

CALCIUM CHANNEL ANTAGONISTS SUPPRESS NICOTINE-INDUCED PLACE PREFERENCE AND LOCOMOTOR SENSITIZATION IN RODENTS

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Calcium channel antagonists suppress nicotine-induced place preference and locomotor sensitization in rodents. G. BIAŁA. Pol. J. Pharmacol., 2003, 55, 327–335.

The influence of calcium channel antagonists on the behavioral sensitization to nicotine-induced hyperlocomotion and place preference was investigated. Locomotor sensitization in mice was produced by injecting nicotine (0.5 mg/kg, *ip*) for 5 consecutive days before placement in an apparatus in which locomotor activity was evaluated for 1 h. One week later, activity of mice was recorded after challenge with the same dose of nicotine. The L-type voltage-dependent calcium channel antagonists: nimodipine (5, 10 and 20 mg/kg, *ip*), verapamil (5, 10 and 20 mg/kg, *ip*) and diltiazem (5, 10 and 20 mg/kg, *ip*) were injected 15 min before each injection of nicotine (induction of sensitization) or acutely 15 min before a challenge nicotine injection (expression of sensitization). It was shown that the calcium channel blockers attenuated both the induction and expression of nicotine-induced locomotor sensitization in a dose-dependent manner. In the place preference paradigm, nicotine produced a place preference to the initially less-preferred compartment paired with its injections during conditioning (0.5 mg/kg, *ip*, 4 drug sessions). Pretreatment with nimodipine (10 mg/kg, *ip*), verapamil (10 mg/kg, *ip*) and diltiazem (10 mg/kg, *ip*) blocked nicotine-induced place conditioning.

These results suggest the common calcium-dependent mechanisms of nicotine-induced behavioral sensitization and place preference.

Key words: *nicotine, place conditioning, sensitization, nimodipine, verapamil, diltiazem, rodents*

INTRODUCTION

Chronic tobacco use has become a major worldwide health problem especially in developed countries. Nicotine, a natural alkaloid present in tobacco leaves, has been accepted as a key agent underlying tobacco addiction. In the animal model, demonstration of nicotine rewarding or reinforcing properties is quite difficult to achieve. For example: nicotine-induced reinforcing effects measured in intravenous self-administration paradigm are modest when compared with other drugs [10]. In the conditioned place preference (CPP) paradigm, divergent results have also been observed. Some reports show nicotine-induced conditioned place aversion [22] or a lack of place conditioning [9]. In some limited cases, the CPP has been observed [8, 34, 39]. These data indicate that nicotine is rather weak reinforcer in animals and its rewarding action can be observed after restricted doses and under specific conditions. An alternative characteristic implicated in the addictive behavior is a phenomenon termed sensitization or reverse tolerance. Evidence available so far suggests that most drugs (i.e. psychostimulants, morphine, ethanol) enhance progressively locomotor activity when given repeatedly [35]. Sensitization-related neuroadaptations have been proposed to be important in the establishment and maintenance of drug addiction and drug-induced psychosis [36].

The dopaminergic projections originating from the ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAC) are considered the main biological substrate of the reinforcing and stimulant effects of drugs of abuse [4, 17]. A number of findings confirm that many addictive drugs, including nicotine, elevate extracellular concentration of dopamine (DA) in the NAC, in particular in its shell, or in the VTA [40]. A role for the mesolimbic systems in nicotine addiction is also supported by a number of findings [12]. Blocking DA release in the NAC with antagonists or lesions abolishes both locomotor activating and rewarding effects of nicotine [3, 12, 42]. Moreover, these effects of nicotine are known to be mediated by the nicotinic cholinergic receptors (nAChRs) located on the dopaminergic neurons [16]. However, the molecular mechanisms that lead to and maintain nicotine addiction are still poorly understood.

Drug addiction is a complex behavioral phenomenon dependent on, besides dopaminergic pathways, several other neural systems. A major involve-

ment of calcium ions and voltage-dependent calcium channels (VDCCs) has previously been suggested but this mechanism is not fully understood. Various classes of calcium channel antagonists (CCAs) have been developed, including 1,4-dihydropyridines (DHP) (e.g. nifedipine, nimodipine), phenylalkylamines (e.g. verapamil) and benzothiazepines (e.g. diltiazem). CCAs are known for their vasodilatory and antiarrhythmic properties. Strong evidence suggests that they can also influence nervous system functions and should be considered psychotropic agents. Some results point to their antidepressant, neuroleptic-like, anxiolytic and anticonvulsant action [32].

Of particular interest were the antireinforcing effects of the L-type CCAs [5, 23]. Little is known of the influence of these drugs on the reinforcing effects of nicotine even though the CCAs are known to modulate the nicotine-evoked DA release from rat striatal synaptosomes [25, 30]. Moreover, the CCAs antagonized some nicotine-induced behavioral responses such as antinociception and discrimination in rats [13, 14, 37]. The changes in extracellular calcium seem to modulate neuronal nAChRs and cholinergic synapses.

The present experiments were undertaken to further evaluate the experimental parameters of nicotine-induced CPP and locomotor sensitization. Additionally, in accordance with the findings suggesting that VDCCs may be important in several aspects of dependence, we investigated the influence of some structurally distinct L-type CCAs on the development and expression of nicotine-induced locomotor sensitization and place conditioning in rodents. The results are discussed in the context of functional association of nicotine with neuronal calcium ions and calcium channels especially in connection with neuroadaptive changes in brain structure and function that ultimately lead to dependence.

MATERIALS and METHODS

Animals

The experiments were carried out on naive male Wistar rats weighing 250–300 g and on naive male Swiss mice (Farm of Laboratory Animals, Warszawa, Poland) weighing 20–25 g at the beginning of the experiments. The animals were kept under standard laboratory conditions (12/12 h light/dark cy-

cle) with free access to tap water and lab chow (Bacutil, Motycz, Poland) and adapted to the laboratory conditions for at least one week. The rats were handled daily during one week preceding the experiments. Each experimental group consisted of 6–12 animals. The experiments were performed between 9.00 a.m. and 5.00 p.m.

All experiments were conducted in accordance with standard ethical guidelines and approved by the local ethical committee (The Medical University of Lublin Committee on the Use and Care of Animals).

Drugs

The compounds tested were: (–)-nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA), nimodipine (RBI, Natick, MA, USA), verapamil (Knoll, Germany), diltiazem hydrochloride (RBI, Natick, MA, USA). Verapamil was diluted to an adequate concentration. Other drugs were dissolved in saline. All agents were administered *ip* in a volume of 10 ml/kg (mice) or 5 ml/kg (rats). Control groups received vehicle injections.

Apparatus

Locomotor activity in mice was measured in a round actometer cages (32 cm in diameter, two light beams) kept in a sound-attenuated experimental room. Two photocell beams located across the long axis measured the animal's movements. The testing apparatus for the CPP test was similar to that used by Spyraiki et al. [41]. Each of six rectangular wooden 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 a neutral masking noise and constant light provided by a 40 W lamp.

Procedure

Acquisition of nicotine sensitization

During the pairing phase (day 1–5) the mice received the following injections: saline + saline, saline + nicotine (0.5 mg/kg), nimodipine (5 and 10 mg/kg) + nicotine (0.5 mg/kg), verapamil (5 and 10 mg/kg) + nicotine (0.5 mg/kg) or diltiazem (5 and 10 mg/kg) + nicotine (0.5 mg/kg). CCAs were ad-

ministered 15 min before each nicotine injection and locomotor activity of animals was measured for 1 h. After one week of withdrawal (day 13), all groups were given a challenge dose of nicotine equal to that previously used to induce behavioral sensitization.

Expression of nicotine sensitization

During the pairing phase (day 1–5), the mice received the injections of saline + saline or saline + nicotine (0.5 mg/kg). On day 13, after one week of withdrawal, the mice were injected with saline + nicotine (0.5 mg/kg), nimodipine (10 and 20 mg/kg) + nicotine (0.5 mg/kg), verapamil (10 and 20 mg/kg) + nicotine (0.5 mg/kg) or diltiazem (10 and 20 mg/kg) + nicotine (0.5 mg/kg) at the intervals of 15 min. Locomotor activity was also recorded for 1 h.

The CPP paradigm consisted of three phases: preconditioning (pre-test), conditioning and postconditioning (test). During the preconditioning phase (the first day) the time that the rats spent in each of the two environments was measured (a baseline preference). Each rat was placed in the central grey area and allowed to explore the compartments for 15 min. The time spent by each animal in the two large compartments was recorded. All subjects showed a moderate preference for the black compartment. The rats were randomized and subsequently conditioned with drug to the less preferred (white) compartment (biased design). The conditioning consisted of two daily 30-min sessions for 4 consecutive days. To measure the effects of CCAs on the acquisition of nicotine-induced CPP, rats were injected with saline before being confined to the black (more preferred) compartment. After 4 h, the animals were pretreated with nimodipine (10 mg/kg, *ip*), verapamil (10 mg/kg, *ip*), diltiazem (10 mg/kg, *ip*) or saline and 15 min later they received injection of nicotine (0.5 mg/kg, *ip*) before being placed in the white compartment. Control group received vehicle every day. During the postconditioning phase, the guillotine doors were removed and the time spent by each rat in the two large compartments was recorded during the session lasting 15 min. To evaluate the effects of CCAs, during the postconditioning phase, the injections of CCAs in the white compartment were followed by the injections of saline.

Statistics

The data were analyzed by the analysis of variance (ANOVA). Post-hoc comparison of means

was carried out with the Bonferroni test, when appropriate. The confidence limit of $p < 0.05$ was considered as statistically significant. Locomotor activity was expressed as a number of photocell beam breaks (means \pm SEM). For the CPP paradigm, the results are expressed as the scores, i.e. the differences (in s) between postconditioning and preconditioning time spent in the drug-associated compartment (means \pm SEM).

RESULTS

Locomotor sensitization

Analysis of the locomotor response to daily administrations of nicotine (0.5 mg/kg, *ip*) or vehicle during the pairing phase revealed a treatment effect ($F_{1,20} = 88.10$, $p < 0.0001$), a day effect ($F_{5,120} =$

5.66, $p < 0.0001$) and a treatment \times day interactions ($F_{5,120} = 2.80$, $p = 0.02$). In nicotine-treated mice, locomotion increased progressively during daily injections (day effect: $F_{5,66} = 5.35$, $p = 0.0003$). After 5 days of repeated nicotine administration and following its 7-day withdrawal, a challenge dose of nicotine (0.5 mg/kg, *ip*) induced marked behavioral sensitization observed as an increase in locomotor activity compared with that after its first injection in the same animal or with the response to acute nicotine challenge in animals treated with repeated saline ($p < 0.001$, Bonferroni test) (Fig. 1 and 2). The locomotor activity of saline-treated mice did not change significantly over time.

Nimodipine, verapamil or diltiazem, at the dose of 10 mg/kg but not 5 mg/kg, administered 15 min prior to each nicotine injection, completely prevented the acquisition of nicotine sensitization

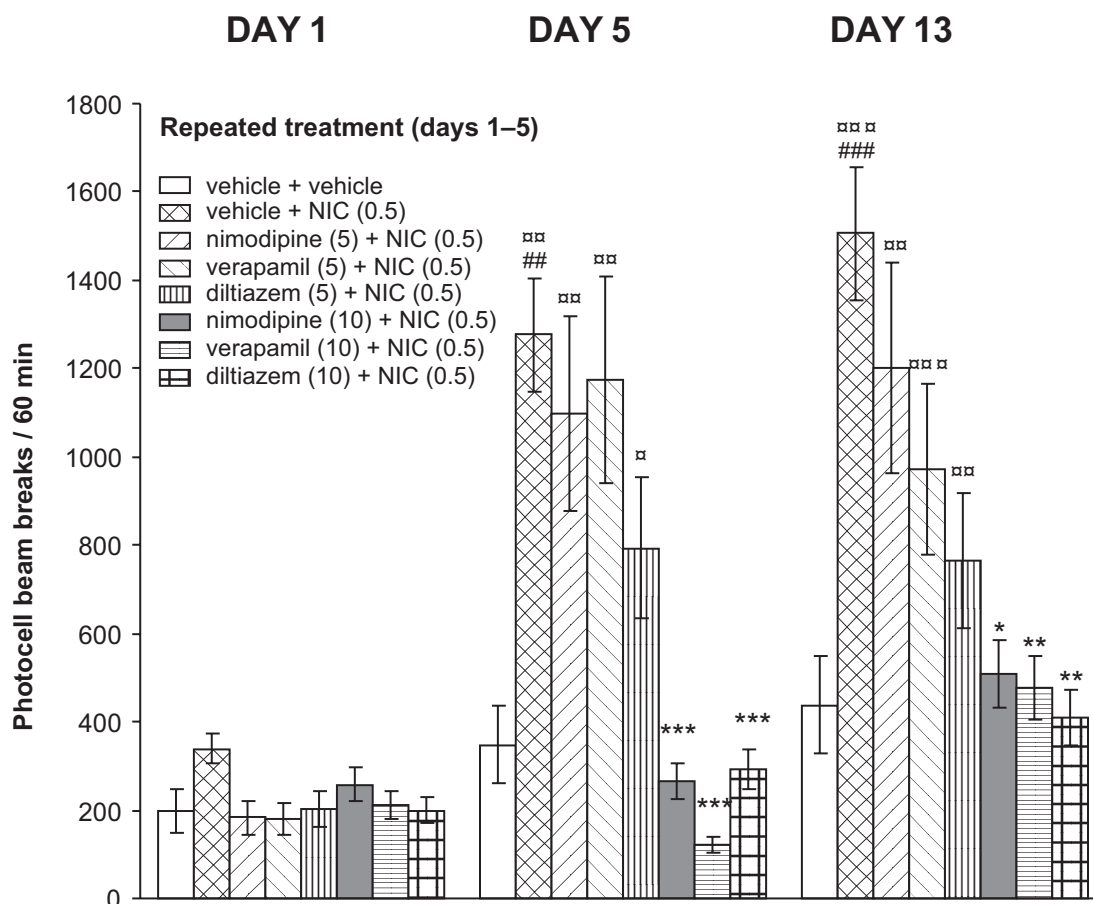


Fig. 1. Effects of nimodipine (5 and 10 mg/kg), verapamil (5 and 10 mg/kg) and diltiazem (5 and 10 mg/kg) challenge with NIC (0.5) on the acquisition of nicotine-induced sensitization in mice. Nimodipine, verapamil, diltiazem or vehicle were injected prior to each nicotine injection (NIC, 0.5 mg/kg) daily for 5 days; on day 13 mice were given a challenge dose of nicotine (0.5 mg/kg). Data represent means \pm SEM; $n = 6-12$. ## $p < 0.01$, ### $p < 0.001$ vs. vehicle + vehicle; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle + NIC; □ $p < 0.05$, □□ $p < 0.01$, □□□ $p < 0.001$ vs. the first pairing day (Bonferroni test)

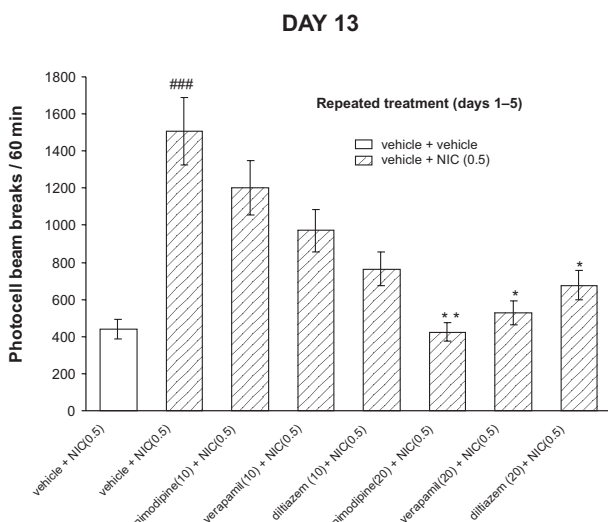


Fig. 2. Effects of nimodipine (10 and 20 mg/kg), verapamil (10 and 20 mg/kg) and diltiazem (10 and 20 mg/kg) on the expression of nicotine-induced sensitization in mice. Nicotine (NIC, 0.5 mg/kg) or vehicle was injected to animals daily for 5 days; on day 13 (a test for expression of sensitization) they were given nicotine (0.5 mg/kg) or a calcium channel antagonist + nicotine. Data represent means \pm SEM; n = 6–12. ### p < 0.001 vs. vehicle-treated and nicotine-challenged mice; * p < 0.05, ** p < 0.01 vs. nicotine-treated and nicotine-challenged mice (Bonferroni test)

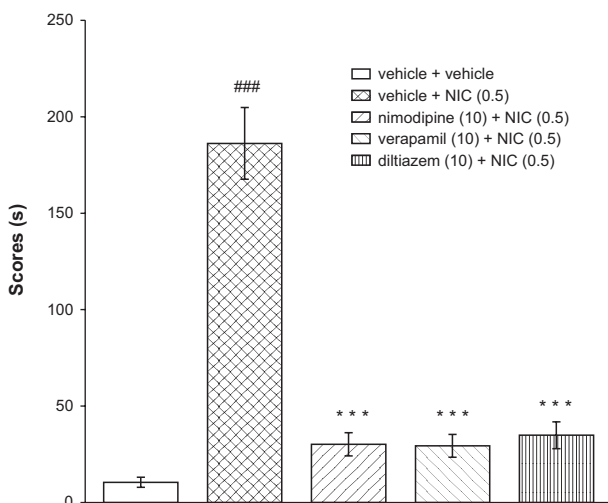


Fig. 3. Effects of nimodipine (10 mg/kg), verapamil (10 mg/kg) and diltiazem (10 mg/kg) on the acquisition of nicotine-induced (0.5 mg/kg) place preference. Preference scores were calculated as the differences (in s) between postconditioning and preconditioning time spent in the compartment associated with the drug in vehicle- and nicotine-treated rats. Data represent means \pm SEM; n = 8. ### p < 0.001 vs. vehicle-treated rats; *** p < 0.001 vs. nicotine-treated rats (Bonferroni test)

tested 7 days after its withdrawal (Fig. 1). ANOVA showed a significant treatment effect: $F_{3,29} = 13.73$, $p < 0.0001$ (day 5), $F_{3,29} = 6.93$, $p = 0.001$ (day 13) vs. saline + nicotine group. Nimodipine, verapamil or diltiazem, at the doses of 20 mg/kg but not 10 mg/kg, injected before the challenge dose of nicotine (0.5 mg/kg, *ip*) attenuated the expression of nicotine sensitization (Fig. 2). ANOVA revealed a significant treatment effect on day 13 vs. the group treated repeatedly with nicotine and challenged with nicotine ($F_{3,28} = 5.81$, $p = 0.003$). None of the CCAs significantly affected basal locomotor activity of mice given acutely or repeatedly (data not shown).

CPP paradigm

The time spent at the initially less preferred (white) and the initially more preferred (black) side, did not significantly differ between groups on the preconditioning day. These side preferences were not significantly changed by the saline injections during the conditioning sessions.

Nicotine administration (0.5 mg/kg, *ip*) induced a clear place preference indicated by a significant increase in the time spent at the drug-associated compartment during the postconditioning phase. Figure 3 shows the results expressed as a score, i.e. postconditioning minus preconditioning time spent in the drug-associated compartment. Pretreatment with nimodipine (10 mg/kg, *ip*), verapamil (10 mg/kg, *ip*) and diltiazem (10 mg/kg, *ip*) inhibited the acquisition of nicotine-induced CPP ($p < 0.001$, Bonferroni test after ANOVA [$F_{4,31} = 22.62$, $p < 0.0001$]).

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 dose tested caused no significant changes in the place preference by themselves (data not shown).

DISCUSSION

The present experiments were undertaken to investigate the effects of some L-type voltage-dependent CCAs on nicotine-induced locomotor sensitization and CPP in rodents. Repeated daily injections of nicotine produced progressively large increases in locomotor activity in mice, especially to a subsequent systemic challenge. This effect is

referred to as behavioral sensitization. Pretreatment with a DHP nimodipine, a phenylalkylamine verapamil and a benzothiazepine diltiazem significantly blocked the acquisition of nicotine-induced locomotor sensitization in a dose-dependent fashion. When mice were injected with these compounds prior to the challenge dose of nicotine, the expression of locomotor sensitization was also attenuated even though this effect was less pronounced and caused by the higher doses. In the second test used, the same dose of nicotine produced a CPP in rats. Pretreatment with nimodipine, verapamil and diltiazem at the dose inactive in producing the place conditioning, completely blocked the establishment of nicotine-induced CPP. These results suggest that both locomotor stimulant and rewarding effects of nicotine are calcium-dependent.

A growing body of evidence indicates that calcium and calcium-mediated second messenger systems play an important role in the reinforcing and hyperlocomotor effects of psychoactive drugs, especially of psychostimulants. It has been reported that nifedipine, diltiazem and verapamil decreased the sensitization response seen after repeated doses of amphetamine [23] as well as amphetamine-induced CPP [31]. The DHPs, isradipine and nimodipine, decreased the self-administration of cocaine [26] and cocaine-induced place preference [29]. When the evidence for the involvement of calcium channels in drug dependence was examined, it has been shown that administration of some CCAs decreased the signs of naloxone-precipitated morphine withdrawal syndrome in rats [2, 6] and the behavioral signs of ethanol-induced hyperexcitability [27]. When CCAs were given concurrently with morphine, the development of tolerance was prevented and the results are similar to the effect of these agents on ethanol tolerance [1, 18].

In the case of nicotine, some studies suggest the calcium-dependent mechanisms of its behavioral effect. The DHP CCAs, given intrathecally, reduced significantly the antinociception induced by nicotine [13]. Pretreatment with isradipine produced also a significant blockade of nicotine discrimination in rats [37]. The results mentioned above and those obtained in the present study suggest the functional association of nAChRs with L-type of VDCCs. Consistent with these data, L-type calcium channels are found on the terminals of dopaminergic afferents in the basal forebrain and activation of nAChRs is known to increase cal-

cium conductance of membranes of central neurons [30]. It is well established that neuronal nAChRs located at pre- and postsynaptic sites within the central nervous system (CNS) are highly permeable to calcium ions [44]. This high Ca^{2+} permeability influences intracellular processes and modulates the release of several neurotransmitters including DA, noradrenaline, adrenaline, serotonin, glutamate and acetylcholine itself [45]. DA systems have received much attention because of their roles in reward. It is well known that nicotine caused its locomotor stimulant and reinforcing effects by stimulating DA release in the NAC and striatum indirectly through the nAChRs on dopaminergic neurons [15, 38]. It is also presumed that intermittent exposure to nicotine may produce the nAChRs up-regulation on presynaptic DA releasing terminals [7]. This effect could, *via* DA release, explain the expression of behavioral sensitization and the reinforcing effects of nicotine on the mesolimbic system. This conclusion is supported by the observation that CCAs were effective blockers of nicotine-evoked DA release from rat striatal synaptosomes [25, 30]. The increase in extracellular DA seems to be dependent on the entry of calcium into the dopaminergic terminals *via* VDCCs.

Taken together, these results suggest that the increases in cytosolic calcium and calcium-mediated second messenger systems influence the behavioral sensitization probably by eliminating the sensitized increase in DA in the NAC and striatum of animals treated repeatedly with psychostimulants and also non-psychostimulant drugs like nicotine.

Behavioral sensitization consists of two separable phenomena: induction (acquisition) and expression. Interestingly, in the present study, the used CCAs (nimodipine, verapamil, diltiazem) were shown to block both phenomena, which can suggest that both induction and expression of behavioral sensitization involves calcium ions and L-type of VDCCs. It is well known that sensitization is a behavioral manifestation of the long-term potentiation (LTP) and Ca^{2+} influx or Ca^{2+} release participates in the induction of LTP [20]. In the case of nicotine, its binding to nAChRs leads to channel opening and depolarization responses with an influx of Ca^{2+} ions. This effect is sufficient to activate calcium-dependent protein kinases like CaM kinase II and induction of LTP. This theory is strongly supported by the data demonstrating that some CCAs inhibi-

ted LTP in the hippocampal CA1 region induced by nicotine [21].

In the present study, it has also been confirmed that nicotine produced a CPP in rats confined to their less-preferred side in a three compartment apparatus (biased design). On the non-drugged test day, the rats spend more time in the compartment paired with nicotine during conditioning. The dose of nicotine was chosen according to the narrow dose range reported by other authors to produce a CPP in rodents [8, 19]. This CPP design has been adapted in accordance with the recent paper demonstrating that rats spent more time in the initially less preferred compartment paired with nicotine, whereas animals that were conditioned with nicotine in their preferred compartment had no significant changes in time spent on that side [8].

Evidence is accumulating that drug and non-drug rewards share the property of activating DA transmission preferentially in the NAC shell. Non-psychostimulant drugs like nicotine, opiates and ethanol have also a μ -opioid component of reward [43]. Our results pointed out the possible role of calcium ions and calcium channels in the acquisition of nicotine-induced place preference providing further evidence for a major role of calcium-dependent mechanisms in mediating the reinforcing properties of nicotine. Recently, the blockade of amphetamine, cocaine and morphine-induced place conditioning and self-administration caused by the CCAs has also been reported [5, 26, 29].

It is worth noting that CCAs, given acutely or repeatedly at the used doses, did not modify the locomotor activity of mice. Moreover, these agents did not provoke any reinforcing effects in the CPP paradigm by themselves. Moreover, in the CPP paradigm, in order to evaluate the influence of CCAs on the acquisition of nicotine place preference, we tested the dose of CCAs in which they blocked the acquisition of nicotine sensitization in our study. Overall, the doses of CCAs were chosen according to those reported in the recent data indicating their ability to prevent morphine- cocaine- and amphetamine-induced place preference, amphetamine-induced hyperactivity as well as conditioned locomotion and behavioral sensitization provoked by cocaine [5, 28, 33].

In summary, it has already been described that nicotine produces effects that are commonly seen with other addictive drugs: self-administration and

place preference, increases in locomotor activity and in reward from brain stimulation [11, 38, 42]. Nicotine cessation also produces a withdrawal syndrome in rodents [24]. Consistent with these data, the present experiments were designed to further study the influence of some CCAs on behavioral sensitization and rewarding effects of nicotine. First, it has been confirmed that nicotine is capable of producing a CPP response in rats, which is considered to represent the rewarding effect of the drug. Concurrent administration of nimodipine, verapamil and diltiazem prevented completely the acquisition of nicotine place conditioning. Secondly, repeated administration of nicotine also results in sensitization to its hyperlocomotor effects. Both induction and expression of this phenomenon were blocked by the systemic administration of nimodipine, verapamil and diltiazem in a dose-dependent manner. Taking all these results together, it is possible to speculate about an influence of the CCAs on the adaptive long-lasting changes, which are observed upon prolonged administration of nicotine. Adaptation in neuronal calcium channels seems to be a common basis for physical dependence on psychoactive drugs. Moreover, the data point out the common calcium-dependent mechanism of nicotine action on both CPP and locomotor sensitization, which may reside in mesolimbic DA system. Our investigations may lead to further strategies for treating nicotine dependence.

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Received: February 24, 2003; in revised form: April 8, 2003.