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ORIGINAL ARTICLE

Effects of varenicline and mecamylamine on the acquisition, expression, and reinstatement of nicotine-conditioned place preference by drug priming in rats

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Abstract Nicotine addiction is a chronic disorder characterized by a relatively high rate of relapse even after long period of abstinence. In the present study, we used the conditioned place preference (CPP) paradigm to investigate the establishment, extinction, reinstatement, and cross-reinstatement of nicotine-induced place conditioning in rats. First, we revealed that nicotine produced a place preference to the initially less-preferred compartment paired with its injections during conditioning (0.175 mg/kg, base, intraperitoneally (i.p.)). Once established, nicotine CPP was extinguished by repeated testing. Following this extinction phase, nicotine-experienced rats were challenged with nicotine (0.175 mg/kg, i.p.) or morphine (10 mg/kg, i.p.). These priming injections of both drugs induced a marked preference for the compartment previously paired with nicotine. Furthermore, given the important role of alpha4beta2 (a4b2) nicotinic receptor subtype in the acquisition and maintenance of nicotine dependence, we evaluated and compared the efficacy of varenicline, a partial a4b2 nicotinic receptor agonist (0.5, 1, and 2 mg/kg, subcutaneously (s.c.)), and mecamylamine (0.5, 1, and 2 mg/kg, s.c.), a non-selective nicotinic receptor antagonist, in blocking nicotine-induced CPP as well as reinstatement of nicotine CPP provoked by nicotine and morphine. It was shown that both nicotinic receptor ligands attenuated the acquisition and expression of nicotine CPP as well as the expression of reinstatement of nicotine CPP provoked by both drugs. Our results indicate similar cholinergic mechanisms, probably through the a4b2 receptors involved in the rewarding effects of nicotine and morphine in rats and may suggest that nicotinic receptors could be a potential target for developing pharmacotherapeutic strategies to treat and prevent nicotine and/or opioid addiction and relapse.

Keywords Nicotine · Morphine · Place conditioning · Varenicline · Mecamylamine · Rats

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Introduction

Drug addiction is a chronic disorder characterized by a relatively high rate of relapse even after long period of abstinence. Among addiction, cigarette smoking is one of the most serious worldwide health problems, and high rates of relapse are very characteristic for people trying to quit tobacco (Kenford et al. 1994). Moreover, withdrawal from nicotine results in a combination of both psychological and physical symptoms leading to cravings and a continued desire to smoke (Hughes 2007; West et al. 2006). Development of tobacco addiction and repeated self-administration of nicotine from cigarettes is due to nicotine's effects on many neurotransmitter systems, especially dopamine released following nicotine's stimulation of neural nicotinic acetylcholine receptors (nAChRs) (Corrigall et al. 1992; Kelley 2002). Among nAChRs, a4b2 subtypes are ligand-gated ion channels found on the dopaminergic neurons and on the gamma-aminobutyric acid (GABA)-containing cells (Potts and Garwood 2007). Biochemical data demonstrate that activation of nAChRs, in particular a4b2 receptor subtypes, indirectly induces dopamine release in the nucleus accumbens (NAC) which is strongly associated with nicotine reward and drug-seeking behavior (Balfour 2002). This neurochemical effect has been shown to be decreased by nAChR subtype antagonists as well (Rahman et al. 2007). In

the preclinical studies, acute pretreatment with nAChR agonists (nicotine itself), antagonists (mecamylamine), and partial agonists (varenicline) decreased nicotine self-administration by 50% (Cohen et al. 2003; Rollema et al. 2007a, b).

Based on the observations that a4b2 nAChRs are thought to play a principal role in the mediation of nicotine addiction, they were identified as a potential target for smoking cessation drugs, especially with their partial agonists (Rollema et al. 2007b). Given evidence such as this, recently approved medication varenicline (Chantix/Champix, Pfizer), a selective partial agonist at a4b2 receptors in the mesolimbic dopamine system (Coe et al. 2005; Rollema et al. 2007a), has been suggested as a relevant and efficacious treatment, as compared with sustained release bupropion or transdermal nicotine patch for the therapy of nicotine addiction (Stapleton et al. 2008). Binding at a4b2 nAChR by a partial agonist is hypothesized to increase the dopaminergic tonus in relevant brain areas which decreases the craving for nicotine and alleviates the symptoms of withdrawal in smokers who try to quit (i.e., agonist effects). In turn, blocking of nicotine's binding at these receptors is thought to reduce nicotine-induced dopamine release, and consequently, reinforcing aspects of tobacco (i.e., antagonist effects) (Coe et al. 2005; Rollema et al. 2007a).

Behavioral responses related to drug addiction and relapse can be measured in various animal models, e.g., in the conditioned place preference (CPP) paradigm (Carr et al. 1989). Considering that nicotine as the main psychoactive component of tobacco is responsible for development and maintenance of addiction and relapse, the present studies were undertaken to further explore a model of nicotine-induced CPP in rats as well as extinction-reinstatement of nicotine place conditioning previously established. In these studies, preference to one distinctive environment associated with a drug administration during conditioning can be extinguished by allowing animals to explore both compartments during a daily session in the absence of the drug. After extinction, a priming dose of drug or the exposure to drug-related environmental stimuli reinstates the extinguished CPP. Together with the fact that there is an interaction between cigarette smoking and abuse of other psychoactive drugs including opioid receptor agonists, and considering the functional interactions between nicotine and morphine within the central nervous system (CNS) already documented (Berrendero et al. 2002; Biala and Weglinska 2006; Zarrindast et al. 1999), cross-reinstatement effect between nicotine and morphine has been also investigated according to a procedure already established in our laboratory (Biala and Budzynska 2006 and 2008). Furthermore, assuming the data reporting that a4b2 receptor partial agonists may represent a new generation of compounds to treat relapse to tobacco addiction, we aimed to investigate and compare the influence of varenicline, a potent and selective a4b2 nAChR partial agonist, and mecamylamine, a non-selective nicotinic receptor antagonist, on the acquisition and expression of nicotine CPP and the expression of the reinstatement of nicotine-induced CPP provoked by nicotine or morphine. Even though varenicline is a recently approved medication for the treatment of tobacco dependence, very little preclinical research on this drug has been published. These experiments may contribute to better understanding of neurobiological mechanisms underlying the relapse to nicotine taking and nicotine/morphine abuse and allow developing more effective methods in the treatment of nicotine dependence with or without concomitant morphine dependence.

Materials and methods

Animals

The experiments were carried out on naive male Wistar rats weighing 250–300 g (Farm of Laboratory Animals, Warszawa, Poland) at the beginning of the experiments. The animals were group-housed, kept under standard laboratory conditions (12/12-h light/dark cycle) with free access to tap water, and adapted to the laboratory conditions for at least 1 week. The rats were handled once a day for 5 days preceding the experiments. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. Each experimental group consisted of 8–12 animals with total number of 580 rats used. The experiments were performed between 9:00 a.m. and 5:00 p.m. All experiments were carried out according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and the European Community Council Directive of 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC) and approved by the local ethics committee.

Drugs

The compounds tested were: morphine hydrochloride (Polfa, Kutno, Poland), (-)-nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA), mecamylamine hydrochloride (Sigma, St. Louis, MO, USA), and varenicline (CP-526555, gift of Pfizer Inc, Groton, USA). All compounds were dissolved in saline (0.9% NaCl). The pH of the nicotine solution was adjusted to 7.0. Fresh drug solutions were prepared on each day of experimentation. Agents were administered subcutaneously (s.c.) or intraperitoneally (i.p.) in a volume of 10 ml/kg, and except for nicotine, drug doses refer to the salt form.

Control groups received saline injections at the same volume and by the same route.

Apparatus

The testing apparatus for the CPP paradigm was already validated in our laboratory (Biala 2003; Biala and Budzynska 2006 and 2008). 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 gray area constituted a "neutral" chamber, which serves as connection and a start compartment. The testing boxes were kept in a soundproof room with neutral masking noise and dim 40-lx illumination. Animal's behavior was observed on a monitor through a digital video camera system, and the amount of time that the rats spent in each of the two large compartments was recorded using a video tracking software (Karnet, Lublin, Poland).

Experimental procedure and treatment

Pre-conditioning

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

Conditioning

One day after pre-conditioning, the rats were randomized and subsequently conditioned with saline paired with the preferred (black) compartment (the morning sessions) and nicotine (0.175 mg/kg, i.p.) with the other (white) compartment (the afternoon sessions) for 30 min. Sessions were conducted twice each day with an interval of 6–8 h for 3 consecutive days (day 2–4). Injections were administered immediately before confinement in one of the two large compartments, as mentioned above. A dose of 0.175 mg/kg nicotine (base) was chosen for conditioning because it is known to produce reliable CPP in rats, also under our experimental conditions (Biala 2003). The control group received saline everyday. The neutral zone was never used during conditioning and was blocked by guillotine doors.

This method (biased design) was similar to that used in our previous experiments (Biala and Budzynska 2006, and 2008) accordingly, to the data indicating that rewarding action of nicotine in the CPP paradigm using three-compartment conditioning boxes can be observed after restricted doses and under specific biased conditions (Calcagnetti and Schechter 1994).

To measure the effects of varenicline and mecamylamine 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 varenicline (0.5, 1, and 2 mg/kg, s.c.), mecamylamine (0.5, 1, and 2 mg/kg, s.c.), or saline, and 30 min later, they received injection of nicotine (0.175 mg/kg, i.p.) or saline before being placed in the white compartment. Control group received saline twice everyday, i.e., 30 min and immediately before each conditioning session.

Post-conditioning (test)

On day 5, conducted one day after the last conditioning trial, animals were placed in the neutral area with the guillotine doors removed and allowed free access to all compartments of apparatus for 15 min. The time spent in the saline- and drug-paired compartments was recorded for each animal.

To evaluate the effects of both nAChR ligands on the expression of nicotine CPP, on the test day (day 5) the rats pretreated with saline or nicotine (as mentioned above) were injected with saline, nicotine (0.175 mg/kg, i.p.), or varenicline (0.5, 1, and 2 mg/kg, s.c.) and mecamylamine (0.5, 1, and 2 mg/kg, s.c.), 30 min before nicotine or saline injection. The time spent by each rat in the two large compartments was recorded during the session lasting 15 min.

The next CPP-reinstatement paradigm took place on 9 consecutive days and consisted of the following phases: pre-conditioning (pre-test), conditioning, post-conditioning (test) (as already described), extinction, and reinstatement.

Extinction

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

Reinstatement

One day after the last extinction trial (day 9), rats that received varenicline (1 and 2 mg/kg, s.c.), mecamylamine (1 and 2 mg/kg, s.c.), or saline, 30 min before a priming injection of nicotine (0.175 mg/kg, i.p.) or morphine (10 mg/kg, i.p.), were immediately tested for reinstatement of CPP. During this reinstatement test, rats were allowed

free access to the entire apparatus for 15 min, and the time spent in each chamber was measured. We have chosen the doses of both agents effective in blocking the acquisition and expression of nicotine CPP.

Statistics

The data are expressed as means ± standard error of mean (SEM) of scores (i.e., the differences between post-conditioning and pre-conditioning time spent in the drug-associated compartment). The statistical analyses for reinstatement paradigm were performed using repeated measure analysis of variance (ANOVA) with treatment as between subjects variables and session as within subjects variable. For other CPP procedures, the statistical analyses were performed using one-way ANOVA with score as variable. Post-hoc comparison of means was carried out with the Tukey's test for multiple comparisons, when appropriate. The confidence limit of $P<0.05$ was considered statistically significant.

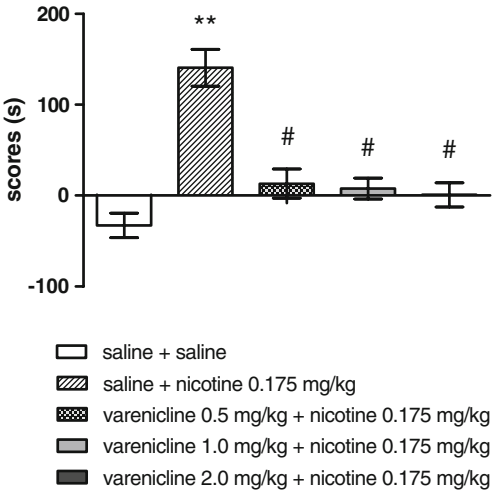

Fig. 1 Effects of varenicline (0.5, 1, and 2 mg/kg, s.c.) on the acquisition of nicotine-induced (0.175 mg/kg, i.p.) place preference. Data represent means ± SEM and are expressed as scores, i.e., differences (in seconds) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; $n=8$–11. (*Double asterisk*) $P<0.01$ vs. saline-conditioned rats; (*Number sign*) $P<0.05$ vs. nicotine-conditioned rats (Tukey's test)

Results

Across all experiments, time (in seconds) spent in the white (e.g., initially less-preferred) compartment during pre-conditioning was 233.5±10.4 compared to 398.1±14.3 in the black (e.g., initially more preferred) side. This side preference was not significantly changed when saline was paired with both compartments during the conditioning sessions.

Influence of varenicline on the acquisition of nicotine-induced CPP

As shown in Fig. 1, on the test day (fifth day, post-conditioning), one-way ANOVA revealed a significant treatment effect [$F(4,43)=4.832$, $P=0.0026$]. Indeed, nicotine administration (0.175 mg/kg) during conditioning induced a clear place preference on the test day, as post hoc analyses showed significant differences in scores between saline-conditioned and nicotine-conditioned groups ($P<0.01$, Tukey's test). Varenicline at the doses of 0.5, 1, and 2 mg/kg administered before each of nicotine injection was effective in blocking the acquisition of nicotine CPP ($P<0.05$ vs. nicotine-conditioned rats; Fig. 1).

Influence of mecamylamine on the acquisition of nicotine-induced CPP

As shown in Fig. 2, on the test day (fifth day, post-conditioning), one-way ANOVA revealed a significant treatment effect [$F(4,46)=4.443$, $P=0.004$]. Indeed, nicotine administration (0.175 mg/kg) during conditioning induced a clear place preference on the test day, as post hoc analyses showed significant differences in scores between saline-conditioned and nicotine-conditioned groups ($P<0.01$, Tukey's test). Pretreatment with mecamylamine (1 and 2 mg/kg, but not 0.5 mg/kg) inhibited the acquisition of nicotine-induced CPP ($P<0.05$ vs. nicotine-conditioned rats; Fig. 2).

Varenicline and mecamylamine injected during conditioning sessions, at the doses tested, did not cause significant changes in place preference by themselves (Table 1).

Influence of varenicline and mecamylamine on the expression of nicotine-induced CPP

As shown in Fig. 3, on the test day (fifth day, post-conditioning), one-way ANOVA revealed a significant treatment effect [$F(8,79)=4.265$, $P=0.0003$]. Indeed, nicotine administration (0.175 mg/kg) during conditioning and after an additional priming injection on the test day induced a clear place preference, as post hoc analyses showed significant differences in scores between saline-conditioned and nicotine-conditioned groups ($P<0.01$, Tukey's test). Both varenicline (0.5, 1, and 2 mg/kg) and mecamylamine (0.5, 1, and 2 mg/kg) administered before an additional nicotine injection on the post-conditioning day were effective in blocking the expression of nicotine CPP (vs. nicotine-conditioned rats given nicotine on the test day) ($P<0.001$ for 2 mg/kg of varenicline; $P<0.01$ for other doses; Fig. 3).

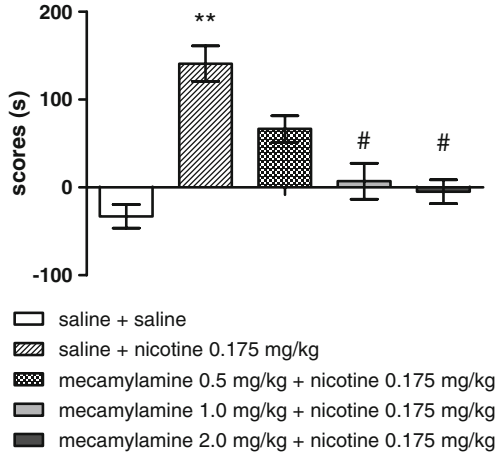

Fig. 2 Effects of mecamylamine (0.5, 1, and 2 mg/kg, s.c.) on the acquisition of nicotine-induced (0.175 mg/kg, i.p.) place preference. Data represent means ± SEM and are expressed as scores, i.e., differences (in seconds) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=8–11. (*Double asterisk*) P<0.01 vs. saline-conditioned rats; (*Number sign*) P<0.05 vs. nicotine-conditioned rats (Tukey's test)

Importantly, when varenicline or mecamylamine injections on the test day were followed by an injection of saline instead of nicotine, they did not cause any significant changes in place preference by themselves at the doses tested. Indeed, the scores calculated for these compounds were not significant as compared with saline-conditioned rats given saline or nicotine injection on the test day (Table 2).

Reinstatement of nicotine-induced CPP

As shown in Fig. 4, in saline- and nicotine-conditioned rats, given saline or nicotine injection on the reinstatement test, two-way ANOVA analyses revealed that there was a significant effect of treatment and session [treatment, F(1,104)=163.7, P<0.0001; session, F(4,104)=6.571, P<0.0001; treatment×session, F(4,104)=13.65, P<0.0001]. On the test day, one-way ANOVA revealed a treatment effect [F(3,44)=39.903, P<0.0001]. Indeed, nicotine administration induced a clear place preference, as post hoc analyses showed that there were significant differences in scores between nicotine-conditioned and saline-conditioned groups (P<0.001, Tukey's test). Figure 4 also shows that the time spent in the nicotine-paired chamber gradually diminished over days of repeated test training. The increase in time spent on the drug-paired compartment on day 6 (first test for extinction, extinction 1, conducted 24 h after the preference test) was still greater for the nicotine-paired animals than for the saline-paired animals; whereas, on day 8 (second test for extinction, extinction 3, 72 h after the initial preference test), there was no difference in the change in time spent on the drug-paired compartment between these two groups, indicating that nicotine CPP had been extinguished by repeated test trials. On Fig. 4, it can be also seen that the priming injection of nicotine (0.175 mg/kg) reinstated the extinguished nicotine CPP (P<0.001 vs. saline-conditioned group given saline injection during reinstatement test, Tukey's test). Furthermore, data show differences in scores between nicotine-conditioned and nicotine-primed rats and saline-conditioned and nicotine-primed rats (P<0.001, Tukey's test), indicating that a prior CPP is necessary for a nicotine prime to produce an increase in time spent on the drug-paired compartment. Moreover, nicotine-conditioned rats show no reinstatement by a saline injection (P<0.001 vs. nicotine-conditioned and nicotine-primed group, Tukey's test).

As shown in Fig. 5, in saline- and nicotine-conditioned rats, given saline or morphine injection on the reinstatement test, two-way ANOVA analyses revealed that there was a significant effect of treatment and session [treatment, F(1,108)=63.17, P<0.0001; session, F(4,108)=4.778, P=0.0014; treatment×session, F(4,108)=5.539, P=0.0004]. On the test day, one-way ANOVA revealed a treatment effect [F(3,42)=14.292, P<0.0001]. Indeed, nicotine administration induced a clear place preference, as post hoc analyses showed that there were significant differences in scores between nicotine-conditioned and saline-conditioned groups (P<0.001, Tukey's test). Figure 5 also shows that the time spent in the nicotine-paired chamber gradually diminished over days of repeated test training as described above. On Fig. 5, it can be also seen that the priming injection of morphine (10 mg/kg, i.p.) reinstated the extinguished nicotine CPP (P<0.001 vs. saline-conditioned group given saline injection during reinstatement test). Furthermore, data show differences in scores between nicotine-conditioned and

Table 1 Effect of varenicline and mecamylamine (0.5, 1, and 2 mg/kg, s.c.) on the acquisition of CPP in rats

Treatment	Scores (in seconds) on the test day
Saline	-32.90±26.89
Varenicline 0.5 mg/kg	67.20±46.35
Varenicline 1 mg/kg	42.73±71.56
Varenicline 2 mg/kg	64.13±32.89
Mecamylamine 0.5 mg/kg	43.88±46.76
Mecamylamine 1 mg/kg	65.38±38.31
Mecamylamine 2 mg/kg	58.80±27.35

Place preference procedure consisted of pre-conditioning, three conditioning sessions (twice each day), and post-conditioning test. Rats were conditioned with saline or both nAChR ligands (as described for nicotine in the "Materials and methods" section). Data represent means ± SEM and are expressed as scores, i.e., the difference (in seconds) between post-conditioning and pre-conditioning time spent in the drug-associated compartment. n=10 rats per group; one-way ANOVA, F(6,63)=0.6523, P=0.6881

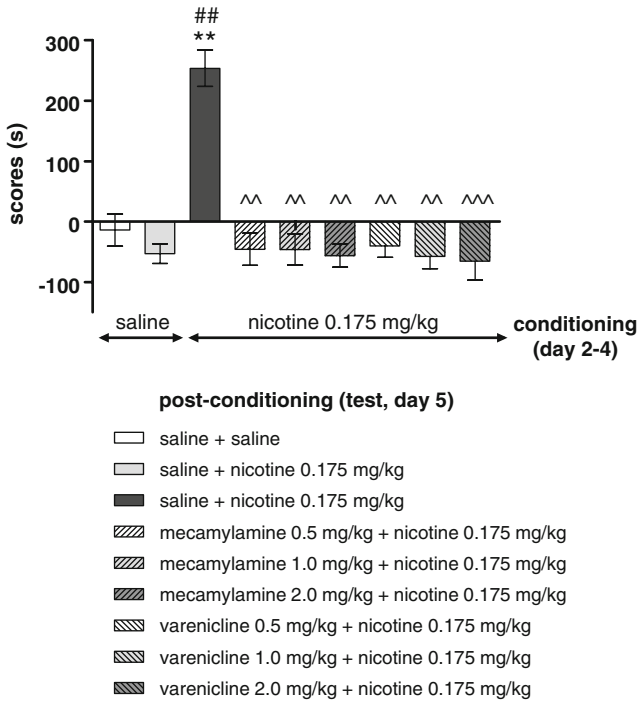

Fig. 3 Effects of varenicline (0.5, 1, and 2 mg/kg, s.c.) and mecamylamine (0.5, 1, and 2 mg/kg, s.c.) on the expression of nicotine-induced (0.175 mg/kg, i.p.) place preference. Data represent means ± SEM and are expressed as scores, i.e., differences (in seconds) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=9–12. (*Double asterisk*) $P<$ 0.01 vs. saline-conditioned rats given saline on the test day; (*Double number sign*) $P<0.01$ vs. saline-conditioned rats given nicotine on the test day; (*Triple circumflex accent*) $P<0.001$, (*Double circumflex accent*) $P<0.01$ vs. nicotine-conditioned rats given nicotine on the test day (Tukey's test)

morphine-primed rats and saline-conditioned and morphine-primed rats ($P<0.001$, Tukey test), indicating that a prior CPP is necessary for a morphine prime to produce an increase in time spent on the drug-paired compartment. Moreover, nicotine-conditioned rats show no reinstatement by a saline injection ($P<0.001$ vs. nicotine-conditioned and morphine-primed group, Tukey's test).

The effect of varenicline and mecamylamine on nicotine- and morphine-induced reinstatement

Pretreatment with varenicline and mecamylamine inhibited the priming effect of nicotine in nicotine-conditioned rats [treatment effect on the reinstatement test, $F(5,59)=19.258$, $P<0.0001$] (Fig. 6). Indeed, post hoc individual comparisons indicated a significant effect of varenicline (1 and 2 mg/kg, s.c.) and mecamylamine (1 and 2 mg/kg, s.c.) ($P<0.001$ vs. nicotine-reinstated group, Tukey's test) which completely abolished the reinstatement of nicotine CPP previously established (Fig. 6). Interestingly, both nAChR ligands also attenuated the priming effect of morphine on nicotine-induced

Table 2 Effect of varenicline and mecamylamine (0.5, 1, and 2 mg/kg, s.c.) injected on the post-conditioning (test) day in saline-conditioned rats

Treatment	Scores (in seconds) on the test day
Saline+saline	−13.83±53.23
Saline+nicotine (0.175 mg/kg)	−52.80±32.78
Varenicline 0.5 mg/kg+saline	−27.20±26.18
Varenicline 1 mg/kg+saline	−28.32±31.66
Varenicline 2 mg/kg+saline	−34.13±22.19
Mecamylamine 0.5 mg/kg+saline	−23.53±36.26
Mecamylamine 1 mg/kg+saline	−27.68±28.81
Mecamylamine 2 mg/kg+saline	−30.36±17.45

Data represent means ± SEM, and are expressed as scores, i.e., the difference (in seconds) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=12 rats per group; one-way ANOVA, $F(7,88)=0.1146$, $P=0.9972$

CPP [treatment effect on the reinstatement test in nicotine-conditioned rats, $F(5,70)=2.613$, $P=0.0318$]. Statistically significant effect was seen for both used doses of varenicline and mecamylamine, i.e., 1 and 2 mg/kg ($P<0.05$ vs. morphine-reinstated group, Tukey's test) (Fig. 7).

Discussion

In the present experiments, we used CPP paradigm including extinction, reinstatement, and cross-reinstatement procedures,

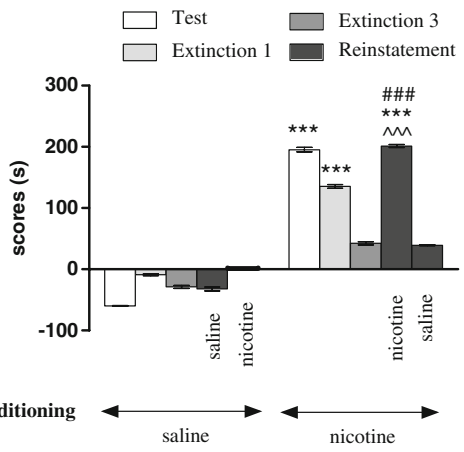

Fig. 4 Reinstatement of nicotine CPP in rats caused by a priming dose of nicotine (0.175 mg/kg, i.p.). Data represent means ± SEM and are expressed as scores, i.e., differences (in seconds) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=12–14. (*Triple asterisk*) $P<0.001$ vs. saline-conditioned group; (*Triple number sign*) $P<0.001$ vs. saline-conditioned rats primed with nicotine; (*Triple circumflex accent*) $P<$ 0.001 vs. nicotine-conditioned rats primed with saline (Tukey's test)

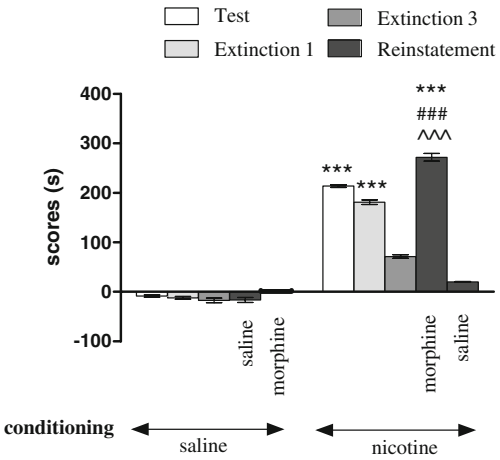

Fig. 5 Reinstatement of nicotine CPP in rats caused by a priming dose of morphine (10 mg/kg, i.p.). Data represent means ± SEM and are expressed as scores, i.e., differences (in seconds) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=10–14. (*Triple asterisk*) P<0.001 vs. saline-conditioned group; (*Triple number sign*) P<0.001 vs. saline-conditioned rats primed with morphine; (*Triple circumflex accent*) P<0.001 vs. nicotine-conditioned rats primed with saline (Tukey's test)

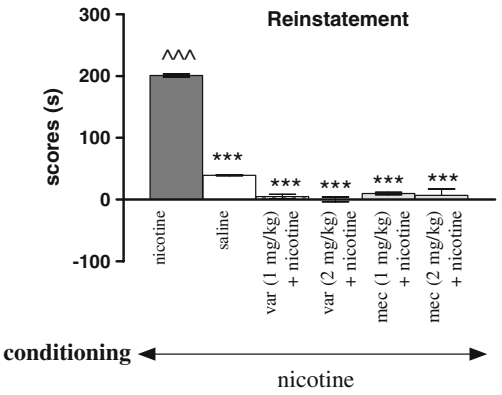

Fig. 6 Effects of varenicline (*var*) (1 and 2 mg/kg, s.c.) and mecamylamine (*mec*) (1 and 2 mg/kg, s.c.) on the reinstatement of nicotine CPP caused by a priming dose of nicotine (0.175 mg/kg, i.p.). Data represent means ± SEM and are expressed as scores, i.e., differences (in seconds) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=9–12. (*Triple circumflex accent*) P<0.001 vs. nicotine-conditioned rats primed with saline; (*Triple asterisk*) P<0.001 vs. nicotine-conditioned rats primed with nicotine (Tukey's test)

a model consistent with drug-seeking behavior. As it was previously shown in our experiments, nicotine at the dose chosen according to the narrow range of doses reported to produce CPP, induced place preference in rats subjected to a biased procedure. On the test day, the rats spend more time in the compartment paired with nicotine during conditioning especially after an additional nicotine priming dose (Biala 2003; Biala and Budzynska 2006 and 2008; Calcagnetti and Schechter 1994). Subsequently, once established, nicotine place preference was extinguished by repeated daily testing and reinstated by a priming dose of nicotine or morphine. Additionally, we investigated and compared the effects of two nAChR ligands, i.e., varenicline, a partial a4b2 agonist, and mecamylamine, a non-selective nAChR antagonist, on these behavioral actions of both drugs. One of the finding of our studies was that concurrent administration of both nAChR ligands with each nicotine injection prevented the acquisition of nicotine place conditioning. Moreover, an acute injection of varenicline and mecamylamine before an additional nicotine administration on the test day also attenuated the expression of nicotine CPP, and this action was probably due to a decrease of the effect of the nicotine priming. It is important to note that at the doses used, both nAChR ligands did not cause any significant effect by themselves as injected during conditioning or on the test day. A major finding of our experiments was that varenicline and mecamylamine significantly prevented the reinstatement effects of previously extinguished nicotine place preference caused by a priming dose of nicotine and morphine. Our data support the hypothesis that similar neural mechanisms, probably through the a4b2 nAChR subtype, can be involved in the rewarding and abuse-producing effects of nicotine and morphine.

Nicotine produces behavioral effects that are potentially related to its interaction with diverse nAChRs populations. Among nAChRs, the heteromeric high-affinity a4b2-containing subtypes are particularly relevant targets for medication development for several reasons. These nAChRs constitute high-affinity nicotine binding sites in the brain (Gotti and Clementi 2004) and are distributed

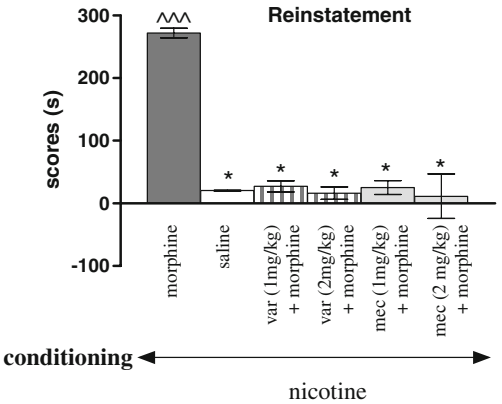

Fig. 7 Effects of varenicline (*var*) (1 and 2 mg/kg, s.c.) and mecamylamine (*mec*) (1 and 2 mg/kg, s.c.) on the reinstatement of nicotine CPP caused by a priming dose of morphine (10 mg/kg, i.p.). Data represent means ± SEM and are expressed as scores, i.e., differences (in seconds) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=12–14. (*Triple circumflex accent*) P<0.001 vs. nicotine-conditioned rats primed with saline; (*Asterisk*) P<0.05 vs. nicotine-conditioned rats primed with morphine (Tukey's test)

throughout the CNS including in dopaminergic areas that have been shown to be important in the rewarding effects of drugs of abuse (Corrigall et al. 1992; Wonnacott 1997; Wonnacott et al. 1997). Studies in humans and rodents have shown that chronic exposure to nicotine increases the density of nicotine binding, mainly to these a4b2 nAChR types (Marks et al. 1992). Convincing evidence for the crucial role of mesolimbic a4b2 nAChRs also came from studies in which deletion of either the a4 or b2 subunit attenuates the pharmacological and behavioral effects of nicotine (Marubio et al. 2003; Picciotto et al. 1998). Additionally, targeted expression of b2 subunits in the ventral tegmental area (VTA) of b2 knock-out mice reinstates nicotine-seeking behavior and nicotine-induced dopamine release (Maskos et al. 2005).

In animal models of addiction and relapse, it was shown that a priming dose of nicotine as well as nicotine-associated cues enhance nicotine reinforcement in rats and reinstate nicotine-seeking behavior after extinction (Chaudhri et al. 2006). Interestingly, our experiments revealed that not only nicotine itself, but also morphine, reinstated nicotine-induced CPP in rats. Recently, similar to our data, studies demonstrated development of crossover effects, i.e., cross-sensitization between nicotine and morphine to their stimulant and rewarding properties as well as development of cross-tolerance to antinociception or hypothermia induced by these drugs (Biala et al. 2005; Biala and Budzynska 2008; Biala and Weglinska 2006; Zarrindast et al. 1999). Several mechanisms underlying this interaction can be proposed. Nicotine reinforces smoking behavior by activating nAChRs in the midbrain dopaminergic reward centers, especially in the VTA (Corrigall et al. 1992). Morphine is known to increase dopamine transmission to the NAC through the inhibition of GABAergic inhibitory interneurons located on the dopaminergic neurons in the VTA (Tzschentke 1998, for review). It can be noted that in the VTA, the majority of dopamine and GABA neurons or glutamatergic terminals express nAChRs (Wooltorton et al. 2003), and several studies have already demonstrated that the regulation of dopamine release by acetylcholine is needed for rewarding effects of drugs (Yeomans and Baptista 1997; Yeomans et al. 1993). It is well known that the cholinergic receptor excitation of the VTA dopaminergic neurons cause release of dopamine in the NAC which plays an important role in the activation of reward systems (Dani et al. 2001; Gronier et al. 2000; Yeomans and Baptista 1997; Yeomans et al. 1993). Moreover, in the context of our study, previous electrophysiological studies have also reported that the nAChRs may be a target through which opioid receptor ligands may regulate directly nicotinic receptor-mediated functions (Almeida et al. 2000; Tome et al. 2001). As such, bilateral microinjection of nicotine into the VTA potentiates, while blockade of these receptors with mecamylamine, inhibits morphine-induced CPP, suggesting the involvement of nAChRs in the VTA in reward-related effects of opioid receptor agonists (Rezayof et al. 2007). In turn, acute morphine administration to rats decreases acetylcholine release in striatum, cortex, and in the core and shell of the NAC (Fiserova et al. 1999; Taguchi et al. 1993).

To further discuss and propose possible neuronal mechanisms implicated, it can be pointed out that our data also showed that concurrent administration of nAChR ligands, varenicline, and mecamylamine not only prevented the acquisition and expression of nicotine CPP but also cross-reinstatement between nicotine and morphine in rats. Consistent with its partial agonist mechanism, varenicline has been shown to produce increases in dopamine release and turnover in the NAC that are significantly lower (40–60%) than those produced by nicotine, and varenicline pretreatment attenuates nicotine-induced increases in dopamine release and turnover acting as an antagonist in the presence of nicotine as compared with mecamylamine (Coe et al. 2005; Rollema et al. 2007a, b). Present data are in line with the clinical data from a double-blind placebo study showing that varenicline (Chantix/Champix, Pfizer) maintains abstinence and reduces rate of relapse (Tonstad et al. 2006). The progressive ratio experiments show that nicotine is a more efficacious reinforcer than varenicline, consistent with the idea that a partial agonist should be less reinforcing and consequently have a significantly lower abuse potential than nicotine. Finally, varenicline reduces nicotine self-administration in rats and supports lower self-administration break points than nicotine. In the drug discrimination paradigm, varenicline substitutes for a nicotine cue, and this effect is blocked by mecamylamine (Rollema et al. 2007b). These data suggest that varenicline can reproduce to some extent the subjective effects of smoking by partially activating a4b2 nAChRs, while preventing full activation of these receptors by nicotine and its rewarding effects. This effect was also confirmed in our study as varenicline, at the doses blocking the nicotine CPP, did not provoke any reinforcing effects by itself in the CPP test. Concerning mecamylamine, a non-competitive and non-selective nAChR antagonist, it has been already known as an agent who attenuates tobacco smoking in humans trying to quit. Some investigations have indicated that the nAChRs of the VTA located on the dopaminergic and GABAergic neurons, pivotal for nicotine dependence-producing effects, can be blocked by mecamylamine (Mansvelder et al. 2002; Wooltorton et al. 2003). Accordingly, it has been found that mecamylamine reduced nicotine self-administration at the same or higher range of doses than used in the present study (Donny et al. 1999; Watkins et al. 1999). Considering the crossover reinforcing effects of nicotine and morphine as well as the inhibitory effects of both nAChR ligands observed in our study, and in

the context of dopamine-related behavior including CPP, it appears that the blockade of VTA nAChRs mainly at the a4b2 subtypes attenuates the excitatory effects of both nicotine and morphine on mesoaccumbens and nigrostriatal dopaminergic transmission (Miller et al. 2005). In addition, possible involvement of other neuronal pathways, including glutamatergic neurotransmission (Reid et al. 2000), can also be suggested.

Relapse is a major characteristic of drug addiction, and the CPP paradigm in rodents can be used to study the potential mechanisms underlying drug craving. Our results showed cross-reinstatement effect of nicotine and morphine providing circumstantial evidence for morphine and nicotine interactions. Altogether, our research provided new insight into the interaction between cholinergic system, especially through the a4b2 nAChR subtypes and nicotine or morphine-rewarding effects. The present studies showed the comparable capacity of a4b2 partial agonist varenicline and nAChR non-selective antagonist mecamylamine to abolish the acquisition and expression of nicotine CPP as well as reinstatement of nicotine-seeking behavior induced by priming dose of the two drugs. This implies that the cholinergic system, especially through the a4b2 nAChRs, plays a pivotal role in the neurobiological processes underlying the relapse to drug addiction. Accordingly, considering polydrug abuse, the antismoking agent varenicline has been already proposed to be beneficial for treating patients with alcohol dependence with or without concomitant nicotine dependence, which is a quite frequent phenomenon (Ericson et al. 2009). Both a4b2 partial agonists like varenicline already registered as a new aid for smoking cessation, and mecamylamine-like nAChR antagonists may also become potential candidates for effective pharmacotherapy and relapse prevention not only in terms of tobacco smoking in abstinent smokers but also nicotine/opioids co-abuse.

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