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Varenicline and mecamylamine attenuate locomotor sensitization and cross-sensitization induced by nicotine and morphine in mice

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ABSTRACT

The present study focused on the evaluation of behavioural sensitization and cross-sensitization induced by nicotine and morphine in mice. First, we revealed that after 9 days of nicotine administration (0.175 mg/kg, free base), every other day and following its 7-day withdrawal, challenge doses of nicotine (0.175 mg/kg) and morphine (5 mg/kg) induced locomotor sensitization in mice. When we examined the influence of varenicline, a partial alpha4beta2 nicotinic receptor agonist (0.5, 1 and 2 mg/kg) and mecamylamine (0.5, 1 and 2 mg/kg), a non-selective nicotinic receptor antagonist, we found that both agents attenuated the acquisition and expression of nicotine sensitization as well as locomotor cross-sensitization between nicotine and morphine. Our results indicate similar cholinergic mechanisms involved in the locomotor stimulant effects of nicotine and morphine in mice, and as such these data may suggest that nicotinic neurotransmission could be a potential target for developing pharmacotherapeutic strategies to treat and prevent nicotine and/or opioid addiction.

1. Introduction

Drug addiction, including polydrug use, is a chronic relapsing brain disease characterized by the compulsive use of addictive substances despite adverse consequences. Dual concomitant drug dependence is becoming increasingly more common, with nicotine and morphine being two of the co-abused psychoactive drugs. Some epidemiological studies revealed that tobacco dependence is more frequent in the opioid-dependent individuals (Frosch et al., 2000; Elkader et al., 2009; Epstein et al., 2010). Despite these epidemiological findings, there have been relatively few animal studies on the neurobiological substrates that may underlie this combined nicotine and morphine exposure.

The dependence-producing effects of nicotine, an alkaloid present in tobacco, are believed to be mediated through the activation of multiple subtypes of neuronal nicotinic acetylcholine receptors (nAChRs), among which the mesolimbic alpha4beta2 subtypes has a pivotal role. Activation of these receptors by nicotine, indirectly increases the release of dopamine in the nucleus accumbens (NAC) and the prefrontal cortex, an effect shared by most substances of abuse with distinct neurochemical targets (Picciotto et al., 2000; Di Chiara, 2000; Dani and De Biasi, 2001). Recent data confirm that the alpha4beta2, but not homomeric alpha7 nAChR subtype plays an important role in modulating the hyperlocomotor (acute and sensitized) or rewarding effects of nicotine, as their antagonists abolish these effects (Grottick et al., 2000; Rahmann et al., 2007). Moreover, nicotine self-administration is reduced in animals given the competitive, and relatively selective (beta2-preferring nAChR) antagonist, dihydro-ß-erythroidine (Watkins et al., 1999). Accordingly, preclinical studies in transgenic mice have shown that elimination of either the alpha4 or beta2 subunit attenuates the pharmacological and behavioural effects of nicotine, including reinforcement (Picciotto et al., 1998; Marubio et al., 2003; Pons et al., 2008).

Given the important role of alpha4beta2 nAChRs in the reinforcement and maintenance of nicotine dependence, modulating the activity of these receptors would be expected to have therapeutic benefits. Specifically, selective partial agonists of alpha4beta2 nAChRs that enhance the activity of these receptors sufficiently to blunt craving and withdrawal, but without abuse potential, have been already proposed as efficacious smoking cessation agents (Buchhalter et al., 2008). Recently, a partial agonist at the alpha4beta2 varenicline (Chantix, Champix, Pfizer) derived from the cytisine compound (Mihalak et al., 2006), was approved as a smoking cessation aid. Varenicline is a partial nAChR agonist that binds to alpha4beta2 nAChRs with greater affinity, but fewer efficacies than nicotine (Coe et al., 2005; Mihalak et al., 2006; Carroll et al., 2008). If such, biochemical studies show that, in the presence of nicotine, varenicline reduces nicotine intake and nicotine-evoked dopamine release in the rat NAC by its antagonist activity, while mimicking the stimulatory effect of nicotine on accumbal dopamine release through its agonist activity (Coe et al., 2005; Rollema et al., 2007a, 2007b). It can be hypothesized that an effective alpha4beta2 partial agonist would, through its intrinsic partial activation, elicit a moderate and sustained increase in mesolimbic dopamine levels, counteracting

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the low dopamine levels encountered in the absence of nicotine during smoking cessation attempts.

Behavioural responses related to drug addiction can be measured in various animal models e.g., in the conditioned place preference (CPP) paradigm (Carr et al., 1989). An alternative characteristic is a phenomenon termed sensitization or reverse tolerance (Robinson and Becker, 1986). Using this paradigm it has been shown that after intermittent chronic exposure to a drug (e.g., psychostimulants and nicotine), animals began to develop addiction-like symptoms including continued drug seeking and an escalation of drug intake, increased motivation to obtain drugs, and a greater propensity to relapse after enforced abstinence (Robinson and Berridge, 1993). Considering that functional interactions between nicotine and morphine within the central nervous system have been already documented (Zarrindast et al., 1999; Berrendero et al., 2002; Biala and Weglinska, 2006), the present studies were undertaken to further investigate behavioural cross-over locomotor effects of both drugs. We used the nicotine-induced locomotor sensitization procedure evaluated in our previous studies (Biala, 2003; Biala and Weglinska, 2004) to examine if nicotine-experienced mice develop sensitization to locomotor stimulating effect of morphine. Additionally, we investigated and compared the influence of varenicline, a partial alpha4beta2 agonist and mecamylamine, a non-selective nicotinic receptor antagonist, on the acquisition and expression of nicotine sensitization and the expression of cross-over effects between nicotine and morphine. Even though varenicline is recently approved medication for the treatment of tobacco dependence, yet very little preclinical research on this drug has been published. It is also plausible that the ability of varenicline to elevate dopamine can provide relief also from withdrawal symptoms and craving related to other drugs of abuse, including morphine, at least in a certain dose range. The antismoking agent varenicline may exhibit properties with respect to its interaction with morphine and nicotine in the brain reward system that may be beneficial for treating patients with nicotine dependence with or without concomitant opioid dependence.

2. Material and methods

2.1. Animals

The experiments were carried out on naive male Swiss mice weighing 20–25 g (Farm of Laboratory Animals, Warszawa, Poland) at the beginning of the experiments. The animals were kept under standard laboratory conditions (12/12-h light/dark cycle, temperature 21 ± 1 °C, humidity 40–50%) with free access to tap water and lab chow (Bacutil, Motycz, Poland), and adapted to the laboratory conditions for at least 1 week. 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. The experiments were performed between 9:00 a.m. and 3:00 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 at the Medical University of Lublin.

2.2. 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. Doses of the nAChR ligands have been chosen accordingly to publish data indicating their influence on drug-induced effects (Liu et al., 2007; Zaniewska et al., 2008; LeSage et al., 2009).

2.3. Apparatus

Locomotion was recorded individually in round actometer cages (Multiserv, Lublin, Poland; 32 cm in diameter) kept in a sound-attenuated experimental room. Two photocell beams located across the axis measured the animal's movements automatically.

2.4. Experimental procedure and treatment

In order to measure locomotor effects of both nAChR ligands, the animals, naive for any drug treatment, were injected with varenicline (0.5, 1 and 2 mg/kg, i.p.), mecamylamine (0.5, 1 and 2 mg/kg, i.p.) or saline for the control group, and immediately placed in the activity chamber. Locomotor activity, i.e., the number of photocell beam breaks was automatically recorded for 60 min.

2.4.1. Influence of varenicline and mecamylamine on the acquisition of nicotine-induced locomotor sensitization

During the pairing phase (days 1–9), mice received the following injections: saline (i.p.) + saline (s.c.) or saline (i.p.) + nicotine (0.175 mg/kg, s.c.) every other day for five sessions. This method was similar to that used in our previous experiments accordingly to the data indicating that this dose of nicotine produces robust locomotor sensitization in mice under our laboratory conditions (Biala and Weglinska, 2004). The mice remained drug free for 1 week and, on day 16, the same groups of mice were further challenged with nicotine (0.175 mg/kg, s.c.), morphine (5 mg/kg, s.c.) or saline, respectively. Locomotor activity was recorded for 60 min during the pairing phase (days 1–9) and on the 16th day, immediately after injections. Next, during the pairing phase (day 1–9) the mice received the following injections: saline + saline, saline + nicotine (0.175 mg/kg), varenicline (0.5, 1 and 2 mg/kg) + nicotine or mecamylamine (0.5, 1 and 2 mg/kg) + nicotine. Both nAChR ligands were administered 30 min before each nicotine injection and locomotor activity of animals was measured for 60 min. After 1 week of withdrawal (day 16), all groups were given a challenge dose of nicotine equal to that previously used to induce behavioural sensitization.

2.4.2. Influence of varenicline and mecamylamine on the expression of nicotine-induced locomotor sensitization

In the next experiment, on the challenge day (day 16) the mice pretreated with saline or nicotine (as mentioned above) were injected with saline + nicotine (0.175 mg/kg), or varenicline (1 and 2 mg/kg, i.p.) and mecamylamine (1 and 2 mg/kg, i.p.) 30 min before nicotine challenge injection. Locomotor activity of mice was also recorded for 60 min. We have chosen the doses of both agents effective in blocking the acquisition of nicotine sensitization.

2.4.3. Influence of varenicline and mecamylamine on the expression of cross-sensitization between nicotine and morphine

In this experiment, on the challenge day (day 16) the mice pretreated with saline or nicotine were injected with saline + morphine (5 mg/kg), or varenicline (1 and 2 mg/kg, i.p.) and mecamylamine (1 and 2 mg/kg, i.p.) 30 min before morphine challenge injection. Locomotor activity of mice was also recorded for 60 min.

2.5. Statistical analysis

The data are expressed as means ± S.E.M. For locomotor sensitization, data were analyzed using repeated measure analysis of variance (ANOVA) with treatment as independent factor and days as repeated measures. The response to drugs on the challenge day was compared using one-way ANOVA. 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.

3. Results

3.1. Influence of varenicline and mecamylamine on the acquisition of nicotine-induced locomotor sensitization

Two-way ANOVA of the locomotor response after administration of nicotine (0.175 mg/kg, s.c.) or saline during the pairing phase (days 1–9 and day 16 — challenge) revealed a treatment effect [$F(4,252)=11.33$, $p<0.0001$)], a day effect [$F(5,252)=6.12$, $p<0.0001$] without interaction effect [$F(20,252)=1.417$, $p=0.1142$] (Fig. 1). On the 1st day, one-way ANOVA did not reveal any treatment effect [$F(4,42)=0.597$, $p=0.6684$]. On the 16th day, after an additional injection of nicotine, one-way ANOVA revealed a significant treatment effect [$F(4,42)=16.825$, $p<0.0001$]. Indeed, after this last nicotine injection (day 16), a significant difference between the response was observed as compared to the first injection of nicotine ($p<0.001$) or with the response to nicotine in animals treated with repeated saline ($p<0.001$, Tukey's test) (Fig. 1). Varenicline at the doses of 1 and 2 mg/kg, but not of 0.5 mg/kg, injected before each of nicotine injection, was effective in blocking the acquisition of nicotine sensitization ($p<0.001$ vs. nicotine-pretreated and nicotine-challenged mice) (Fig. 1). Similarly, in the 2nd experiment in which mecamylamine influence was measured, two-way ANOVA of the locomotor response after administration of nicotine (0.175 mg/kg, s.c.) or saline during the pairing phase (days 1–9 and day 16 — challenge) revealed a treatment effect [$F(4,240)=7.844$, $p<0.0001$], a day effect [$F(5,240)=25.14$, $p<0.0001$] and an interaction effect [$F(20,240)=8.548$, $p<0.0001$] (Fig. 2). On the 1st day, one-way ANOVA revealed a significant treatment effect [$F(4,40)=6.106$, $p=0.0006$]. Accordingly, 2 mg/kg of mecamylamine blocked the locomotor effect of nicotine injected on the 1st day of the pairing phase ($p<0.01$). On the 16th day, after an additional injection of nicotine, one-way ANOVA revealed a significant treatment effect [$F(4,40)=56.763$, $p<0.0001$]. Indeed, after this last nicotine injection (day 16), a significant difference between the response was observed as compared to the first injection of nicotine ($p<0.001$) or with the response to nicotine in animals treated with repeated saline ($p<0.001$, Tukey's test) (Fig. 2). Mecamylamine at the doses of 1 and 2 mg/kg, but not of 0.5 mg/kg, injected before each of nicotine injection, was effective in blocking the acquisition of nicotine sensitization ($p<0.001$ vs. nicotine-pretreated and nicotine-challenged mice) (Fig. 2). In can be noted that neither varenicline nor mecamylamine at doses tested (0.5, 1 and 2 mg/kg) caused no statistically significant changes in the locomotor activity of mice measured 60 min after injection (Table 1) as compared with the control saline-injected group.

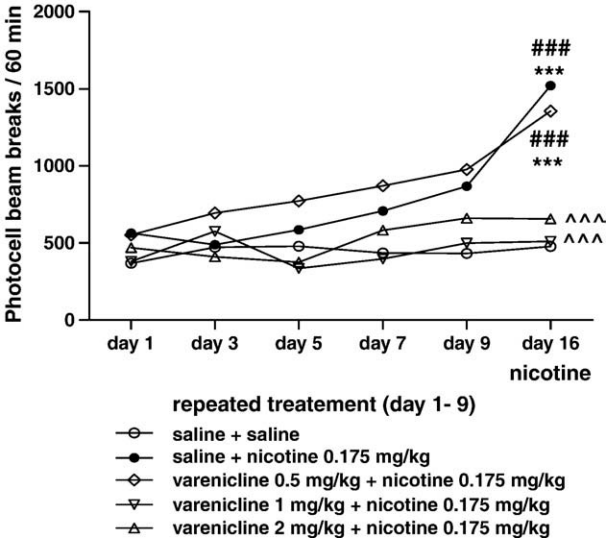

Fig. 1. Effects of varenicline (0.5, 1 and 2 mg/kg, i.p.) on the acquisition of locomotor sensitization to nicotine in mice. Saline or varenicline were administered 30 min before each nicotine injection (0.175 mg/kg, base, s.c.) daily for 9 days, every other day; on day 16 (a test for expression of sensitization) mice were given nicotine (0.175 mg/kg, s.c.) challenge injection. Data represent means ± S.E.M.; $n=9$–10 mice per group. ###$p<0.001$ vs. the first pairing day; ***$p<0.001$ vs. saline-pretreated and nicotine-challenged mice; ^^^$p<0.001$ vs. nicotine-pretreated and nicotine-challenged mice (Tukey's test).

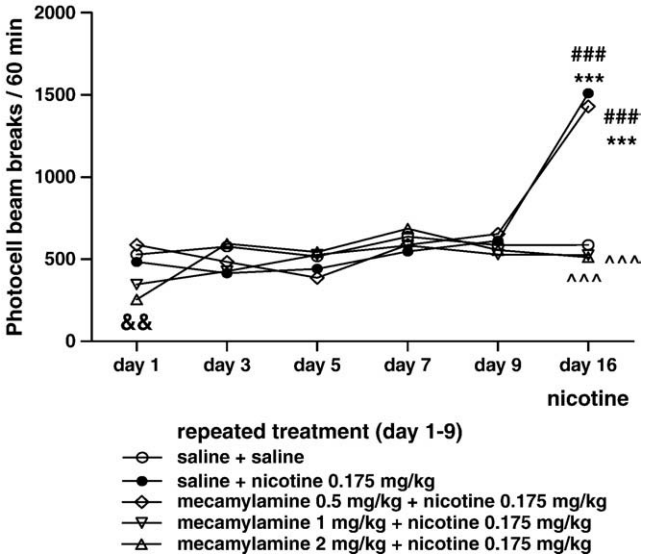

Fig. 2. Effects of mecamylamine (0.5, 1 and 2 mg/kg, i.p.) on the acquisition of locomotor sensitization to nicotine in mice. Saline or mecamylamine were administered 30 min before each nicotine injection (0.175 mg/kg, base, s.c.) daily for 9 days, every other day; on day 16 (a test for expression of sensitization) mice were given nicotine (0.175 mg/kg, s.c.) challenge injection. Data represent means ± S.E.M.; $n=8$–10 mice per group. ###$p<0.001$ vs. the first pairing day; ***$p<0.001$ vs. saline-pretreated and nicotine-challenged mice; ^^^$p<0.001$ vs. nicotine-pretreated and nicotine-challenged mice; &&$p<0.01$ vs. nicotine injected mice on the first day (Tukey's test).

3.2. Influence of varenicline and mecamylamine on the expression of nicotine-induced locomotor sensitization

Two-way ANOVA of the locomotor response after administration of nicotine (0.175 mg/kg, s.c.) or saline during the pairing phase (day 1 and day 16 — challenge) revealed a treatment effect [$F(5,90)=18.94$, $p<0.0001$], a day effect [$F(1,90)=62.05$, $p<0.0001$] and an interaction effect [$F(5,90)=18.94$, $p<0.0001$] (Fig. 3). On the 1st day, one-way ANOVA did not reveal any significant treatment effect [$F(5,45)=0.07253$, $p=0.9960$]. On the 16th day, after an additional injection of nicotine, one-way ANOVA revealed a significant treatment effect [$F(5,45)=42.491$, $p<0.0001$]. Indeed, after this last nicotine injection (day 16), a significant difference between the response was observed as compared to the first injection of nicotine ($p<0.001$) or with the response to nicotine in animals treated with repeated saline ($p<0.001$, Tukey's test) (Fig. 3). Both

Table 1
Effect of varenicline and mecamylamine (0.5, 1 and 2 mg/kg, i.p.) on locomotor activity (means ± S.E.M., photocell beambreaks) of mice measured during 60 min after injection; one-way ANOVA: $F(6,47)=2.295$, $p=0.054$.

Treatment	Means	SEM	n
Saline	670.43	86.65	7
Varenicline 0.5 mg/kg	505.13	66.68	8
Varenicline 1 mg/kg	448.00	75.17	8
Varenicline 2 mg/kg	352.86	50.86	7
Mecamylamine 0.5 mg/kg	420.88	54.28	8
Mecamylamine 1 mg/kg	498.38	71.06	8
Mecamylamine 2 mg/kg	380.38	60.10	8

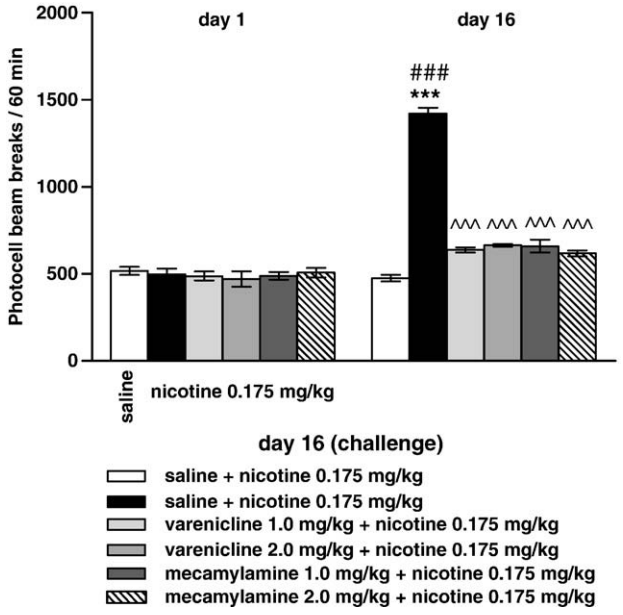

Fig. 3. Effects of varenicline and mecamylamine (1 and 2 mg/kg, i.p.) on the expression of locomotor sensitization to nicotine in mice. Nicotine (0.175 mg/kg, base, s.c.) or saline were injected daily for 9 days, every other day; on day 16 (a test for expression of sensitization) mice were given nicotine (0.175 mg/kg, s.c.) and varenicline or mecamylamine, 30 min before nicotine challenge injection. Data represent means ± S.E.M.; $n = 8$–10 mice per group. ###$p<0.001$ vs. the first pairing day; ***$p<0.001$ vs. saline-pretreated and nicotine-challenged mice; ^^^$p<0.001$ vs. nicotine-pretreated and nicotine-challenged mice (Tukey's test).

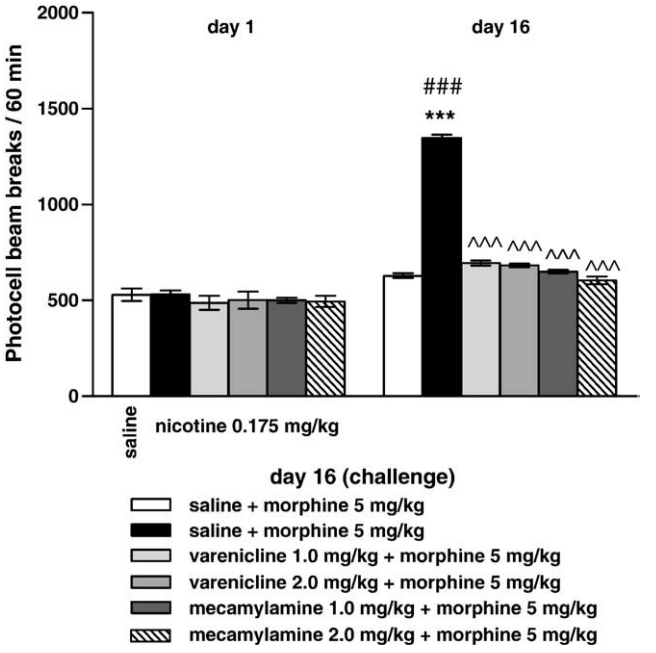

Fig. 4. Effects of varenicline and mecamylamine (1 and 2 mg/kg, i.p.) on the expression of locomotor cross-sensitization between nicotine and morphine in mice. Nicotine (0.175 mg/kg, base, s.c.) or saline were injected daily for 9 days, every other day; on day 16 (a test for expression of sensitization) mice were given morphine (5 mg/kg, s.c.) and varenicline or mecamylamine, 30 min before morphine challenge injection. Data represent means ± S.E.M.; $n = 8$–12 mice per group. ###$p<0.001$ vs. the first pairing day; ***$p<0.001$ vs. saline-pretreated and morphine-challenged mice; ^^^$p<0.001$ vs. nicotine-pretreated and morphine-challenged mice (Tukey's test).

varenicline and mecamylamine, at the doses of 1 and 2 mg/kg, injected before nicotine challenge dose, were effective in blocking the expression of nicotine sensitization ($p<0.001$ vs. nicotine-pretreated and nicotine-challenged mice) (Fig. 3).

3.3. Influence of varenicline and mecamylamine on the expression of cross- sensitization between nicotine and morphine

Two-way ANOVA of the locomotor response after administration of nicotine (0.175 mg/kg, s.c.) or saline during the pairing phase (day 1 and day 16 — challenge) revealed a treatment effect [$F(5,106) = 19.38$, $p<0.0001$], a day effect [$F(1,106) = 89.56$, $p<0.0001$] and an interaction effect [$F(5,106) = 16.7$, $p<0.0001$] (Fig. 4). On the 1st day, one-way ANOVA did not reveal any significant treatment effect [$F(5,53) = 0.09355$, $p = 0.9929$]. On the 16th day, after an additional injection of morphine, one-way ANOVA revealed a significant treatment effect [$F(5,53) = 115.63$, $p<0.0001$]. Indeed, after this additional morphine injection (day 16), a significant difference between the response was observed as compared to the first injection of nicotine ($p<0.001$) or with the response to morphine in animals treated with repeated saline ($p<0.001$, Tukey's test) (Fig. 4). Both varenicline and mecamylamine, at the doses of 1 and 2 mg/kg, injected before morphine challenge dose, were effective in blocking the expression of cross-sensitization between nicotine and morphine to their locomotor effects ($p<0.001$ vs. nicotine-pretreated and morphine-challenged mice) (Fig. 4).

4. Discussion

Given that the neural systems mediating locomotor sensitization overlap those mediating reward (Robinson and Becker, 1986), the present study focused on the evaluation of behavioural sensitization and cross-sensitization induced by nicotine and morphine in mice. Additionally, we investigated and compared the effects of two nAChR ligands, i.e., varenicline, a partial alpha4beta2 agonist and mecamylamine, a non-selective nAChR antagonist, on these behavioural actions of both drugs. The present results indicated 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 locomotor cross-sensitization occurred between nicotine and morphine. Indeed, nicotine-experienced mice showed an enhanced response to morphine injection compared with both the first pairing day and the response to acute morphine challenge in animals pre-exposed to saline. Interestingly, we found that both varenicline and mecamylamine, administered before every nicotine injection or prior to a challenge dose of nicotine and morphine, attenuated the acquisition and expression of nicotine-induced sensitization as well as the expression of cross-sensitization between nicotine and morphine to their locomotor stimulant effects. The ability of both agents to block these effects did not reflect a general suppression of activity because they had no locomotor effect on naive mice. Our findings support the hypothesis that similar neural mechanisms, probably through the alpha4beta2 nAChR subtype, can be involved in the psychomotor effects of nicotine and morphine.

Several reports suggest that sensitization, a phenomenon dependent on the functioning of the mesolimbic dopamine system, plays a crucial role in the reacquisition of drug-seeking behaviour. This animal model reflects the long-lived behavioural abnormalities induced by chronic drug exposure and the changes in synaptic plasticity at the molecular or cellular levels (Robinson and Berridge, 1993). Accordingly, nicotine has been reported to be less effective in activating the mesolimbic dopamine system in drug-naive rats compared with nicotine-experienced animals (Corrigall et al., 1994). Our results which also described a phenomenon of cross-sensitization between nicotine and morphine are in accordance with other animal experimental studies showing interactions between both drugs (Zarrindast et al., 1999; Biala and Weglinska, 2004; Berrendero et al., 2002; Biala and Weglinska, 2006; Vihavainen et al., 2008). The mechanism underlying these interactions is still not well known, but it is suggested that nicotine and morphine share similar neurochemical mechanisms of action in the brain. One possibility could be that nicotine exerts its effect through direct action on the nAChRs,

especially of alpha4beta2 subtype, which can interact with opiate receptor signalling. An interaction between nicotinic receptors and opioid system has been already described, especially activation of endogenous opioid peptides release and biosynthesis in discrete brain nuclei after nicotinic receptor stimulation (Houdi et al., 1991). In preclinical studies, it has been shown that morphine reversed withdrawal signs in nicotine-dependent rats (Malin, 2001), while nicotine abolished naloxone-precipitated opioid withdrawal as well as place aversion induced by naloxone in morphine-dependent rats (Zarrindast and Farzin, 1996; Araki et al., 2004). As nicotinic receptor stimulation induces release of opioid peptides, after prolonged nicotine exposure an upregulation of mu-opioid receptors may cause an opioid-like dependence state (Wewers et al., 1999). In turn, 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 (Tome et al., 2001). It has been largely reported that many drugs of abuse, including nicotine and morphine exert their rewarding effect via the activation of a common neuronal substrate, especially in the mesolimbic dopamine pathways. Nicotine reinforces smoking behaviour by activating nAChRs in the midbrain dopaminergic reward centres, especially in the ventral tegmental area (VTA) (Wonnacott, 1997; Dani et al., 2001). One could argue these drugs can prime responding to one another because they share the property of an activating reward system, which becomes sensitized after repeated drug use. An additive or synergistic effect, especially on the release of accumbal dopamine induced by simultaneous administration of nicotine and morphine, could further contribute to their co-abuse. Thus, the observed in present and our previous experiments cross-over effects between nicotine and morphine may also result from mentioned above mechanism (i.e. release of dopamine in the reward system by the activation of the cholinergic system).

Among nAChRs, alpha4beta2 subtype are heteromeric ligand-gated ion channels high-affinity nicotine binding sites in the brain found on the dopaminergic neurons and on the gamma-aminobutyric acid (GABA)-containing cells. These receptors are thought to play a principal role in the mediation of nicotine addiction as biochemical data demonstrate that their activation indirectly induces dopamine release in the NAC which is strongly associated with nicotine reward and drug-seeking behaviour, as already mentioned (Dani and De Biasi, 2001). This neurochemical effect has been shown to be decreased by nAChR subtype antagonists as well (Rahman et al., 2007). Evidence from gene deletion studies showed that mice lacking beta2 subunits do not discriminate nicotine (Shoaib et al., 2002), and nicotine does not elicit dopamine release in these animals (Grady et al., 2001). Based on these observations, the alpha4beta2 receptor was identified as a potential target for a smoking cessation drug, especially with partial agonists at this receptor subtype (Rollema et al., 2007b). Generally, a partial agonist binds to and activates a receptor, but has only partial efficacy at the receptor compared to a full agonist. In addition, a partial agonist can act as a competitive antagonist by competing with the full agonist for receptor occupancy. Varenicline (Chantix/Champix, Pfizer) was developed to have a high affinity for alpha4beta2 nAChRs in the mesolimbic dopamine system (Coe et al., 2005; Rollema et al., 2007a) and to act as a selective partial agonist of the alpha4beta2 nAChR in effects similar to, but of lesser magnitude than those of nicotine. Consistent with its partial agonist mechanism and accordingly to the dopamine-dependent phenomenon observed in our study, 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, while varenicline pretreatment attenuates nicotine-induced increases in dopamine release and turnover blocking the nicotine response both in vivo and in vitro and limiting craving and withdrawal (Coe et al., 2005). Although, it should be noted that varenicline has effects, at lower affinity, on other high-affinity nAChRs, such alpha7 and alpha3beta4 (Coe et al., 2005; Mihalak et al., 2006). Further research is necessary to determine which of these nicotinic receptors are critically involved in the effects of varenicline on nicotine-induced sensitized response.

It has been well established that long-term exposure to nicotine can modify the function and expression of alpha4beta2 nAChRs through diverse mechanisms, including receptor desensitization, posttranslational modifications and receptor upregulation, all of which could have a role in nicotine addiction (Gentry and Lucas, 2002; Buisson and Bertrand, 2002). For instance, prolonged exposure to low levels of agonist can desensitize nAChRs, resulting in inhibition of nAChR function. Consistent with this, it has been shown that, at low concentrations, (partial) agonists can act as antagonists at alpha4beta2 nAChRs (Rollema et al., 2007a; Paradiso and Steinbach, 2003). This suggestion can be further confirm in our study showing the similar effects of varenicline and mecamylamine, the nAChR antagonist. Mecamylamine was selected because it has been reported to completely inhibit several nicotine effects, i.e., its discriminative stimulus effects (Varvel et al., 1999), and decrease nicotine self-administration in animals (Donny et al., 1999; Watkins et al., 1999). In general, nicotinic receptor antagonists have been found to block nicotine-induced dopaminergic signalling and neuronal excitation, as well as the locomotor effects of nicotine (Nisell et al., 1994; Hamada et al., 2004). Moreover, nAChR antagonists precipitate nicotine withdrawal syndrome and decrease rates of nicotine self-administration (Shoaib et al., 1997; Donny et al., 1999; Watkins et al., 2000). Mecamylamine, a non-competitive and non-selective nAChR antagonist, has been already known as an agent who attenuates tobacco smoking in humans trying to quit.

Concerning morphine, through the mu-opioid receptor activation, this drug is known to excite dopamine neurons in the VTA by the inhibition of the GABA-ergic inhibitory interneurons and thereby increase dopamine transmission to the NAC (Rezayof et al., 2007). Recent studies also revealed that bilateral microinjection of nicotine into the VTA potentiated while blockade of the VTA nAChRs with mecamylamine inhibited morphine-induced CPP, suggesting the involvement of VTA nAChRs in reward-related processes (Rezayof et al., 2007). It is also possible that long-term consumption of morphine and/or nicotine alters the subtype composition of nAChRs toward an increased significance of alpha4beta2 nAChRs also in morphine reinforcement. Dual nicotine/morphine interactions can be strengthened by the data which reveal that extensive overlapping of mu-opioid and nicotinic responsiveness in cortical interneurons (Férézou et al., 2007). The antismoking agent varenicline exhibits properties with respect to its interaction with opioid receptor agonists and nicotine in the brain reward system that may be beneficial for treating patients with opioid dependence with (and possibly also without) concomitant nicotine dependence. Additionally, as in our study, mecamylamine also blocked the expression of cross-sensitization between nicotine and morphine, it can be suggested that this nAChR antagonist may become a potentially effective anti-craving agent for dependence and relapse prevention for not only tobacco smoking but also concomitant morphine use.

A common element in the phenomenon of addiction is polysubstance abuse of several different drugs. Relapse is a major characteristic of drug addiction and could be used to study the neuronal mechanisms underlying drug craving. The present findings, which reveal the development of nicotine locomotor sensitization show analogies with similar phenomenon described in ex-smokers and support the addictive role of nicotine in tobacco smoking. Our results also showed cross-sensitization to the locomotor stimulant effects of nicotine and morphine providing circumstantial evidence for morphine and nicotine interactions. One of the main findings of the present paper was that concurrent administration of nAChR ligands, varenicline and mecamylamine completely prevented the acquisition and expression of nicotine sensitization or cross-sensitization between nicotine and morphine. These data suggest that nicotinic neurotransmission may be a potential target for developing

pharmacotherapeutic strategies to treat and prevent nicotine and/or opioid addiction. As the alpha4beta2-containing nAChR subtypes are particularly relevant targets for medication development, recent attention has been paid to the development of alpha4beta2-directed ligands for the treatment of tobacco dependence (Rollema et al., 2007b). Targeting these receptor subtypes with a partial agonist would provide improved efficacy, since the agonist and antagonist properties of a partial agonist would both relieve craving and withdrawal symptoms in smokers who try to quit, and reduce or eliminate the reinforcing aspects of tobacco (Rose and Levin, 1991; Kelley, 2002; Cohen et al., 2003). An increased understanding of the mechanisms through which varenicline, a drug with good oral bioavailability and predictable pharmacokinetics, facilitates smoking cessation could provide information valuable to the development of medication for nicotine and/or morphine dependence and offers a novel and well-validated pharmacotherapeutic approach.

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