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Monoamine oxidase A rather than monoamine oxidase B inhibition increases nicotine reinforcement in rats

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Abstract

Although nicotine is considered to be responsible for the addictive properties of tobacco, growing evidence underlines the importance of non-nicotine components in smoking reinforcement. It has been shown that tobacco smoke contains monoamine oxidase (MAO) A and B inhibitors and decreases MAO-A and MAO-B activity in smokers. Here, we investigated the effects of clorgyline hydrochloride (irreversible MAO-A inhibitor; 2 mg/kg/day), selegiline (irreversible MAO-B inhibitor; 4 mg/kg) and the beta-carboline norharmane hydrochloride (reversible MAO-B inhibitor; 5 mg/kg/day) treatments on nicotine self-administration (30 μg/kg/infusion, free base) in rats. Independent of the responsiveness to novelty and locomotor activity stimulation, only clorgyline hydrochloride treatment increased the intake of nicotine in a fixed-ratio schedule (FR5) of reinforcement. When a progressive-ratio schedule was implemented, both clorgyline hydrochloride and norharmane hydrochloride treatments potentiated the reinforcing effects of nicotine, whereas selegiline had no effect. Taken together, these results indicate that MAO-A inhibition interacts with nicotine to enhance its rewarding effects in rats and suggest that other compounds present in tobacco, such as beta-carboline, may also play an important role in sustaining smoking behavior in humans.

Introduction

Nicotine (Nic) is commonly believed to be the primary agent motivating tobacco smoking and maintenance of tobacco addiction (Jaffe & Kanzler, 1979; Stolerman & Jarvis, 1995; Pontieri *et al.*, 1996). However, its critical role in the regulation of cigarette smoking remains in question due to the weak reinforcing properties of Nic in rodents (Corrigall & Coen, 1989; Donny *et al.*, 1995; Stolerman & Jarvis, 1995; Manzardo *et al.*, 2002) and the modest success of Nic replacement therapy as a smoking cessation treatment (Balfour & Fagerström, 1996; Pierce & Gilpin, 2002). These findings have led to the hypothesis that other substances present in tobacco smoke may contribute to its reinforcing actions.

Studies of positron emission tomography imaging have reported that tobacco smokers have lower brain monoamine oxidase (MAO) A and B activity compared with non-smokers, which normalizes during abstinence (Berlin *et al.*, 1995; Fowler *et al.*, 1996a,b; Berlin & Anthenelli, 2001). Smokers also exhibit a low MAO activity in platelets (Oreland *et al.*, 1981; Norman *et al.*, 1987) and reduced peripheral MAO-B levels (Fowler *et al.*, 2003). This MAO inhibition is probably due to a direct action of inhaled smoke. Whereas Nic and its metabolite cotinine produce no inhibition of MAO (Yong & Perry, 1986), a recent study has reported that the amount of the two beta-carbolines [norharmane hydrochloride (NOR) and harmane] inhaled in the smoke contributes substantially to the inhibition of the MAO enzyme as observed in positron emission tomography studies in smokers (Poindexter & Carpenter, 1962; Breyer-Pfaff *et al.*, 1996; Rommelspacher *et al.*, 2002).

Monoamine oxidase, a flavin-adenosine-dinucleotide-containing enzyme, appears as two isozymes (MAO-A and MAO-B) distinguished by their differences in substrates and inhibitor selectivities (Johnson, 1968; Kalgutkar *et al.*, 2001). MAO-A is selectively inhibited by clorgyline hydrochloride (CLOR) and preferentially catalyses the oxidation of serotonin and norepinephrine, whereas MAO-B is selectively inhibited by selegiline (SEL) and preferentially catalyses the oxidation of phenylethylamine and benzylamine (Knoll & Magyar, 1972). Both forms of MAO metabolize dopamine (DA) and tyramine (Finberg & Youdim, 1985).

We have recently shown that rats pre-treated with mixed MAO inhibitors self-administered a larger amount of Nic [fixed-ratio (FR)5] and worked more to obtain the drug when tested under a progressive-ratio (PR) schedule of reinforcement, and that these effects were more prominent in rats selected for high responsiveness to novelty compared with those with low responsiveness (Guillem *et al.*, 2005). However, because non-selective monoamine oxidase inhibitors (MAOIs) were used in this study, definitive conclusions regarding the respective role of each MAO subtype in the reinforcing and motivational properties of Nic remain to be investigated.

Thus, the aim of the present study was to examine the potential differential role of MAO-A and MAO-B on Nic reinforcement by determining the effects of chronic CLOR (an irreversible selective MAO-A inhibitor), SEL (an irreversible selective MAO-B inhibitor)

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and NOR (a reversible MAO-B inhibitor contained in smoke) treatments during intravenous Nic self-administration (SA) in a subpopulation of rats selected on the basis of their spontaneous level of locomotor activity in response to novelty exposure.

Materials and methods

Animals

Three hundred and three male Sprague Dawley rats (Iffa-Credo, Lyon, France) weighing 175–200 g at the beginning of the experiment were used. They were housed in groups of four and maintained in rooms at 20–22 °C with a reverse light/dark cycle (light off from 09:00 to 21:00 h). Daily food rations were limited to 20 g delivered after the SA session. Experiments were performed in accordance with the European Communities Council Directives (86/609/EEC, 24 November 1986) and the French Directives concerning the use of laboratory animals (no. 87-848, 19 October 1987).

Drugs

(–)Nicotine hydrogen tartrate, CLOR, R-(–)-deprenyl hydrochloride (SEL) and NOR were purchased from Sigma Aldrich (St Louis, MO, USA) and dissolved in isotonic NaCl (0.9% w/w saline in water). MAOIs (CLOR, an irreversible selective MAO-A inhibitor; SEL, an irreversible selective MAO-B inhibitor and NOR, a reversible MOA-B inhibitor contained in smoke) were administered intraperitoneally (1.0 mL/kg body weight) at the following doses expressed as free base: CLOR, 0.5 (CLOR-0.5), 1, 2 or 4 mg/kg every day; SEL, 2 (SEL-2), 4 or 6 mg/kg every day and NOR, 2.5 (NOR-2.5), 5, 10 or 12.5 mg/kg every day. Control rats received vehicle. Based on determined dose–response relationships on locomotor activity for which no psychostimulant effects had been detected from the MAOIs alone, the doses of MAOIs selected for the Nic SA were 2 mg/kg for CLOR, 4 mg/kg for SEL and 5 mg/kg for NOR. Treatments with MAOIs began the first day of the experiment and occurred 1 h prior to each daily session.

Locomotor activity recording

Apparatus

Locomotor activity was measured in activity cages (41 × 26 × 28 cm) with wire mesh floors and 10-mm Plexiglas side walls (IMETRONIC, Pessac, France). Two infrared photoelectric cells were located 14 cm apart and 3 cm above the floor. The activity cages were kept in a dimly lit room with white noise continuously present. Total motor activity (total number of beam interruptions) was recorded every 10 min during the light cycle for locomotor response to novelty and for acute effects of MAOI or every 24 h (full light/dark cycle) for chronic MAOI treatments.

Locomotor activity following acute MAOI treatments

In order to have a low activity baseline, activity recordings were performed during the light phase. All rats ($n = 82$) were previously habituated to the activity cages. At 3 h following lights on, rats were habituated to the cages for 2 h, subsequently injected with vehicle (1 mL/kg) and activity recorded for 2 h. Rats were then injected with either vehicle ($n = 12$), CLOR (0.5, 1, 2 or 4 mg/kg, $n = 6$ for each group), SEL (2, 4 or 6 mg/kg; $n = 7$, 8 and 6, respectively) or NOR (2.5, 5, 10 or 12.5 mg/kg; $n = 6$, 7, 6 and 6, respectively) and locomotor activity recorded for 3 h.

Locomotor activity following chronic MAOI treatments

The experiment lasted for 14 days. Doses of CLOR, SEL and NOR were chosen according to their inability to modify locomotor activity following acute injection. Twenty-four animals completed the experiment (vehicle, $n = 15$; CLOR-2, $n = 4$; SEL-4, $n = 5$; NOR-5, $n = 5$). Animals were permanently housed in eight individual cages allowing continuous recording of locomotor activity. Temperature, light/dark cycle, and food and water availability were identical to the animal colony housing conditions. Baseline locomotor activity was established during a 3-day habituation period. During the following 8 days rats received once a day vehicle, CLOR-2, SEL-4 or NOR-5 (chronic MAOI phase). Treatments were then interrupted and locomotor activity was recorded for 3 days (withdrawal phase).

Effects of chronic MAOI treatments on nicotine-induced locomotion and behavioral sensitization

The experiment lasted for 6 days. Each day, rats were pre-treated with vehicle, CLOR-2, SEL-4 or NOR-5. At 1 h later they were injected with vehicle or several doses of Nic (Nic-0, Nic-0.2 and Nic-0.4 mg/kg, s.c.) and locomotor activity was immediately recorded for 20 min. The following groups were used: Vehicle-Nic-0, CLOR-2-Nic-0, SEL-4-Nic-0, NOR-5-Nic-0, Vehicle-Nic-0.2, CLOR-2-Nic-0.2, SEL-4-Nic-0.2, NOR-5-Nic-0.2, Vehicle-Nic-0.4, CLOR-2-Nic-0.4, SEL-4-Nic-0.4 and NOR-5-Nic-0.4 ($n = 10$ for each group).

Locomotor response to novelty

Animals were tested in activity cages for 2 h (light phase) and their locomotor responses to this novel environment recorded in a free-drug situation. The novel context consisted of an activity cage (41 × 26 × 28 cm) equipped with two parallel horizontal infrared beams positioned 2 cm above the floor and spaced 13.5 cm apart along the longitudinal axis. Photocell beam interruptions were monitored and recorded via a microcomputer system. The activity cages were kept in a dimly lit room with white noise continuously present. According to their total activity scores in 2 h, rats were then allocated to one of two groups: a group showing locomotor activity scores in the upper third and designated high responders (HRs), and a group showing locomotor scores in the lower third and designated low responders (LRs). Rats in the middle third were discarded. Animals were then assigned to one of the following eight experimental groups: LR-Vehicle ($n = 9$), LR-CLOR ($n = 7$), LR-SEL ($n = 7$), LR-NOR ($n = 7$), HR-Vehicle ($n = 8$), HR-CLOR ($n = 6$), HR-SEL ($n = 8$) and HR-NOR ($n = 7$).

Blood sampling, nicotine and cotinine assays

The purpose of this study was to evaluate the effects of MAOI treatments on Nic clearance and its accumulation following repeated intravenous injections of Nic (30 μg/kg/injection, free base) at 12-min intervals to mimic Nic SA. Rats were pre-treated for 5 days with vehicle ($n = 4$), CLOR (2 mg/kg/day, i.p., $n = 5$) or NOR (5 mg/kg/day, i.p., $n = 4$). On the fifth day, two catheters were implanted, one into the external jugular vein and one into the femoral vein for Nic injections and blood sampling. Rats then received MAOI

treatments followed 60 min later by intravenous injections of Nic. Collection of blood samples (200 μL) was performed at 3, 7, 11 and 15 min post-Nic injections. Second, third, fourth and fifth Nic injections were performed at 20, 32, 44 and 56 min following the first injection, and blood was sampled at 31, 43, 55 and 67 min. Following dichloromethane extraction, Nic and cotinine levels were determined using liquid chromatography-mass spectrometry, as previously described (Guillem *et al.*, 2005).

Nicotine self-administration

Surgery

Fifty-nine animals were deeply anesthetized with chloral hydrate (350 mg/kg, i.p.; J-T Baker, the Netherlands) and an indwelling catheter (Silastic tubing, 0.012 inch i.d., 0.025 inch o.d.; Dow Corning Corporation, Midland, MI, USA) was surgically implanted into the external jugular vein. The catheter was secured to the vein with surgical silk sutures and passed subcutaneously to the top of the back where it exited into a connector (modified 22-gauge cannula). After surgery, animals were flushed daily with 0.2 mL of an ampicillin solution (0.1 g/mL; Totapen, ConvaTec, Paris, France) containing heparin (300 IU/mL) to maintain patency.

Apparatus

Each experimental chamber (30 × 40 × 37 cm, Imetronic) was equipped with two nose-poke operanda. During drug SA sessions, the animals' catheters were connected by spring-covered Tygon tubing through a fluid swivel to a 10-mL syringe pump (Razel, Bioblock Scientific, France) located outside the chamber. Pokes in one hole, defined as the active hole, delivered intravenous Nic infusions (30 μg/kg/infusion, free base) in a volume of 100 μL over 3 s. Pokes in the other hole, defined as the inactive hole, had no scheduled consequence. Each infusion was paired with a 3-s cue light located above the active hole and followed by a 20-s time out period during which responding was recorded but not reinforced.

Procedure

Experimental sessions started at the beginning of the dark cycle on Day 6 of recovery from surgery. Acquisition of Nic SA was established on a FR schedule of reinforcement (Days 1–10, FR1; Days 11–13, FR2; Days 14–23, FR5) in daily 2-h sessions. Following completion of the FR phase, animals were switched to a PR (Days 24–28) schedule of reinforcement under which the number of nose pokes required to obtain each successive infusion was increased according to the following sequence: 1, 3, 6, 10, 15, 20, 25, 32, 40, 50, etc. (Depoortere *et al.*, 1993). The PR sessions lasted for a maximum of 10 h or until 1 h elapsed without a drug infusion. The last ratio attained (breaking point) was recorded. All of the above subgroups (LR-Vehicle, LR-CLOR, LR-SEL, LR-NOR, HR-Vehicle, HR-CLOR, HR-SEL and HR-NOR) went through the entire SA procedure (FR1, FR2, FR5 and PR, Days 1–28).

Data analyses

Locomotor activity data following acute and chronic MAOI treatments were subjected to ANOVAs with group as the between subject factor and time or day as the within subject factors. Analyses of Nic SA data were performed using ANOVA with treatment (vehicle, CLOR, SEL and NOR) and novelty (HR–LR) as between-subject factors, and days and hole as within-subject factors. For the FR study, only the last 3 days were analysed because they best characterized stable responding at a particular phase and were less susceptible to the transitional instability produced by changing the FR schedule. For responding (hole visits), treatment and novelty were between-subject factors, and hole (active–inactive) was the within-subject factor. For analyses of Nic infusions, treatment and novelty were between-subject factors. For the PR study, treatment and novelty were between-subject factors for the final ratio attained. Whenever main factors or interaction effects were found, post-hoc comparisons were performed using the Newman Keuls test. $P > 0.05$ was considered as not statistically significant (NS).

Results

Locomotor activity following acute MAOI treatments

Levels of locomotor activity recorded following vehicle injections were identical between groups. After MAOI treatments (Fig. 1), ANOVA indicated main effects of group ($F_{11,70} = 2.6$, $P < 0.01$) and time ($F_{5,350} = 53.1$, $P < 0.001$) but no group by time interaction ($F_{1,68} = 1.03$, NS). When compared with the vehicle group, only SEL at the dose of 6 mg/kg increased locomotor activity (Vehicle vs. SEL-6, $P < 0.05$).

Locomotor activity following chronic MAOI treatments

Locomotor activity baselines were not significantly different between groups ($F_{3,25} = 1.4$, NS). When compared with vehicle-treated rats, levels of locomotor activity in CLOR-2, SEL-4 and NOR-5 animals were not significantly different during MAOI treatment (8 days, $F_{3,25} = 0.10$, NS) or during the withdrawal phase ($F_{3,25} = 0.84$, NS) (Fig. 2).

Effects of chronic MAOI treatments on nicotine-induced locomotion and behavioral sensitization

The three-way ANOVA revealed a significant effect of Nic ($F_{2,108} = 71.2$, $P < 0.001$) but no effect of treatments ($F_{3,108} = 0.5$, NS) and no Nic by treatment interaction ($F_{6,108} = 2.2$, NS), indicating that MAOI treatments did not modify the psychostimulant effects of

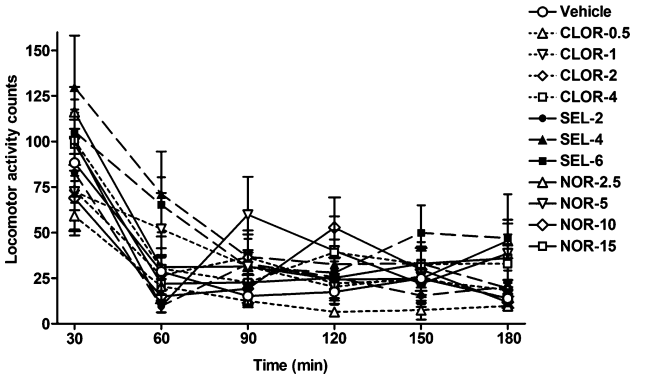

FIG. 1. Time-course representation of the dose–response effects of acute MAOI treatments on spontaneous locomotor activity [vehicle, clorgyline hydrochloride (CLOR), selegiline (SEL) and norharmane hydrochloride (NOR) pre-treatment]. Animals were injected with vehicle ($n = 12$), CLOR (0.5, 1, 2 or 4 mg/kg; $n = 6$ for each group), SEL (2, 4 or 6 mg/kg; $n = 7$, 8 and 6, respectively) or NOR (2.5, 5, 10, or 12.5 mg/kg; $n = 6$, 7, 6 and 6, respectively). Locomotor activity (mean photocell interruptions ± SEM) was recorded for 3 h.

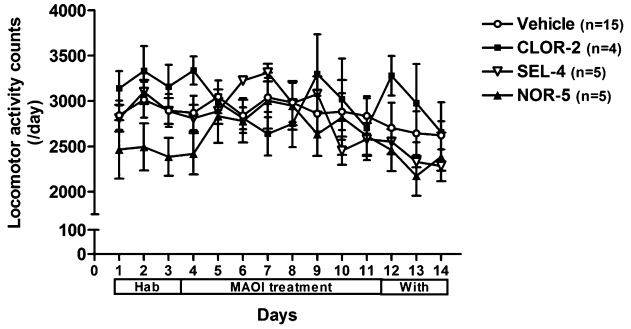

FIG. 2. Effects of vehicle (white circles), clorgyline hydrochloride (CLOR) (black squares, 2 mg/kg/day), selegiline (SEL) (white downward triangles, 4 mg/kg/day) and norharmane hydrochloride (NOR) (black upward triangles, 5 mg/kg/day) on daily locomotor activity on each of the 14 days of testing. After 3 days of habituation (Hab), animals received MAOI treatments once a day for 8 days (MAOI treatment). Treatment was then interrupted and locomotor activity was recorded for 3 days (With). Locomotor activity (mean photocell interruptions ± SEM) was recorded for 24 h.

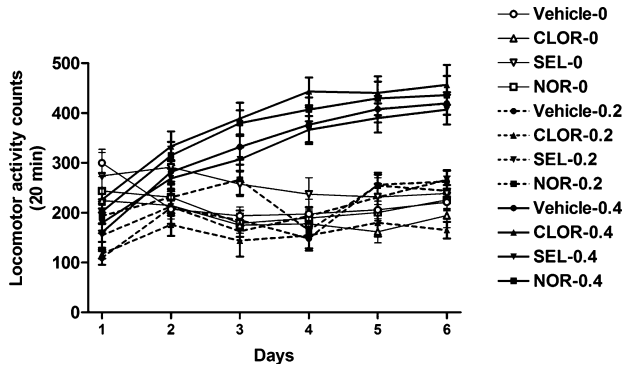

FIG. 3. Effects of vehicle, clorgyline hydrochloride (CLOR) (2 mg/kg/day), selegiline (SEL) (4 mg/kg/day) and norharmane hydrochloride (NOR) (5 mg/kg/day) on behavioral sensitization to nicotine. Once a day, for 6 days, rats received MAOI treatments followed 60 min later by an injection of nicotine (0, 0.2 or 0.4 mg/kg, s.c.). Locomotor activity (mean photocell interruptions ± SEM) was recorded for 20 min.

Nic (Fig. 3). Moreover, there was a significant Nic by day interaction ($F_{10,540} = 37.3$, $P < 0.001$) but no treatment by day interaction ($F_{15,540} = 0.9$, NS) and no Nic by treatment by day interaction ($F_{30,540} = 1.2$, NS), indicating that MAOI treatments did not affect Nic-induced locomotor activation or the development of Nic sensitization.

Effects of MAOI treatments on nicotine metabolism

As indicated in Fig. 4, MAOIs did not modify either clearance of Nic or its accumulation following repeated Nic injections (one injection every 12 min). Overall three-factor ANOVA indicated no group effect ($F_{2,10} = 2.1$, NS), a Nic/cotinine difference ($F_{1,10} = 41.7$, $P < 0.001$), a time-course effect ($F_{7.70} = 42.5$, $P < 0.001$) but no group by Nic/cotinine by time interaction ($F_{14,70} = 0.41$, NS). Concerning the clearance observed following the first injection, Nic decreased monotonically (Fig. 4a), whereas cotinine increased (Fig. 4b). Following the fifth injection of Nic, there was no significant difference between groups in terms of either Nic (Vehicle, 79.5 ± 11.5 ng/mL; CLOR, 70.6 ± 12.6 ng/mL; NOR, 94.5 ± 17.9 ng/mL; $F_{2,10} = 1.49$, NS) (Fig. 4a) or cotinine (Vehicle,

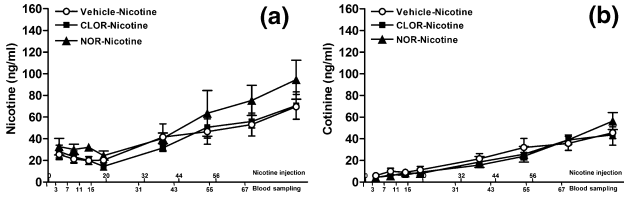

FIG. 4. Plasma nicotine (a) and cotinine (b) (ng/mL) following repeated intravenous injections of nicotine (30 μg/kg/injection, free base) to mimic nicotine self-administration. Rats were pre-treated for 5 days with vehicle (circles, $n = 4$), clorgyline hydrochloride (CLOR) (squares, 2 mg/kg/day, $n = 5$), or norharmane hydrochloride (NOR) (triangles, 5 mg/kg/day, $n = 5$). On the fifth day rats received MAOI treatment followed 60 min later by intravenous injections of nicotine.

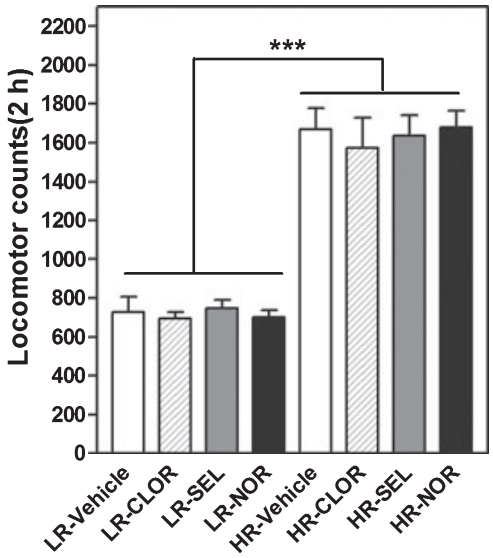

FIG. 5. Evaluation of locomotor reactivity to novelty of the rats which will be used in nicotine self-administration. Reactivity to novelty was assessed prior to any pharmacological treatment [vehicle, clorgyline hydrochloride (CLOR), selegiline (SEL) and norharmane hydrochloride (NOR)] by measuring locomotor activity for 2 h in a novel environment (mean photocell interruptions ± SEM). Low-responder (LR) and high-responder (HR) rats corresponded, respectively, to the lower third and higher third scores of the subject sample. The middle score subjects were discarded from the study. LR-Vehicle ($n = 9$), LR-CLOR ($n = 7$), LR-SEL ($n = 7$), LR-NOR ($n = 7$), HR-Vehicle ($n = 8$), HR-CLOR ($n = 6$), HR-SEL ($n = 8$) and HR-NOR ($n = 7$). ***$P < 0.001$, significant difference between groups as revealed by Newman-Keuls post-hoc test.

45.6 ± 5.4 ng/mL; CLOR, 43.6 ± 9.6 ng/mL; NOR, 56.3 ± 7.9 ng/mL; $F_{2,10} = 0.75$, NS) (Fig. 4b).

Response to novelty

All animals were first screened for their locomotor responses to a novel environment in a free-drug situation (Fig. 5). On the basis of their mean locomotor responses over 120 min, two main groups, LR (708 ± 30 beam interruptions; $n = 30$) and HR (1647 ± 65 beam interruptions; $n = 29$), were designated as LRs and HRs. Each of these main LR and HR groups were then subdivided into four subgroups with equal activity scores. These subgroups were then tested in the Nic SA paradigm with different pharmacological treatments (vehicle, CLOR, SEL and NOR). These subgroups were designated as follows: LR-Vehicle ($n = 9$), LR-CLOR ($n = 7$), LR-

SEL ($n = 7$), LR-NOR ($n = 7$) ($F_{2,29} = 0.21$, NS) and HR-Vehicle ($n = 8$), HR-CLOR ($n = 6$), HR-SEL ($n = 8$), HR-NOR ($n = 7$) ($F_{2,28} = 0.15$, NS).

Effects of MAOI treatments on nicotine self-administration on a fixed-ratio schedule of reinforcement

In the first experiment, animals were tested for acquisition of Nic SA (Fig. 6). In vehicle-treated animals, although both groups demonstrated a preference for the active hole (LR rats, $F_{1,8} = 8.25$, $P < 0.05$; HR rats, $F_{1,7} = 10.38$, $P < 0.01$), HR animals performed better than LR animals. Only HR rats showed evidence for a progressive acquisition of Nic SA, indicated by the increasing number of active vs. inactive responses over the 23 days of testing (LR rats, $F_{22,176} = 1.50$, NS; HR rats, $F_{22,154} = 1.63$, $P < 0.05$), and this increase in responding was specific to the active hole (active hole, $F_{22,154} = 1.8$, $P < 0.05$; inactive hole, $F_{22,154} = 0.9$, NS). However, no significant difference between LR and HR vehicle-treated rats in the number of infusions obtained was detected at any time during this period (novelty by days, $F_{22,330} = 0.99$, NS). Concerning MAOI treatments, the ANOVA revealed a significant main effect of treatment ($F_{3,51} = 3.88$, $P < 0.05$) as well as significant day by treatment ($F_{66,1122} = 2.44$, $P < 0.001$) and day by hole by treatment ($F_{66,1122} = 1.41$, $P < 0.05$) interactions.

Further analysis revealed that MAOI treatments had no effect on Nic responding on either FR1 ($F_{3,51} = 1.49$, NS) or FR2 ($F_{3,51} = 1.81$, NS), indicating that the primary reinforcing properties of Nic are unchanged by MAOIs in both LR and HR animals. In contrast, under an FR5 schedule, CLOR treatment increased responding for Nic, whereas SEL and NOR had no effect ($F_{3,51} = 4.61$, $P < 0.01$; CLOR vs. Vehicle, $P < 0.01$; SEL vs. Vehicle, NS; NOR vs. Vehicle, NS).

The analysis of the mean of the last 3 days of FR5 (representing stable Nic SA) (Fig. 7a) revealed a significant effect of CLOR treatment ($F_{3,51} = 3.72$, $P < 0.05$; CLOR vs. Vehicle, $P < 0.01$; SEL vs. Vehicle, NS; NOR vs. Vehicle, NS) as well as a significant hole by treatment interaction ($F_{3,51} = 2.87$, $P < 0.05$). When active and inactive hole visits were analysed separately, it appeared that CLOR-increased responding was specific to the active hole ($F_{3,51} = 3.2$, $P < 0.05$; CLOR vs. Vehicle, $P < 0.05$; SEL vs. Vehicle, NS; NOR vs. Vehicle, NS), whereas SEL specifically decreased responding in the inactive hole ($F_{3,51} = 5.7$, $P < 0.01$; CLOR vs. Vehicle, NS; SEL vs. Vehicle, $P < 0.01$; NOR vs. Vehicle, NS). However, there was no effect of novelty ($F_{1,51} = 0.5$, NS) and no treatment by novelty interaction ($F_{3,51} = 0.4$, NS), indicating that this treatment produced similar effects in both LR and HR rats.

As a consequence, only animals treated with CLOR showed a higher rate of Nic infusions than vehicle-treated rats ($F_{3,51} = 5.5$, $P < 0.01$; CLOR vs. Vehicle, $P < 0.01$; SEL vs. Vehicle, NS; NOR vs. Vehicle, NS) (Fig. 7b). Moreover, there was no effect of novelty ($F_{1,51} = 0.5$, NS) and no significant treatment by novelty interaction ($F_{3,51} = 0.45$, NS), indicating that CLOR increased Nic infusions similarly in LR and HR rats.

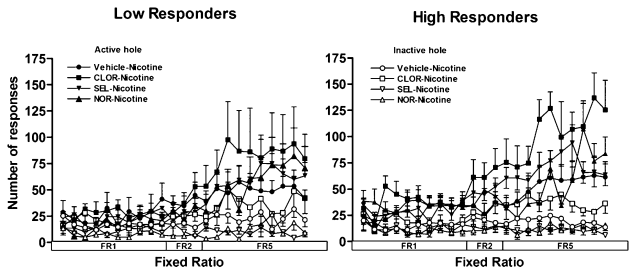

FIG. 6. Effects of vehicle (circles), clorgyline hydrochloride (CLOR) (squares, 2 mg/kg/day), selegiline (SEL) (downward triangles, 4 mg/kg/day) and norharmane hydrochloride (NOR) (upward triangles, 5 mg/kg/day) pre-treatment on acquisition of nicotine self-administration (SA) in low-responder (LR) and high-responder (HR) animals on each of the 23 days of testing under fixed-ratio (FR) schedules of reinforcement (FR1, FR2 and FR5). Data are shown as mean responding (± SEM) on the active (filled symbols) and inactive (open symbols) holes.

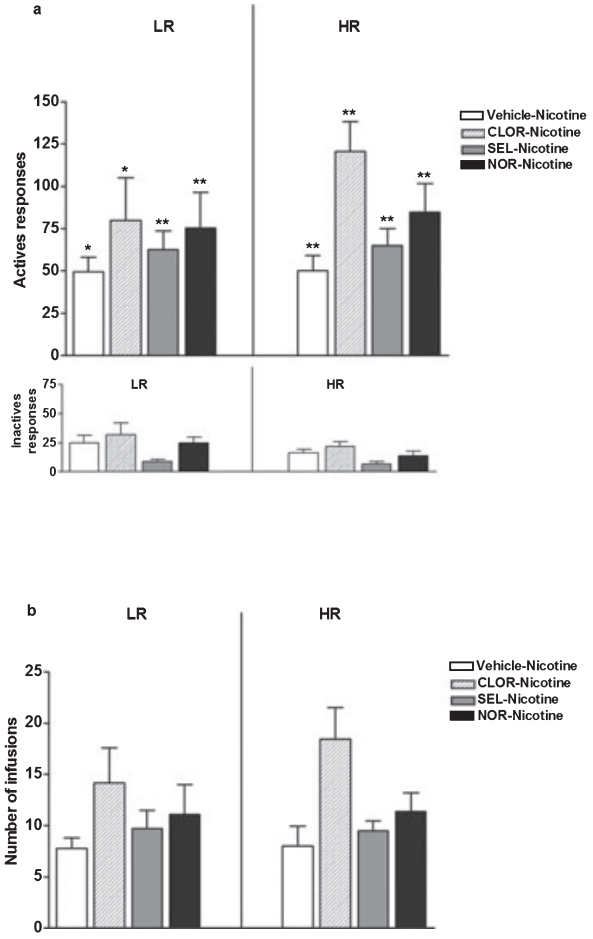

FIG. 7. Effects of vehicle, clorgyline hydrochloride (CLOR) (2 mg/kg/day), selegiline (SEL) (4 mg/kg/day) and norharmane hydrochloride (NOR) (5 mg/kg/day) pre-treatment on stable nicotine self-administration under the fixed-ratio (FR)5 schedule of reinforcement in low-responder (LR) and high-responder (HR) animals. Mean scores (± SEM) during the last 3 days of the FR5. Each self-administration session lasted 2 h. (a) Effects of MAOI pre-treatment (Vehicle, CLOR, SEL and NOR pretreatment, respectively, white, black, grey and hatched bars) on the number of responses on the active (a, upper panel) and inactive (a, lower panel) hole. *$P < 0.05$, **$P < 0.01$, significant difference in response rates between the two holes in the same group of rats. (b) Effects of MAOI pre-treatment on the number of nicotine infusions (30 μg/kg/infusion).

Effects of MAOI treatments on nicotine self-administration on a progressive-ratio schedule of reinforcement

To further test the motivational significance of an interaction between MAOIs and Nic, the behavior of the animals was studied in a more demanding task, i.e. a PR schedule of reinforcement (Fig. 8). Under PR schedules, the number of responses required to earn the next

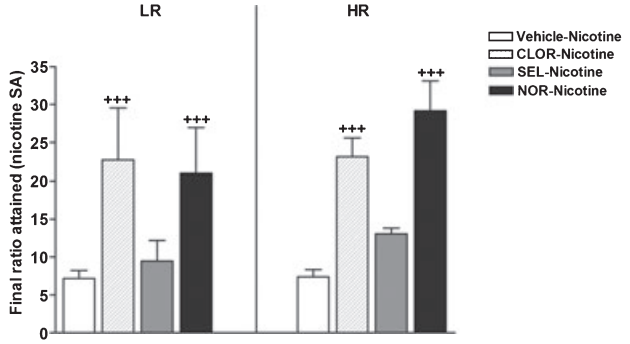

FIG. 8. Effects of vehicle (white bars), clorgyline hydrochloride (CLOR) (2 mg/kg/day, black bars), selegiline (SEL) (4 mg/kg/day, grey bars) and norharmane hydrochloride (NOR) (5 mg/kg/day, hatched bars) pre-treatment under the progressive-ratio (PR) schedule of reinforcement on nicotine self-administration in low-responder (LR) and high-responder (HR) animals. Values represent the mean number of nose-poke responses (± SEM) for nicotine self-administration (SA), corresponding to the final ratio attained (breaking point) during the 5 days of the PR schedule of reinforcement. $^{+++}P < 0.001$, significant difference between each subgroup as revealed by the Newman Keuls post-hoc test.

infusion increases with a constant factor and the measure of the final ratio attained (breaking point) allows assessment of the amount of effort that an animal is willing to expend to obtain the reinforcer. In this task, all rats treated either with CLOR or NOR showed an increased level of motivation to obtain Nic compared with vehicle-treated rats, whereas no effect was found in SEL-treated animals ($F_{3,49} = 12.82$, $P < 0.001$; CLOR vs. Vehicle, $P < 0.001$; SEL vs. Vehicle, NS; NOR vs. Vehicle, $P < 0.001$). However, there was no effect of novelty ($F_{1,49} = 1.59$, NS) and no significant treatment by novelty interaction ($F_{3,49} = 0.57$, NS), indicating that CLOR and NOR treatments produced similar increases in both LR and HR rats.

Discussion

The present results confirm our previous findings that MAO inhibition potentiated the reinforcing properties of Nic. Independent of their reactivity to novelty, rats pre-treated with either CLOR or NOR showed an increased level of motivation to self-administer Nic under a PR schedule of reinforcement, whereas SEL had no effect. Furthermore, under the FR schedule (FR5), only CLOR-treated rats self-administered a higher amount of Nic.

The specificity of these results was supported by the finding that these MAOI treatments were devoid of psychostimulant effects, did not modify the development of behavioral sensitization to Nic and did not increase responding in the inactive hole. Therefore, the effects of MAOI reflect the heightened incentive motivational properties of Nic rather than a general stimulatory effect on operant behavior.

Clorgyline hydrochloride and SEL are irreversible inactivators of MAO and have been used extensively due to their selectivity for MAO-A and MAO-B, respectively (Johnson, 1968; Knoll & Magyar, 1972). The drug doses used in our study are consistent with previous investigations on the MAO-inhibiting effects of these drugs. Indeed, chronic treatment with CLOR (2 mg/kg/day) affords complete inhibition of MAO-A in rats (MAO-A, 90%; MAO-B, 10%) (Fagervall & Ross, 1986; Todd & Baker, 1995) and is sufficient to irreversibly inhibit MAO-A for more than 24 h (Lamensdorf *et al.*, 1996). Moreover, non-selective inhibition of MAO-B activity was not observed at doses less than 10 mg/kg (Felner & Waldmeier, 1979). Chronic treatment with selective low doses of SEL (0.5–5 mg/kg in rats) results in the almost complete inhibition (90%) of MAO-B activity (Paterson *et al.*, 1991; Shimazu *et al.*, 2005). Increasing the dose of SEL to 10 mg/kg did not induce additional inhibition of MAO-B but rather induced about 50% non-selective inhibition of MAO-A activity in rat brains (Waldemeier *et al.*, 1981; Paterson *et al.*, 1991). Thus, the doses of CLOR and SEL used in our study were specific for MAO-A and MAO-B, respectively.

The results showed that CLOR-treated animals demonstrated a higher Nic intake during FR schedules of reinforcement and worked more than vehicle rats to obtain the drug under a PR schedule, indicating that this treatment increased the reinforcing efficacy as well as the motivational effects of Nic. In contrast to CLOR, SEL treatment had no effect on either Nic intake or performance under a PR schedule, suggesting that MAO-A inhibition rather than MAO-B inhibition might be involved in the reinforcing effects of Nic SA in rats. Consistent with this hypothesis, it has been proposed that MAO-B inhibition is not crucial for the addictive potential of Nic (Stolerman & Shoaib, 1991). SEL alone does not appear to have addiction potential and neither does it increase the reinforcing potencies of cocaine or methamphetamine in monkeys (Goldberg *et al.*, 1994; Winger *et al.*, 1994). Moreover, it has been shown that daily treatment with 6.4 mg/kg/day of SEL did not modify SA of food under a PR schedule in rats (Grasing & He, 2005).

The present result indicating that a CLOR pre-treatment increases responding for Nic under an FR5 schedule, whereas SEL does not, is consistent with recent findings that MAO-B knockout mice have similar amounts of oral Nic intake to wild-type mice (Lee *et al.*, 2004). Furthermore, it has been shown that chronic treatment with low doses of CLOR (3 mg/kg/day) did not modify SA of ethanol in rats (Cohen *et al.*, 1999), suggesting that the CLOR effects were specific to Nic.

This result is also consistent with previous findings indicating that serotonin and DA play a critical role in the control of Nic SA (Balfour *et al.*, 1986; Corrigall & Coen, 1989; Corrigall, 1992; Olausson *et al.*, 2002). However, because both DA and serotonin are metabolized by the MAO-A form in the rat brain, the present results do not allow an evaluation of the relative contribution of these neurotransmitters in the regulation of Nic intake.

The results obtained with NOR showed that this treatment had no significant effect on Nic intake during FR schedules of reinforcement. Nevertheless, both HR and LR NOR-treated animals worked more than vehicle rats to obtain the drug when tested under a PR schedule. It has been shown that NOR plasma levels increased following acute smoking (Breyer-Pfaff *et al.*, 1996), and that NOR readily crossed the blood–brain barrier and was accumulated in the brain (partition factor ~3, Fekkes & Bode, 1993). Moreover, NOR, which is present in tobacco smoke in remarkably high concentrations (12.6 μg NOR/g tobacco; Poindexter & Carpenter, 1962), has been shown to preferentially inhibit MAO-B in a reversible way ($K_i = 730$ nM in rat brain tissue) (May *et al.*, 1991).

It has been suggested that only an irreversible blockade of both MAO-A and MAO-B initiates locomotor response in mice (Villegier *et al.*, 2005). Thus, it is possible that the difference observed in Nic intake between CLOR and NOR treatments was independent of their role on MAO-A or MAO-B but was rather linked to their irreversible or reversible properties. However, SEL, which is an irreversible MAO-B inhibitor, had no effect on Nic intake under the FR schedule or on motivation in the PR schedule. Thus, the difference in the present study between CLOR and NOR treatments may not account for the irreversible or reversible properties. One reason for this discrepancy between the two studies could be that MAO activity differs between rats and mice. Indeed, MAOs are less efficient in rats than in mice, thus allowing Nic effects on extracellular levels of

monoamine to be prolonged (Tassin *et al.*, 1992; Vezina *et al.*, 1992; Di Chiara, 2000).

An explanation for the differences observed between NOR and SEL treatments could be the relative non-selective inhibition of NOR. Indeed, although it has been shown to preferentially inhibit MOA-B one cannot exclude that, at the dose used, NOR affected Nic SA by blocking both MAO-A and MAO-B (May *et al.*, 1991; Herraiz & Chaparro, 2005, 2006).

Another explanation resides in the fact that NOR treatment possesses other pharmacological properties in addition to reversible MAO-B inhibition. Indeed, a recent study has demonstrated that a single dose of NOR decreased cocaine SA in a U-shaped manner with the dose of 10 mg/kg having the most potent effect (Cappendijk *et al.*, 2001), suggesting that several receptor mechanisms mediate the effects of NOR. NOR is also a monoamine reuptake blocker and it has been shown that monoamine uptake blockers with prominent effects on either DA or serotonin neurotransmission can decrease cocaine SA in monkeys (Kleven & Woolverton, 1993) and rats (Tella, 1995).

Moreover, several β-carbolines, including NOR, have been shown to bind in the low micromolar range to benzodiazepine receptors acting as inverse agonists (Müller *et al.*, 1981). Thus, it is possible that the present dose of NOR used could also increase the efflux of DA by a benzodiazepine receptor-mediating mechanism rather than by the MAO-B inhibition. Indeed, it has been shown that a high dose of SEL (10 mg/kg, i.p.), a specific MAOI-B, does not change the 3-methoxytyramine concentration in the striatum or those of 3,4-dihydrophenylacetic acid, homovanillic acid and 5-hydroxyindoleacetic acid (Kato *et al.*, 1986). However, the low affinity of NOR for benzodiazepine receptors makes it unlikely that this mechanism plays a role.

Norharmane hydrochloride has also been shown to potently displace [^{3}H]-2-(2-benzofuranyl)-2-imidazoline binding to I_2 sites in rat brain, whereas SEL did not (Hudson *et al.*, 1999; Husbands *et al.*, 2001; MacInnes & Handley, 2002; Miralles *et al.*, 2005). Imidazoline receptor ligands and β-carbolines share the ability to interact with the opioid system in the central nervous system (Garcia-Sevilla *et al.*, 1999; Robinson *et al.*, 2003; Miralles *et al.*, 2005). Moreover, several lines of evidence suggest that opioid receptors may play an important role in Nic dependence (Carboni *et al.*, 2000; Watkins *et al.*, 2000; Walters *et al.*, 2005; Galeote *et al.*, 2006; Zhang *et al.*, 2006). Thus, another possible mechanism that should be considered includes the capacity of NOR to interact with opioid systems.

Evidence is accumulating that Nic reinforcement is particularly dependent on non-pharmacological stimuli such as conditioned stimuli (Goldberg *et al.*, 1981; Caggiula *et al.*, 2001, 2002; Donny *et al.*, 2003). It has been suggested that the primary role of increased DA release in the nucleus accumbens is to facilitate the strengthening of stimulus–reward (incentive learning) and stimulus–response (habit learning) associations (Di Chiara, 1998) or the attribution of positive incentive salience to previously neutral cues associated with reward (Berridge & Robinson, 1998). More recently, several studies from Balfour and collaborators have hypothesized the role of extrasynaptic DA in these effects (Balfour *et al.*, 2000; Balfour, 2002). Thus, it is possible that MAO treatments, by increasing the extracellular levels of DA in the brain, potentate the attribution of positive incentive salience to conditioned cues.

Most of the Nic is metabolized to its inactive metabolite cotinine (Benowitz *et al.*, 1994) by the genetically variable enzyme CYP2A6 (Nakajima *et al.*, 1996; Messina *et al.*, 1997) and MAOIs have recently been described in *in vitro* experiments as potential inhibitors of CYP2A6 (Kuhn-Velten, 1993; Zhang *et al.*, 2001). Thus, it is possible that MAOI increased the motivational properties of Nic through its interaction with and inhibition of Nic metabolism. However, our results indicate that there was no difference in the pharmacokinetics of Nic and cotinine under either CLOR or NOR treatment. Thus, these findings exclude that the observed effects of these MAOIs are elicited by Nic metabolism inhibition.

We have previously shown that the effects of mixed MAO inhibitors were more prominent in rats selected for high responsiveness to novelty (HR) than in those with low responsiveness to novelty (LR) (Guillem *et al.*, 2005). However, in the present work, pretreatments with selective MAOI did not differentially affect HR and LR animals. An explanation of this discrepancy could reside in the smaller potentiation of the reinforcing and motivational properties of Nic with selective MAO inhibitors than mixed MAO inhibitors. Indeed, rats pre-treated with selective MAO inhibitors consumed less Nic and reached a smaller breaking point than those treated with mixed MAO inhibitors. Thus, it seems that selective inhibition of either MAO-A or MAO-B activity is much less efficient at enhancing the reinforcing and motivational properties of Nic, and thus discriminating between HR and LR rats.

A large number of studies have shown that MAOI increases the levels of three principal monoamines (DA, NA and serotonin) in the rat brain (Waldmeier & Baumann, 1983; Kumagae *et al.*, 1991; Curet *et al.*, 1996). Moreover, all three of these monoamines have been implicated at different times in Nic reinforcement (Mitchell, 1993; Lucas & Spampinato, 2000; Seth *et al.*, 2002) and this may contribute to the differences observed between propargylamine inhibitors and NOR.

At this point, it is important to mention that the activities and distributions of MAO isoforms in the central nervous system show regional and species-specific differences (Saura *et al.*, 1992; Fowler *et al.*, 2001). Studies of MAO in rodents are characterized by differences in the distribution, abundance and substrate binding affinity of each MAO isoform, relative to those seen in humans (Weyler *et al.*, 1990; Krueger *et al.*, 1995; Inoue *et al.*, 1999). In humans, MAO-A preferentially oxidizes serotonin and norepinephrine, whereas MAO-B oxidizes DA, phenylamine and benzylamine (Lan *et al.*, 1989; Gerlach *et al.*, 1996; Shih *et al.*, 1999). However, in rodents, MAO-A oxidizes DA, serotonin and norepinephrine, whereas MAO-B oxidizes phenylethylamine and benzylamine (Johnson, 1968; Garrett & Soares-da-Silva, 1990; Cases *et al.*, 1995; Grimsby *et al.*, 1997). Moreover, it has recently been shown that the conformation of the active loop of rat MAO-A is unlike that found in human MAO-A but is the same as the homologous loop in human MAO-B (De Colibus *et al.*, 2005). Therefore, it seems reasonable to assume that in humans MAO-B has the same functions as MAO-A in rats on the metabolism of monoamines. Because smoking behavior has been shown to be correlated to MAO-B inhibition (Rose *et al.*, 2001), the finding that CLOR treatment potentiates Nic SA in rats suggests the potential use of MAO-B inhibitors in smoking cessation treatment.

Taken together, these findings reveal a greater role for MAO-A than MAO-B inhibition in the reinforcing effects of Nic in rats. Moreover, these results suggest that other compounds present in tobacco such as NOR, which can act on other neurotransmitter systems, may also play an important role in the reinforcing properties of smoking. Animal models that more fully reflect the pharmacological profile of tobacco smoke should be helpful for developing more effective treatments for smoking cessation.

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Abbreviations

CLOR, clorgyline hydrochloride; DA, dopamine; FR, fixed-ratio; HR, high responder; LR, low responder; MAO, monoamine oxidase; MAOI, monoamine oxidase inhibitor; Nic, nicotine; NOR, norharmane hydrochloride; NS, not statistically significant; PR, progressive-ratio; SA, self-administration; SEL, selegiline.

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