kg ($P<0.05$) and 10 mg/kg ($P<0.05$, Tukey test), injected i.p. 15 min before the priming dose of morphine, prevented the reinstatement of previously established nicotine place preference in rats (Fig. 2). Neither of the calcium channel antagonists, at the doses tested, caused significant changes in place preference by themselves (data not shown).

4. Discussion

In the present experiments, we used the conditioned place preference paradigm to study the extinction and reinstatement of extinguished nicotine place conditioning, a model consistent with drug-seeking behaviour. 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 conditioned place preference in rodents (Calcagnetti and Schechter, 1994; Biala, 2003). Subsequently, once established, nicotine place preference was extinguished by repeated daily testing. Rats were then given one priming injection of either nicotine or morphine in order to investigate the phenomenon of reinstatement and

cross-reinstatement. Our results show that both drugs are able to induce reacquisition of extinguished nicotine-conditioned place preference, causing the reappearance of a preference for the compartment previously paired with nicotine. In the second step, in order to further examine the role of calcium-dependent mechanisms in drug relapse, we investigated the effect of two calcium channel blockers, nimodipine and flunarizine, which act at L-type voltage-gated calcium channels, on the expression of nicotine-or morphine-induced reinstatement of nicotine-induced place conditioning. Interestingly, we found that both antagonists, administered prior to the priming injections of nicotine or morphine, attenuated, in a dose-dependent manner, the reinstatement effect of these drugs. Our findings support the hypothesis that calcium ions and calcium channels play an important role in modulating the reacquisition of drug-seeking behaviour following the extinction phase.

Relapse is an important phenomenon for identifying the neurobiological mechanisms responsible for long-term neuroadaptive changes in the brain during chronic drug use, changes that sustain drug craving and may contribute to compulsive drug use (Weiss, 2005). One of the contextual reinstatement procedures is based on learning of a place preference for an environmental stimulus paired with experience of the drugs (Mueller et al., 2002; Mueller and Stewart, 2000). Following extinction, which leads to a decline in the behavioural significance of the drug-related stimuli, the renewal of acquired drug preference by non-contingent re-exposure to drug priming or drug-associated cues can be considered a measure of relapse and craving. It has been suggested that priming injections of drugs reinstate drug-seeking behaviour after extinction because they activate brain systems involved in their discriminative stimulus properties and/or their rewarding properties (Di Chiara, 1995; Shaham et al., 2003).

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. 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, according to studies which described nicotine-induced conditioned place preference as a rather weak phenomenon (Calcagnetti and Schechter, 1994; Biala, 2003), 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). Thus, also in our experiments, only three daily extinction trials were sufficient to eliminate previously established nicotine-conditioned place preference.

Very little is known about the brain mechanisms involved in the relapse to nicotine, the most widely used addictive substance. We suggest 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 observed in our study. Nicotine reinforces smoking behaviour by activating nicotinic acetylcholine receptors in the midbrain dopaminergic reward centres, especially in the ventral tegmental area. The data also implicate the dopaminergic (and glutamatergic) projection from the ventral tegmental area to the nucleus accumbens in the conditioned reinforcing effects and reinstatement induced by priming injections of drugs, such as psychostimulants or nicotine (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. Moreover, 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. It is possible that reinstatement of conditioned place preference 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. Additionally, several reports suggest that sensitization, a phenomenon dependent on the functioning of the mesolimbic dopamine system, plays a crucial role in the reacquisition of drug-seeking behaviour. This animal model reflects the long-lived behavioural abnormalities induced by chronic drug exposure and the changes in synaptic plasticity at the molecular or cellular levels (Robinson and Berridge, 1993). Accordingly, nicotine has been reported to be less effective in activating the mesolimbic dopamine system in drug-naïve rats compared with nicotine-experienced animals (Corrigall et al., 1994). In view of our results, it is likely that sensitization in the induction of conditioned place preference may develop following repeated drug administration, and the neuroadaptation resulting from behavioural sensitization may play an important role in drug relapse and the enhanced vulnerability to drug abuse.

Our results also showed cross-reinstatement between morphine and nicotine after short-term extinction. Thus, nicotine place conditioning was reactivated by the single administration of both nicotine and morphine. Recently, we revealed the development of cross-sensitization between nicotine and morphine to their stimulant and rewarding properties (Biala and Weglinska, 2004). In addition to sensitization and cross-sensitization, tolerance develops to some of the pharmacological effects of nicotine and morphine, such as antinociception or hypothermia, and cross-tolerance to these effects has been shown (Zarrindast et al., 1999). One could argue that drugs can prime responding to one another because they share the property of activating the mesolimbic dopamine system, which becomes sensitized after repeated drug use. Such an effect is consistent with the common molecular and neurobiological mechanisms shared by nicotine and morphine.

Evidence is accumulating that drug and non-drug rewards share the property of activating dopamine transmission preferentially in the shell of the nucleus accumbens (Di Chiara and

Imperato, 1988). Non-psychostimulant drugs such as nicotine, opiates and ethanol have also a mu-opioid component of reward (Tanda and Di Chiara, 1998). One possibility could be that nicotine exerts its effect through a direct action on nicotinic cholinergic receptors, which have been shown to interact with opiate receptor signalling. An interaction between nicotine receptors and the opioid system has been described, especially activation of endogenous opioid peptide release and biosynthesis in discrete brain nuclei after nicotinic receptor stimulation (Houdi et al., 1991). In an animal model, it has been shown that morphine reverses withdrawal signs in nicotine-dependent rats (Malin, 2001), while nicotine abolishes naloxone-precipitated opioid withdrawal as well as place aversion induced by naloxone in morphine-pretreated rats (Araki et al., 2004; Zarrindast and Farzin, 1996). As nicotinic cholinergic receptor stimulation induces the release of opioid peptides, after prolonged nicotine exposure up-regulation of mu opioid receptors (Wewers et al., 1999) may cause an opioid-like dependence state. Interestingly, previous electrophysiological studies have also reported that nicotinic receptors may be a target through which opioid compounds might directly regulate nicotinic receptor-mediated functions (Tome et al., 2001). It is likely that an interaction between nicotine and opioid systems may be involved in the control of dopamine release and, subsequently, in the induction of both tolerance and sensitization induced by nicotine and morphine as well as the cross-reinstatement revealed in our study.

The present experiments were also designed to further evaluate the possible role of calcium ions and calcium-mediated second messenger systems in the nicotine-induced reinstatement and cross-reinstatement between nicotine and morphine. We found that systemic administration of nimodipine and flunarizine dose dependently attenuated conditioned nicotine-seeking behaviour in the conditioned place preference model, by blocking the reinstatement effect of nicotine or morphine priming. It is important to note that neither of the calcium channel antagonists, given acutely or repeatedly at the doses used, had any effects in naïve rats and did not provoke any reinforcing effects in the conditioned place preference 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 to locomotor and conditioned rewarding effects between nicotine and morphine (Biala, 2003; 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 conditioned place preference (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). When these compounds were given concurrently with morphine, the development of tolerance (and sensitization) was prevented, and the results are similar to the effect of these agents on ethanol tolerance (Dolin and Little, 1989). The close relationship between opioid action and intracellular calcium level in the central nervous system has been documented already. It is worth mentioning that opioid receptors are functionally coupled to voltage-dependent calcium channels and their effects involve a reduction in calcium ion conductance (North, 1986).

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 also produced a significant blockade of nicotine discrimination in rats (Schechter and Meehan, 1992). The mechanisms by which the calcium channel blockers affect behavioural sensitization and reinstatement are more complex. It is well established that neuronal nicotinic receptors located at pre- and postsynaptic 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 addiction, it has been already pointed out that calcium channel antagonists are effective blockers of nicotine-evoked dopamine release from rat striatal synaptosomes (Kulak et al., 2001; Prince et al., 1996). As already mentioned, the increase in extracellular dopamine seems to be dependent on the entry of calcium into dopaminergic terminals via voltage-gated calcium channels. Calcium channel antagonists acting at L-type calcium channels, 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, 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.

Moreover, it is worth noting that both sensitization and reinstatement are behavioural manifestations of synaptic plasticity (i.e., long-term potentiation, LTP) and that Ca^{2+} influx or Ca^{2+} release participates in the induction of LTP (Grover and Teyler, 1990). Actually, nicotine binds to nicotinic receptors, leading to channel opening and depolarization responses with an influx of Ca^{2+} ions. This effect is sufficient to activate calcium-dependent protein kinases such as calcium/calmodulin-dependent protein kinase II (CaM kinase II) and to induce LTP. This theory is strongly supported by data demonstrating that some calcium channel blockers inhibit LTP in the hippocampal CA1 region induced by nicotine (He et al., 2000).

Relapse is a major characteristic of drug addiction and is thought to serve as a useful animal model of plasticity and neuroadaptation associated with repeated administration of drugs. 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. It is possible to speculate on the influence of calcium channel antagonists in the adaptive long-lasting changes, observed upon prolonged administration of nicotine. As concurrent administration of nimodipine and flunarizine completely prevented the reinstatement effects of nicotine and morphine 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. Taking all these results together, it is possible to speculate that adaptation of neuronal calcium channels, especially in the mesolimbic dopamine system, seems to be a common basis for physical dependence on psychoactive drugs and their compulsive use. 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.

Our data also confirm that the conditioned place preference procedure may be useful to investigate the neurobiological mechanisms of drug-induced reinstatement of drug-seeking behaviour and vulnerability to relapse, providing support for the utility of this animal model as a tool for investigating specific aspects of addiction. This technique may serve as an alternative to the traditional intravenous self-administration method as an animal model of relapse. This extinction/reinstatement model shows good predictive validity for the clinical efficacy of new compounds, including calcium channel antagonists acting at L-type voltage-dependent calcium channels, for the treatment of addiction, including nicotine dependence.

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