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Calcium channel antagonists attenuate cross-sensitization to the rewarding and/or locomotor effects of nicotine, morphine and MK-801

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Abstract

The present study focused on the evaluation of behavioural cross-sensitization, particularly in locomotor activities and conditioned rewarding effects, between nicotine and morphine, cocaine, amphetamine or MK-801. Nicotine (0.5 mg kg^{-1})-experienced mice manifested an enhanced locomotor response to morphine (5 mg kg^{-1}) or MK-801 (0.3 mg kg^{-1}). No cross-sensitization was observed between nicotine and amphetamine (2 mg kg^{-1}) or cocaine (15 mg kg^{-1}). Additionally, the L-type voltage-dependent calcium-channel antagonists, nimodipine and verapamil, but not diltiazem, at a dose of 20 mg kg^{-1} injected before morphine or MK-801 challenge, blocked the expression of this cross-sensitization. In the second test, an enhancement of morphine place conditioning in rats pre-exposed to nicotine (0.5 mg kg^{-1}, injected daily for 5 days) was demonstrated. After two conditioning sessions, morphine (5 mg kg^{-1}) induced a clear place preference only in animals that had previously received nicotine injections. The administration of nimodipine (10 and 20 mg kg^{-1}), verapamil (10 and 20 mg kg^{-1}) and diltiazem (10 and 20 mg kg^{-1}) prior to nicotine dose-dependently prevented this sensitization to the rewarding effect of morphine produced by prior injections of nicotine. These findings support the hypothesis that similar neural calcium-dependent mechanisms are involved in the appetitive effects of nicotine and morphine and in the sensitized locomotor stimulant effects of nicotine and morphine or MK-801.

Introduction

Drug addiction is a complex behavioural phenomenon dependent on several neural systems. There is considerable evidence that the rewarding properties of drugs in humans and animals may be owing to their common properties of facilitating (directly or indirectly) dopaminergic transmission, especially in the mesolimbic pathways (Di Chiara & Imperato 1988). This increase of extracellular dopamine (DA) concentration is a major neurobiological substrate of addictive properties of drugs of abuse. DA release within the nucleus accumbens (NAC) is preferentially increased following administration of many drugs commonly abused by humans, including amphetamine, cocaine, morphine and nicotine. Although these drugs share this ability to increase DA turnover in the NAC, their mechanisms of action differ. For instance, amphetamine and cocaine block the reuptake of monoamines and/or enhance their release from non-vesicular stores (Seiden et al 1993). Morphine stimulation of mu receptors in the ventral tegmental area enhances mesolimbic DA transmission, presumably by inhibiting GABAergic interneurons and consequently increasing somatodendritic and axonal DA release (Klitenick et al 1992). Nicotine is thought to increase DA transmission in the NAC by stimulating the nicotinic cholinergic receptors (nAChRs) located on the dopaminergic neurons in this area (Di Chiara 2000).

Motivational behavioural responses related to drug addiction can be measured in various animal models. For instance, the conditioned place preference (CPP) paradigm is a sensitive behavioural model used to measure the rewarding properties of different drugs of abuse (Carr et al 1989). This model utilizes the phenomenon of secondary conditioning in which a neutral stimulus that has been paired with a drug acquires the ability to serve as a reward itself. An alternative characteristic implicated in the

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addictive behaviour is a phenomenon termed sensitization or reverse tolerance. Behavioural sensitization is the progressive and enduring enhancement of certain drug-induced effects such as locomotor activity, which develops following repeated, intermittent treatment with psychostimulants or opioids. This phenomenon represents a model of long-lasting adaptive changes after chronic drug treatment and is directly related to drug seeking and reinstatement behaviour (Robinson & Becker 1986; Koob 1992).

Although behavioural sensitization induced by psychostimulants has been widely described, relatively less is known about the behavioural consequences of repeated exposure to other drugs, including nicotine, and particularly whether cross-sensitization to some effects of various psychoactive substances may develop. Based on the finding that similar neural substrates are involved in the psychomotor and rewarding actions of the drugs, the present studies were undertaken to investigate behavioural sensitization and cross-sensitization to their locomotor and rewarding effects. We first examined if nicotine-experienced mice develop sensitization to locomotor stimulating effect of amphetamine, cocaine, morphine or dizocilpine (MK-801), a representative non-competitive antagonist of *N*-methyl-D-aspartate (NMDA) receptor. In the second step, we evaluated the rewarding effects of morphine in rats with a prior history of nicotine exposure using the CPP model. Additionally, since calcium ions and L-type voltage-dependent calcium channels (VDCCs) may be important in several aspects of drug reward and addiction (Biala & Langwinski 1996), we investigated the influence of some structurally distinct calcium-channel antagonists (CCAs) on the expression of this cross-sensitization. Nimodipine, verapamil and diltiazem were chosen as representative of three major clinically available subclasses of VDCC blockers (dihydropyridines, phenylalkylamines and benzothiazepines). The results are discussed in the context of long-lasting neural and behavioural changes induced by chronic drug treatment that ultimately lead to addiction, especially in connection with neural calcium ions and calcium channels.

Materials and Methods

Animals

The experiments were carried out on naive male Wistar rats, 250–300 g, and on naive male Swiss mice, 20–25 g (Farm of Laboratory Animals, Warszawa, Poland). The animals were kept under standard laboratory conditions (12/12 h light–dark cycle) with free access to tap water and laboratory 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 before the experiments. Each experimental group consisted of 8–14 animals. The experiments were performed between 0900 and 1700 hours.

All experiments were carried out according to the standard ethical guidelines (National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and to the European Community Council Directive for Care and Use of Laboratory Animals) and approved by the local ethics committee.

Drugs

The compounds tested were: (–)-nicotine hydrogen tartrate (Sigma, St Louis, MO, USA), nimodipine (RBI, Natick, MA, USA), verapamil (Knoll, Germany), diltiazem hydrochloride (RBI), d-amphetamine sulfate (Sigma), morphine hydrochloride (Polfa Kutno, Poland), cocaine hydrochloride (Sigma), (+) MK-801-{[+]-5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyklohepten-5,10-imine hydrogen maleate, dizocilpine} (RBI). Verapamil was diluted to an adequate concentration using saline (0.9% NaCl). Other drugs were dissolved in saline. All agents were administered intraperitoneally in a volume of 10 mL kg^{-1} (mice) or 5 mL kg^{-1} (rats). Control groups received injections of the vehicle.

Apparatus

Locomotor activity in mice was measured in round actometer cages (32 cm in diameter, two light beams; Multiserv, Lublin, Poland) kept in a sound-attenuated experimental room. Two photocell beams measured the animal's displacements. The testing apparatus for the CPP paradigm was similar to that used by Spyraki et al (1982). Each of six rectangular boxes ($60 \times 35 \times 30$ 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). The walls and floor of one of the compartments was painted white and the other compartment was painted black. The central grey area constituted a "neutral" chamber. The testing boxes were kept in a soundproof room with a neutral masking noise and a dim 40 lx illumination.

Locomotor sensitization

During the pairing phase (Days 1–5), the two groups of mice received the following injections: saline + saline or saline + nicotine (0.5 mg kg^{-1}). This dose of nicotine (hydrogen tartrate) is often used in order to produce robust conditioned hyperactivity. Immediately after each saline or nicotine injection, mice were confined to the apparatus and their locomotor activity was recorded for 30 min. Subsequently, animals remained drug free for 1 week and, on Day 13, the distinct saline- or nicotine-treated groups were challenged with one of the following injections: nicotine (0.5 mg kg^{-1}), d-amphetamine (2 mg kg^{-1}), morphine (10 mg kg^{-1}), cocaine (15 mg kg^{-1}) or MK-801 (0.3 mg kg^{-1}). Locomotor activity was recorded for 30 min. The second experiment was designed to investigate whether pretreatment with some of the CCAs modifies the expression of the locomotor cross-sensitization to the effects of nicotine and other drugs. For this purpose, on the challenge day, mice were injected with nimodipine (20 mg kg^{-1}), verapamil (20 mg kg^{-1}) or diltiazem (20 mg kg^{-1}) 15 min before nicotine, morphine,

MK-801 or saline injection. This dose was chosen according to our recent data indicating that, at the dose of 20 mg kg^{-1}, all of these CCAs attenuated the expression of nicotine sensitization in mice in the experimental conditions described above (Biala 2003).

The CPP procedure

The CPP procedure (biased design) was similar to that used in previous experiments (Biala & Langwinski 1996). The biased design is one of the procedures still commonly used by other authors (Bespalov et al 1994; Tzschentke 1998). During the preconditioning phase, the time spent by rats in each of the two large compartments was measured (a baseline preference) for 15 min. All subjects spent more time in the black compartment (>480 s) than in the white one (<60 s). The rats were randomized and subsequently conditioned with saline paired with the preferred (black) compartment (morning sessions), and drug with the other (white) compartment (afternoon sessions) for 30 min. Sessions were conducted twice each day 6–8 h apart. The control group received saline before each conditioning session. During the post-conditioning phase, the guillotine doors were removed and the time spent by each rat in the two large compartments was recorded for 15 min.

The procedure of sensitization to the rewarding effects of drugs was carried out according to the method described by Shippenberg et al (1996). Animals received the following injections once daily for 5 days: saline + saline, saline + nicotine (0.5 mg kg^{-1}), nimodipine (10 and 20 mg kg^{-1}) + nicotine (0.5 mg kg^{-1}), verapamil (10 and 20 mg kg^{-1}) + nicotine (0.5 mg kg^{-1}) and diltiazem (10 and 20 mg kg^{-1}) + nicotine (0.5 mg kg^{-1}). The CCAs used are injected 15 min before nicotine. The CPP preconditioning session (Day 0) took place 2 days after the termination of the pre-exposure phase as described above. During the conditioning phase, separate groups of rats received an injection of saline before being placed in the black compartment and morphine (5 mg kg^{-1}) before being confined to the white compartment for 2 consecutive days. Test of conditioning was conducted on the third day: uninjected rats were allowed free access to both compartments for 15 min.

Statistics

The data are expressed as mean ± s.e.m. The statistical analyses of locomotor activity were performed using two-factor repeated measure analysis of variance, with treatment as independent factor and days as repeated measures. Locomotion was expressed as a number of photocell beam breaks. To evaluate behavioural sensitization, the response to drugs on Day 13 was compared with the acute drug response to the first injection (Day 1) in the same animal, or with the response to the challenge drug injection (Day 13) in animals treated with repeated saline, using one-way analysis of variance. For the CPP paradigm, the results were analysed using one-way analysis of variance with score (i.e. the differences between post-conditioning and pre-conditioning time spent in the drug-associated compartments) as the dependent factor. Post-hoc comparisons of means were carried out with the Tukey test for multiple comparisons when appropriate. The confidence limit of $P < 0.05$ was considered as statistically significant.

Results

Locomotor sensitization

To induce sensitization to nicotine, the mice were given nicotine (0.5 mg kg^{-1}, injected daily for 5 days). Seven days after cessation of treatment, the mice were challenged with 0.5 mg kg^{-1} nicotine. Nicotine sensitization, that is an enhanced locomotor response, was developed in mice compared with that seen after the first injection of nicotine in the same animal ($P < 0.001$) or with the response to acute nicotine challenge in animals treated with repeated saline ($P < 0.01$, Tukey test) (Figures 1 and 2). The locomotor activity of saline-treated mice did not change significantly over time. When nicotine-pretreated mice received a morphine challenge (10 mg kg^{-1}), a significant difference between the response was observed on Day 13 compared with the first injection of nicotine ($P < 0.001$)

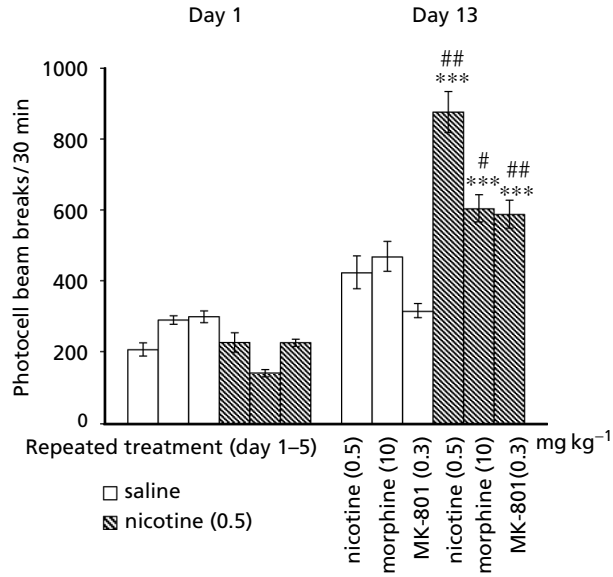

Figure 1 Effects of a challenge dose of nicotine (0.5 mg kg^{-1}), morphine (10 mg kg^{-1}) or MK-801 (0.3 mg kg^{-1}) injections on locomotor activity of mice treated with saline or nicotine (0.5 mg kg^{-1}). Nicotine or saline was injected daily for 5 days: on Day 13, the distinct saline- or nicotine-experienced mice were given a challenge dose of morphine, MK-801 or nicotine. Locomotor activity was recorded for 30 min. Data represent the mean ± s.e.m. of activity after the first injection (Day 1) and on the day of drug challenge (Day 13). Two-way analysis of variance showed a significant treatment effect [$F(1,84) = 71.92$, $P < 0.0001$], day effect [$F(5,84) = 3.77$, $P = 0.004$] and treatment-day interaction [$F(5,84) = 6.11$, $P < 0.0001$)]. ***$P < 0.001$ vs the first pairing day; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs saline-treated mice (Tukey test).

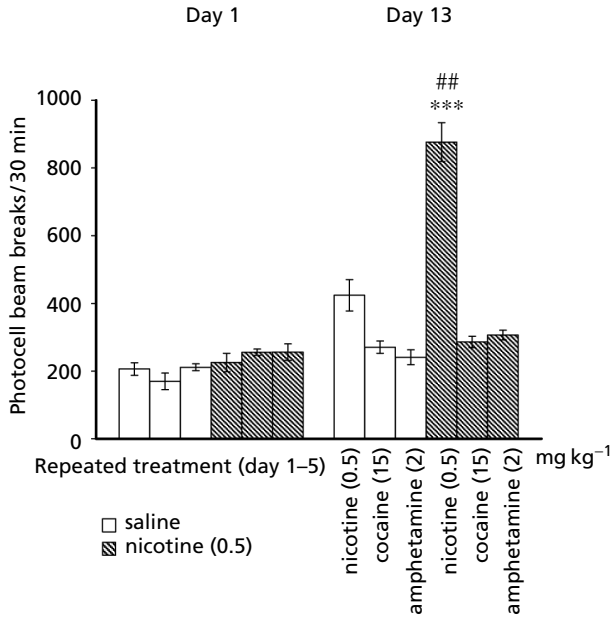

Figure 2 Effects of a challenge dose of nicotine (0.5 mg kg^{-1}), cocaine (15 mg kg^{-1}) or amphetamine (2 mg kg^{-1}) injections on locomotor activity of mice pretreated with saline or nicotine (0.5 mg kg^{-1}). Nicotine or saline was injected daily for 5 days: on Day 13, the distinct saline- or nicotine-experienced mice were given a challenge dose of cocaine, amphetamine or nicotine. Locomotor activity was recorded for 30 min. Data represent the mean ± s.e.m. of activity after the first injection (Day 1) and on the day of drug challenge (Day 13). Two-way analysis of variance showed a significant treatment effect [F (1,84) = 30.99, $P < 0.0001$], day effect [F (5,84) = 9.62, $P < 0.0001$] and treatment-day interaction [F (5,84) = 9.28, $P < 0.0001$]. ***$P < 0.001$ vs the first pairing day; $^{\#\#}P < 0.01$ vs saline-treated mice (Tukey test).

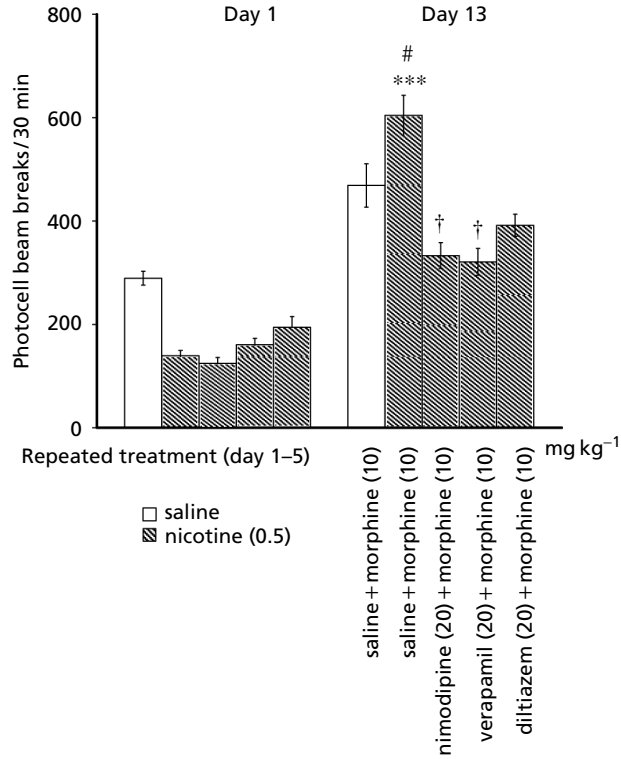

Figure 3 Effects of nimodipine (20 mg kg^{-1}), verapamil (20 mg kg^{-1}) or diltiazem (20 mg kg^{-1}) on the expression of locomotor cross-sensitization between nicotine (0.5 mg kg^{-1}) and morphine (10 mg kg^{-1}) in mice. Nicotine (0.5 mg kg^{-1}) or saline were injected daily for 5 days: on Day 13 (a test for expression of sensitization) they were given saline + morphine or a calcium channel antagonist + morphine. Locomotor activity was recorded for 30 min. Data represent the mean ± s.e.m. of activity after the first injection (Day 1) and on the day of drug challenge. (Day 13) Two-way analysis of variance showed a significant treatment effect [F (1,70 = 60.91, $P < 0.0001$)], day effect [F (4,70) = 4.16, $P = 0.0044$] and treatment-day interaction [F (4,70) = 3.29, $P = 0.016$]. $^{\dagger}P < 0.05$ vs nicotine-treated and morphine-challenged mice; ***$P < 0.001$ vs the first pairing day; $^{\#}P < 0.05$ vs saline-treated and morphine-challenged mice (Tukey test).

or with the response to acute morphine challenge in animals treated with repeated saline ($P < 0.05$) (Figure 1). Similarly, the administration of MK-801 (0.3 mg kg^{-1}) to nicotine-treated mice caused a marked increase in locomotion compared with the first injection of nicotine ($P < 0.001$) or with the response to acute MK-801 challenge in animals pretreated with saline ($P < 0.01$, Tukey test) (Figure 1). These findings indicated that pre-exposure to nicotine results in locomotor sensitization to nicotine itself as well as in sensitization to morphine and MK-801 challenges. Administration of cocaine (15 mg kg^{-1}) or amphetamine (2 mg kg^{-1}) challenge to nicotine-pretreated mice caused no significant increase in locomotion, indicating the lack of cross-sensitization (Figure 2).

Nimodipine and verapamil, but not diltiazem, injected at a dose of 20 mg kg^{-1} before the challenge dose of morphine, attenuated the increase of locomotion, that is the expression of cross-sensitization between nicotine and morphine (Figure 3). One-way analysis of variance revealed a significant treatment effect on Day 13 [F (4,35) = 3.41, $P = 0.015$]. Post-hoc individual comparisons showed that in morphine-challenged mice, locomotor activity was decreased in mice pretreated with nimodipine or verapamil ($P < 0.05$, Tukey test). When the CCAs were injected before the MK-801 challenge, nimodipine and verapamil, but not diltiazem (20 mg kg^{-1}), prevented the expression of cross-sensitization between nicotine and MK-801 (Figure 4). One-way analysis of variance revealed a significant treatment effect on Day 13 [F (4,35) = 5.16, $P = 0.0036$]. Post-hoc individual comparisons indicated a significantly lower locomotor activity in mice challenged with MK-801 and pretreated with nimodipine or verapamil ($P < 0.01$; Tukey test). None of the CCAs, given acutely or repeatedly, significantly affected basal locomotor activity of mice (data not shown).

CPP paradigm

The time spent at the initially less preferred (white) and the initially more preferred (black) side did not significantly

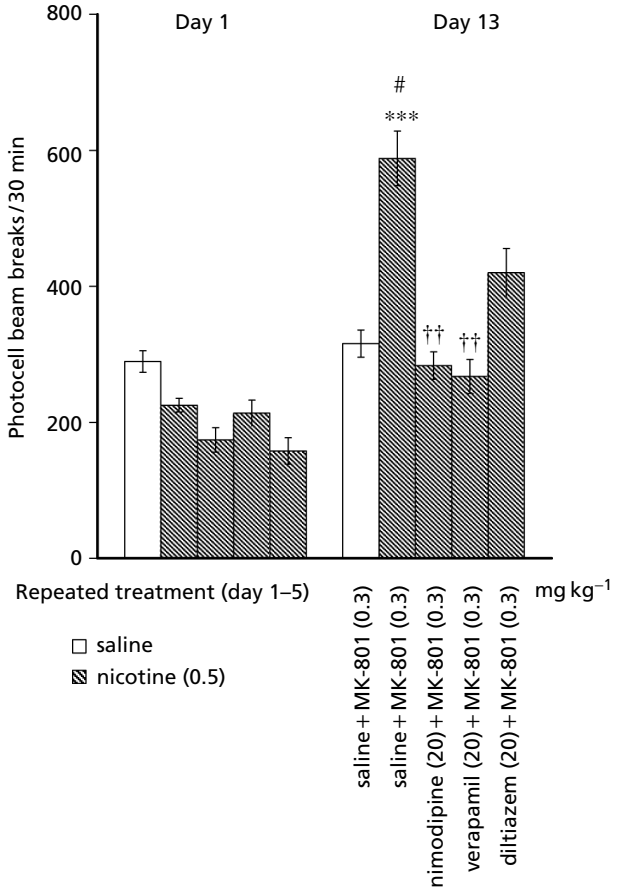

Figure 4 Effects of nimodipine (20 mg kg^{-1}), verapamil (20 mg kg^{-1}) or diltiazem (20 mg kg^{-1}) on the expression of locomotor cross-sensitization between nicotine (0.5 mg kg^{-1}) and MK-801 (0.3 mg kg^{-1}) in mice. Nicotine (0.5 mg kg^{-1}) or saline were injected daily for 5 days: on Day 13 (a test for expression of sensitization) they were given MK-801 or a calcium-channel antagonists + MK-801. Locomotor activity was recorded for 30 min. Data represent the mean ± s.e.m. of activity after the first injection (Day 1) and on the day of drug challenge (Day 13). Two-way analysis of variance showed a significant treatment effect [F (1,70) = 28.80, $P < 0.0001$], day effect [F (4,70) = 4.31, $P = 0.004$] and treatment-day interaction [F (4,70) = 4.51, $P = 0.0027$]. $^{\dagger\dagger}P < 0.01$ vs nicotine-treated and MK-801-challenged mice; $^{***}P < 0.001$ vs the first pairing day; $^{\#}P < 0.05$ vs saline-treated and MK-801-challenged mice (Tukey test).

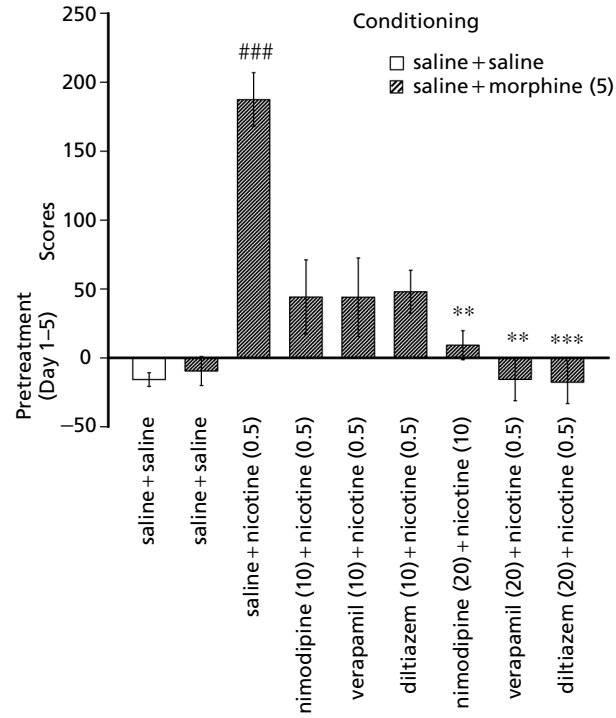

Figure 5 Morphine-induced conditioned place preference in rats that had previously received nicotine (0.5 mg kg^{-1}, injected daily for 5 days) in combination with nimodipine (10 and 20 mg kg^{-1}), verapamil (10 and 20 mg kg^{-1}), diltiazem (10 and 20 mg kg^{-1}) or saline. Place preference procedure (i.e. pre-conditioning, two conditioning sessions with 5 mg kg^{-1} morphine, post-conditioning) commenced 2 days after the last injection. Data represent the mean ± s.e.m. and are expressed as the scores, that is the differences (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment. Analysis of variance indicated a treatment effect [F (8,71) = 4.69, $P < 0.0001$]. $^{**}P < 0.01$, $^{***}P < 0.001$ vs nicotine-pretreated and morphine-conditioned rats; $^{\#\#\#}P < 0.001$ vs saline-treated and saline-conditioned rats (Tukey test).

differ between groups on the pre-conditioning day. This side preference was not significantly changed when saline was paired to both compartments during the conditioning sessions.

Figure 5 shows that, after two conditioning sessions, morphine (5 mg kg^{-1}) induced a clear place preference only in animals that had previously received nicotine injections, indicated by a significant increase in the time spent in the drug-associated compartment during the post-conditioning phase. No enhanced response to morphine, after only two conditioning trials, was seen in rats pretreated with saline. Animals that had received nicotine (0.5 mg kg^{-1}) in combination with nimodipine, verapamil or diltiazem at the doses of 20 mg kg^{-1}, but not 10 mg kg^{-1}, for 5 days developed no significant place preference in response to morphine ($P < 0.01$, $P < 0.01$, $P < 0.001$, respectively; Tukey test) (Figure 5). An additional experiment was carried out to assess the reinforcing effects of the CCAs used, as measured in the CPP procedure. Any of the three compounds, paired with the more or less preferred compartment, at the doses tested caused no significant changes in the place preference by themselves (data not shown).

Discussion

The present study focused on the evaluation of behavioural sensitization and cross-sensitization processes, particularly in locomotor activity and appetitive effects of some drugs in rodents. Additionally, we investigated

the effects of some L-type voltage-dependent CCAs on this sensitization. In accordance with a previous study, the present results indicate that repeated daily injections of nicotine produced progressive increases in locomotor activity in mice, especially to a subsequent nicotine challenge. One of the main findings of the present study is that, among the various psychoactive substances tested, locomotor cross-sensitization occurred between nicotine and morphine, or MK-801, but not between nicotine and cocaine or amphetamine. Indeed, nicotine-experienced mice showed an enhanced response to morphine or MK-801 challenge compared with both the first pairing day and the response to acute morphine or MK-801 challenge in animals pre-exposed to saline. In the second step, morphine-induced conditioned place preference was enhanced in rats with a prior history of nicotine administration. Interestingly, pretreatment with nimodipine, verapamil or diltiazem dose-dependently prevented this sensitization by nicotine to the appetitive effect of morphine. These results support the hypothesis that similar neural substrates can be involved in the psychomotor and rewarding effects of nicotine and morphine and that this mechanism is calcium-dependent.

A variety of drugs of abuse appear to exert their rewarding effect via the activation of a common neuronal substrate, especially in the mesolimbic DA pathways. There is evidence suggesting that nicotine increases dopaminergic transmission indirectly through the presynaptic nAChRs located on dopaminergic neurons, preferentially in the ventral tegmental area, and that such activation underlies the reinforcing and locomotor stimulant effect of this drug (Dani et al 2001). It is well known that systemic injections of the nAChRs antagonist, mecamylamine, which crosses the blood–brain barrier, attenuated both the acute and sensitized mesoaccumbens DA responses to nicotine (Di Chiara 2000). Although nicotine shares most of the characteristics of other addictive drugs, its motivational effects in the behavioural paradigms are quite difficult to demonstrate. For instance, nicotine-induced reinforcing effects as measured by intravenous self-administration are modest when compared with psychostimulants (Corrigall 1999). In the CPP paradigm, some reports show nicotine-induced conditioned place aversion (Jorenby et al 1990) or a lack of place conditioning (Clarke & Fibiger 1987). However, in spite of these negative results, place preference has been also reported with nicotine under biased conditions (Calcagnetti & Schechter 1994). Moreover, repeated daily exposure to nicotine results in behavioural sensitization, manifested by a progressive increase in locomotor activity (Shim et al 2001; present study).

In a set of experiments we investigated cross-sensitization to the locomotor stimulant effects of nicotine and some psychoactive substances, a process whereby repeated administration of one drug leads to an enhancement of the effects of other drugs. Interestingly, the cross-sensitization was observed only between nicotine and morphine or MK-801. There was no cross-sensitization between nicotine and amphetamine or cocaine. Accordingly, other studies have shown no cross-sensitization between the mesoaccumbens DA responses to nicotine and cocaine (Henry et al 1989). It seems unlikely that the mechanisms involved in the nicotine- and psychostimulants-induced sensitized 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 are in effect less sensitive (Desai & Philip 2003).

Our data support the notion that similar mechanisms may underlie the development of sensitization to nicotine compared with morphine and MK-801. An interaction between nicotine receptors and the opioid systems has already been described, especially activation of endogenous opioid peptides release and biosynthesis in discrete brain nuclei after nAChRs stimulation (Houdi et al 1991). Nicotine could attenuate some of the naloxone-precipitated withdrawal signs in morphine-dependent animals (Zarrindast & Farzin 1996). In addition to sensitization, tolerance develops to some of the pharmacological effects of nicotine and morphine, such as antinociception or hypothermia, and a cross- tolerance to these effects has been revealed (Zarrindast et al 1999). It is likely that an interaction of nicotine with opioid systems may be involved in the control of DA release and in the induction of both tolerance and sensitization induced by nicotine and morphine.

In addition, our results showed a cross-sensitization between nicotine and MK-801, a representative non-competitive antagonist of NMDA receptor, which can suggest similar drug-induced neuroadaptations underlying that phenomenon. Actually, an in-vitro study indicates that nAChRs agonists displace [^{3}H]MK-801 from its binding sites (Aizenman et al 1991). Moreover, MK-801 induced hyperactivity and its repeated administration caused sensitization to its own locomotor stimulant effect. Interestingly, low doses of MK-801 blocked the acquisition and expression of nicotine-induced locomotor sensitization in rats (Kelsey et al 2002) and these results can suggest that sensitized responses to nicotine depend on co-stimulation of NMDA receptors. The interpretation of these data is complicated by the fact that MK-801 can also act as an antagonist at central nAChRs (Amador & Dani 1991). Some reports suggest the involvement of DA in this stimulant action of MK-801 through an effect on nAChRs located presynaptically on glutamate-secreting terminals (Gray et al 1996), but other studies do not support this notion (Kashihara et al 1990). Further studies are necessary to determine if a cross-sensitization occurs between nicotine and MK-801 or nicotine and morphine.

In the CPP paradigm, the repeated administration of nicotine sensitized animals to the appetitive values of morphine. We observed that morphine failed to induce place preference after two conditioning sessions in control animals. Only in animals with prior administration of nicotine, a significant place preference in response to morphine was shown. As already suggested, the common ability of nicotine and morphine to stimulate DA transmission in the shell of the NAC may underlie this enhanced rewarding response to morphine.

The present study further examined the role of calcium ions and calcium-mediated second messenger system in the cross-sensitization described above. We have shown that a pretreatment with the CCAs nimodipine and verapamil, but not diltiazem, which decrease the intracellular calcium level, attenuated the expression of cross-sensitization to the locomotor stimulant effects between nicotine and morphine, as well as between nicotine and MK-801. It is worth noting that at this dose CCAs were also effective in blocking locomotor sensitization to nicotine in mice (Biala 2003). In the CPP paradigm, CCAs co-administered with nicotine before the conditioning sessions prevented the development of sensitization to the rewarding effects of morphine. It is important to note that none of the CCas, given acutely or repeatedly at the used doses, had any effects in naive animals. Our results are in accordance with the recent data demonstrating that CCAs, including nimodipine and verapamil, inhibited the development of morphine-induced sensitization in mice (Zhang et al 2003), which further suggests an involvement of calcium-dependent mechanisms in morphine-induced sensitized responses. The close relationship between opioid action and intracellular calcium level in the central nervous system has been already documented. It is worth mentioning that opioid receptors are functionally coupled to VDCC and their effects involve reduction in calcium ions conductance (North 1986).

Considerable evidence exists for central calcium channels to be involved in at least some of the effects of drugs commonly abused by humans. Some studies demonstrate the anti-reinforcing properties of the CCAs acting at the L-type VDCC. For example, it has been reported that CCAs decreased the induction and expression of behavioural sensitization and place conditioning induced by amphetamine (Karler et al 1991; Pucilowski et al 1993), as well as cocaine and morphine self-administration (Kuzmin et al 1992). In the context of addiction, it has been shown that some CCAs decreased naloxone-precipitated morphine withdrawal syndrome in rats (Antkiewicz-Michaluk et al 1993) and ethanol-induced hyperexcitability (Littleton et al 1990). CCAs are also known to modify the behavioural effects of MK-801, relevant to its abuse potential (Sukhotina et al 1999). Some studies suggested the influence of CCAs on the behavioural effects of nicotine. Pretreatment with CCAs reduced nicotine antinociception and discrimination in rats (Schechter & Meehan 1992; Damaj & Martin 1993). Taking into account the reinforcing effects of nicotine, it is worth mentioning that the CCAs are known to modulate the nicotine-induced release of DA from rat striatal synaptosomes so that this effect seems to be calcium-dependent (Kulak et al 2001).

The mechanisms by which the CCAs affect behavioural sensitization and cross-sensitization are complex and not fully understood. CCAs acting at L-type calcium channels, with their antidopaminergic properties, are capable of eliminating the sensitized increase in accumbal and striatal DA by blocking the fusion of the synaptic vesicles and the releasing of neurotransmitters or impairing the activation of calcium-mediated second messengers.

Conclusion

Drugs with addictive potential are often co-abused by humans. The present experiments were designed to further evaluate the possible mechanisms of behavioural sensitization to locomotor and rewarding effect of drugs. Sensitization is thought to serve as a useful animal model of plasticity and neuroadaptation associated with repeated administration of drugs.

Our findings indicate a common neuronal pathway and similar calcium-dependent mechanisms involved in the development of behavioural sensitization to the rewarding effects of nicotine and morphine, as well as in the sensitized locomotor stimulant effects of nicotine and morphine or MK-801, but not of nicotine and cocaine or amphetamine. Since the CCAs acting at the L-type VDCC can reduce the appetitive and stimulant properties of addictive drugs, this class of compounds offers an interesting approach for the pharmacotherapy of addiction including nicotine dependence and dependence on the combination of nicotine and other drugs. It is reasonable to conclude that calcium channels may play an important role in drug-induced neural and behavioural plasticity underlying the development of addiction.

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