Monoamine Oxidase Inhibition Dramatically Prolongs the Duration of Nicotine Withdrawal-Induced Place Aversion

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Background: Long-lasting effects of withdrawal from nicotine are hypothesized to contribute to relapse and persistence of tobacco habits, and significant evidence supports a role of monoamine oxidase inhibitors (MAOI) contained in cigarette smoke as potent modulators of the rewarding effects of tobacco.

Methods: With quantification of somatic signs of withdrawal and the place aversion conditioning paradigm, we assessed the effects of MAOI pretreatment on both somatic and aversive motivational components of mecamylamine-induced nicotine withdrawal in rats rendered dependent on nicotine by the subcutaneous implantation of osmotic minipumps (vehicle or nicotine tartrate 9 mg/kg/day).

Results: In nicotine-infused rats, mecamylamine induced a place aversion that lasted 6 weeks. When nicotine-infused rats were also treated with a MAOI, mecamylamine-induced conditioned place aversion persisted for at least 8 months of abstinence. The MAOI treatment slightly decreased ratings of somatic signs induced by mecamylamine administration but had no effect on the threshold or the magnitude of mecamylamine-induced conditioned place aversion.

Conclusions: These results show that MAOI pretreatment induces a long-lasting conditioned placed aversion associated with nicotine withdrawal, possibly through a potentiation of learning and memory process, and provides some indications on protracted abstinence that might be useful for delineating the neurobiological substrate of relapse.

Key Words: Conditioned place aversion, nicotine, rats, phenelzine somatic withdrawal, tranylcypromine

It is widely accepted that a majority of habitual tobacco smokers become dependent upon nicotine present in tobacco smoke and that this accounts for the problems many smokers experience when they try to quit (1,2). Although it is clear that the primary reinforcing effects of the drug trigger the initiation of drug consumption, once dependence is initiated the negative consequences of drug abstinence might motivate the continued administration of drug to prevent the appearance of a withdrawal syndrome (3,4).

Nicotine withdrawal might be evoked after cessation of chronic nicotine exposure (5–8) and is characterized by both somatic and affective negative symptoms. Although the somatic symptoms of nicotine withdrawal might contribute to smoking behavior, it has been hypothesized that affective signs are of greater motivational significance in contributing to relapse and continued use (9,10). Clinical studies indicate that the negative affective states experienced during drug withdrawal can become associated with previously neutral environmental stimuli and that these conditioned stimuli gain motivational significance in the maintenance of drug use and relapse during periods of abstinence (11–13).

In rodent models of nicotine withdrawal, somatic signs such as abdominal constrictions, facial fasciculation, and ptosis can be observed (6–8). The aversive state associated with withdrawal can be modeled with the conditioned place aversion paradigm. Indeed, it has been shown that precipitated nicotine withdrawal in rats, with the nicotinic receptor antagonist mecamylamine, produces a place aversion to a previously neutral environment paired with precipitated nicotine withdrawal (14,15). Nevertheless, remarkably little is known about the duration of the motivational aspects of nicotine withdrawal.

Tobacco smoke is known to contain a number of compounds among which monoamine oxidase inhibitors (MAOIs) have been the focus of special interest (16–18). Current smokers have lower brain MAO A and B activity, which normalizes during prolonged abstinence (19–22). Moreover, we have recently demonstrated that MAOI treatment increases the motivation to self-administer nicotine in rats and might thus contribute to the development of tobacco addiction (23). The purpose of the present study was to determine the effects of chronic MAOI treatment on both the intensity of the somatic signs and the aversive motivational state associated with precipitated nicotine withdrawal in rats and the duration of conditioned place aversion, once established, with repeated measures of conditioned place aversion during nicotine abstinence. We report that MAOI treatment dramatically prolonged the aversive state associated with nicotine withdrawal but had little effect on the somatic signs of nicotine withdrawal.

Methods and Materials

Animals

A total of 258 male Sprague Dawley rats (Charles River, Lyon, France) weighing 101–125 g at the beginning of the experiment were used. Animals were housed in groups of four and maintained in rooms at 20°–22°C with a reverse light/dark cycle (lights off from 7:00 AM to 7:00 PM). Animals had ad libitum access to food and water throughout the experiment. Experiments were performed in accordance with the declaration of Helsinki, the European Communities Council Directives (86/609/EEC, Novem-

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ber 24, 1986) and the French Directives concerning the use of laboratory animals (décret n° 87-848, October 19, 1987).

Drugs

(-)Nicotine hydrogen tartrate, mecamylamine hydrochloride, and the two mixed irreversible MAO inhibitors (MAOI-A/B) tranylcypromine hydrochloride and phenelzine sulfate were purchased from Sigma Aldrich (St. Louis, Missouri) and were dissolved in isotonic sodium chloride (.9% w/w saline in water). Treatments with MAOI began the 1st day of pump implantation and were administered twice a day (9:00 AM and 7:00 PM) intraperitoneally (1 mL/kg body weight) at the following doses expressed as free base: tranylcypromine 1.5 mg/kg/day, and phenelzine 2 mg/kg/day. Control rats received vehicle. Mecamylamine (2 or 4 mg/kg; [15]) was injected subcutaneously. Doses of MAOIs were chosen on the basis of previously determined dose–response relationships on locomotor activity for which no psychostimulant effects had been detected (23). The MAOI treatments began the 1st day of pump implantation and occurred every day until the end of the conditioning phase in the place aversion experience or at the end of the somatic evaluation of nicotine withdrawal.

Induction of Nicotine Dependence

Osmotic minipumps (Alzet, model 2 ML2 [14 days]; Alza Corporation, Palo Alto, California) filled with either saline (n = 63) or nicotine tartrate dissolved in saline (n = 195) were implanted subcutaneously under halothane oxygen mixture (1%–3% halothane) anesthesia. The concentration of nicotine was adjusted to compensate for differences in body weight to deliver a dose of 9 mg/kg/day (3.16 mg/kg/day, free base) for 14 days.

Conditioned Place Aversion

The apparatus and place aversion paradigm used to produce a reliable conditioned place aversion have been described in detail elsewhere (24). Briefly, the apparatus consisted of three rectangular boxes (40 × 33 × 34 cm, 120° to each other), distinguished by distinctive visual and tactile cues and accessible from a triangular central compartment. Treatments with MAOI began the 1st day of pump implantation and occurred twice a day (9:00 AM and 7:00 PM) until the end of the conditioning phase.

In the preconditioning phase (day 3 after implantation), animals were allowed to freely explore the apparatus for 20 min. For each rat, the two compartments with the most similar time allotments were chosen. One side was randomly chosen to be paired with mecamylamine (D0) and the other side with vehicle (S0). The third compartment was not paired with any injection (N0). After compartment assignment there were no differences between the time spent in the drug- and saline-paired compartments during the preconditioning phase (D0 vs. S0). This procedure eliminated possible bias before conditioning.

The conditioning phase consisted of five pairings over 5 consecutive days (days 4, 5, 6, 7, and 8 after implantation). In the morning, before being confined to their preselected saline-paired compartment for 20 min, rats received an injection of saline. In the afternoon, rats received mecamylamine immediately before being confined to the preselected mecamylamine-paired compartment for 20 min.

The testing phase consisted of a 20-min free exploration of the entire apparatus, without any MAOI treatment. The first test was conducted 24 hours after conditioning (day 10 after implantation, test 1 [D1, S1, N1]). The same day, minipumps were removed in all rats. Tests for place aversion conditioning were then conducted every 2 weeks, at days 14–224 after conditioning ([D14, S14, N14] to [D224, S224, N224]). The difference (D-D0) in time spent in the mecamylamine-paired compartment during the testing phase (D) and the preconditioning phase (D0) served as a measure of place aversion.

Ratings of Somatic Signs of Withdrawal

Forty-eight naive rats were implanted with minipumps filled with either saline (n = 12) or nicotine tartrate dissolved in saline (n = 36). Treatments with MAOI began the 1st day of pump implantation and occurred twice a day (9:00 AM and 7:00 PM) until the end of the experiment. Animals were habituated to the observation glass box (31 × 29 × 40 cm) in which the rat could move freely for 15 min for 3 consecutive days.

On days 4–10 after implantation, the effects of various doses of mecamylamine on somatic signs were examined. Mecamylamine doses (0, .29, .57, 1.14, 1.72, 2.29 and 3.43 mg/kg SC [15]) were presented according to a within-subjects Latin square design (repeated measures). Five minutes after the antagonist injection, each animal was placed in the observation glass box and filmed for 10 min with a video recording device. The frequency and time of occurrence of the following signs were recorded: body shakes, chews, cheek tremors, foot licks, gasps, genital licks, head shakes, scratches, teeth chattering, writhes, and yawns (6). The categories of "abdominal constrictions" included gasps and writhes; "facial fasciculation" included cheek tremors, chews, and teeth chattering; and "miscellaneous other signs" included shakes, escape attempts, licks, scratches, and yawns (15). If present continuously, ptosis was only counted once/minute. The total number of somatic signs/10-min observation period was defined as the sum of individual occurrences of the aforementioned withdrawal signs.

Data Analyses

Induction of place aversion conditioning, evaluating 1 day after the end of the conditioning phase, was analyzed with a four-way analysis of variance (ANOVA) with place aversion (comparison between the time spent in the antagonist-paired compartment after vs. before conditioning; D vs. D0) as a within factor and nicotine treatment (nicotine-infused or vehicle-infused), MAOI treatments (vehicle, tranylcypromine, or phenelzine), and mecamylamine doses (2 or 4 mg/kg) as between-subjects factors, followed by Newman-Keuls post hoc tests when necessary. Long-term place aversion conditioning was analyzed with a four-way ANOVA with nicotine treatment, MAOI treatments as between-subjects factors, and place aversion and repeated tests (17 levels) as within factors, followed by Newman-Keuls post hoc tests when necessary.

Somatic withdrawal data were analyzed with a three-way repeated measures ANOVA with nicotine treatment, MAOI treatment and mecamylamine doses (6 levels) as between-subjects factors, followed by Newman-Keuls post hoc tests when necessary.

Results

Induction of Place Aversion Conditioning

The induction of place aversion conditioning was evaluated 1 day after the end of the conditioning phase (Table 1). Overall ANOVA indicated a significant effect of place aversion [F (1,183) = 50.8, $p < .001$], nicotine treatment [F(1,183) = 9.7, $p < .01$], and mecamylamine treatment [F (1,183) = 15.9, $p < .001$] and a significant place aversion × mecamylamine interac-