Reboxetine: Attenuation of Intravenous Nicotine Self-Administration in Rats

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ABSTRACT

The ability of reboxetine, a selective inhibitor of the norepinephrine transporter and noncompetitive antagonist at neuronal nicotinic receptors, to alter nicotine self-administration in rats was compared with that of mecamylamine, a classical noncompetitive antagonist at nicotinic receptors. The ability of reboxetine to alter sucrose-maintained responding was also examined to assess the specificity of the effect on nicotine self-administration. Rats were trained on a fixed ratio 5 schedule to self-administer nicotine (0.02 mg/kg/infusion i.v.) or to respond for sucrose pellets. Upon reaching a stable baseline, rats were pretreated 15 min before the session with vehicle, reboxetine (racemic), (+)-(S,S)-reboxetine (0.3–30 mg/kg s.c.) or mecamylamine (0.5–4 mg/kg s.c.). To assess the effect of repeated administration, reboxetine (5.6 mg/kg) was injected once daily for 14 consecutive sessions before either nicotine self-administration or sucrose-maintained responding. Specificity was further assessed by examining the ability of repeated administration of reboxetine (5.6 mg/kg) to alter nicotine-induced hyperactivity (0.8 mg/kg). Reboxetine, (+)-(S,S)-reboxetine, and mecamylamine dose dependently decreased nicotine self-administration by ~60%, whereas reboxetine and (+)-(S,S)-reboxetine decreased sucrose-maintained responding to a lesser extent (~20%). Repeated administration of reboxetine (5.6 mg/kg) decreased nicotine self-administration and sucrose-maintained responding across the 14 sessions, suggesting that tolerance did not develop to these effects of reboxetine. Additionally, reboxetine did not alter baseline locomotor activity, indicating that the decrease in operant responding for nicotine and sucrose was not the result of a nonspecific decrease in activity. The reboxetine-induced decrease in nicotine self-administration and sucrose-maintained responding may be the result of inhibition of norepinephrine transporters and/or neuronal nicotinic receptor function.

Clinical and epidemiological evidence has linked smoking and depression. The incidence of major depression is higher among smokers than nonsmokers (Glassman, 1993; Kendler et al., 1993; Breslau et al., 1998). The likelihood of successful smoking cessation decreases in those individuals with a history of major depression or depressive symptoms at the initiation of smoking cessation (Hall et al., 1991; Glassman, 1993). Furthermore, depressive symptoms associated with nicotine withdrawal are more severe in depressed patients compared with patients without histories or symptoms of depression (Glassman, 1993). The onset of smoking cessation may initiate an acute depressive episode in certain individuals (Stage et al., 1996; Covey et al., 1997). Furthermore, nicotine has been reported to have antidepressant properties in depressed individuals (Glassman, 1993; Salin-Pascual and Drucker-Colin, 1998) and in animal models of depression (Tizabi et al., 1999). Such evidence has led to the self-medication hypothesis of nicotine dependence, such that individuals may use tobacco, at least in part, to ameliorate depression or depressive symptoms (Markou et al., 1998).

The self-medication hypothesis of nicotine dependence is further strengthened by the observation that the antidepressant, bupropion, serves as an efficacious smoking cessation pharmacotherapy (Ferry et al., 1992; Hurt et al., 1997; Jorenby et al., 1999). The pharmacological mechanism of action by which bupropion reduces smoking has not been elucidated but has been attributed to inhibition of both dopamine and norepinephrine transporters (Ferris et al., 1983; Shiffman et al., 2000). However, bupropion has been reported recently to also act as a noncompetitive antagonist at nicotinic receptors (nAChRs), and in this respect, nAChR antagonism may contribute to the therapeutic efficacy of bupropion as a smoking cessation agent (Slemmer et al., 2000; Miller et al., 2002a).

Since bupropion acts as an inhibitor of dopamine and norepinephrine transporters as well as nAChRs, it was of interest to assess the potential utility of reboxetine as a smoking cessation agent. Reboxetine is an efficacious and well toler-
ated antidepressant in clinical use in Europe (Berzewski et al., 1997), and its therapeutic efficacy has been attributed to selective inhibition of the norepinephrine transporter (Montgomery, 1999; Sacchetti et al., 1999; Wong et al., 2000; Miller et al., 2002b). However, recent studies have shown that reboxetine, like other antidepressants, including bupropion (Fryer and Lukas, 1999; Hennings et al., 1999; Slemmer et al., 2000; Miller et al., 2002a), acts as a noncompetitive inhibitor of nAChRs (Miller et al., 2002b).

The purpose of the present series of experiments was to determine the ability of reboxetine to alter i.v. nicotine self-administration in rats. Reboxetine has been shown to produce a behavioral profile characteristic of an antidepressant using several animal models (Harkin et al., 1999). Consistent with these preclinical studies, several double-blind, placebo-controlled studies have found reboxetine to be a clinically efficacious antidepressant with efficacy comparable to that of desipramine, fluoxetine, and imipramine (for review, see Montgomery, 1999). In the present study, the ability of reboxetine and (+)-(S,S)-reboxetine to alter nicotine self-administration was determined. Since reboxetine acts as a noncompetitive inhibitor of nAChRs (Miller et al., 2002b), the ability of reboxetine to alter nicotine self-administration was compared with that of the classic non-competitive nAChR antagonist, mecamylamine (experiment 1). To assess the specificity of the effects of reboxetine on nicotine self-administration, the ability of reboxetine (racemic) and (+)-(S,S)-reboxetine to alter sucrose-maintained responding also was determined (experiment 2). Tolerance to the effects of reboxetine on nicotine self-administration and sucrose-maintained responding was evaluated after 14 daily injections of reboxetine 15 min before either nicotine self-administration sessions (experiment 3) or sucrose-maintained responding sessions (experiment 4). In experiment 5, the specificity of the effects of reboxetine on nicotine self-administration and sucrose-maintained responding was assessed by determining the ability of reboxetine to alter nicotine-induced hyperactivity.

Drugs
S-(+-)Nicotine ditartrate was purchased from Sigma/RBI (Natick, MA) and is referred to throughout as nicotine. The nicotine solution for i.v. self-administration was prepared in 0.9% physiological saline, adjusted to pH 7 by addition of sodium hydroxide (5 M) solution. Nicotine was administered i.v. in a volume of 60 μl for the self-administration experiments. In the locomotor activity experiment, nicotine was given s.c. in a volume of 1 ml/kg body weight. Reboxetine (a racemic mixture of R,R- and S,S-(2-[α(2-ethoxyphenoxy) benzyl]-morpholine sulfate)) and (+)-(S,S)-reboxetine methanesulfonate were provided by Pharmacia Corp. (Kalamazoo, MI). The latter drugs were prepared in phosphate-buffered saline and administered s.c. in a volume of 1 ml/kg body weight. Mecamylamine HCl (Sigma/RBI) was prepared in 0.9% physiological saline and administered s.c. in a volume of 1 ml/kg body weight. Nicotine doses administered i.v. or s.c. are expressed as the free base; doses of reboxetine and mecamylamine are expressed as the salt weight. Morphine HCl (15 mg/kg salt weight) was prepared in saline and administered i.v. in a volume of 1 ml/kg body weight.

Apparatus
For the nicotine self-administration and sucrose-maintained responding experiments, standard rat operant conditioning chambers (ENV-001; MED Associates, St. Albans, VT) were used. The side-walls of the chamber were aluminum, and the front and back walls were clear Plexiglas. The floor consisted of 18 stainless steel rods, with newspaper under the floor. A recessed food tray (5 × 4.2 cm) was located at the bottom-center of one of the sidewalls of the chamber. Response levers were located on either side of the recessed food tray. Responses made on the active lever were reinforced, and responses made on the other, inactive lever had no scheduled consequence (i.e., were not reinforced). A 28-V cue light was located 6 cm above each lever. Completion of the fixed ratio requirement resulted in the simultaneous activation of the cue light and either the infusion pump or the pellet dispenser. All stimulus and response events were controlled and recorded by a computer.

For the locomotor activity experiment, a custom-made wooden chamber (30 × 28 × 43 cm high) was used. The interior walls of the chamber were painted white and contained a wire-mesh floor. Pine wood-chip bedding (P. J. Murphy Forest Products, Montville, NJ) was placed in a tray beneath the floor. Two photo beams, located 4 cm above the base of the floor, divided the chamber into four equal quadrants. Each break of a photo beam was scored as an activity count and recorded by a computer located in a control room adjacent to the test room. A speaker located in the test room provided an ambient white noise (70 dB).

General Procedure
Surgery. Rats were anesthetized by an i.p. injection of ketamine (80 mg/kg) and diazepam (5 mg/kg). A silastic catheter was inserted into the jugular vein with the free end of the catheter exiting through the skin and secured to an acrylic head mount, which was attached to an infusion pump prior to self-administration sessions.

Nicotine Self-Administration. Nicotine self-administration procedures were based on published methods (Corrigall and Coen, 1989). Before commencement of behavioral testing, rats were deprived to 85% of their free-feeding body weight. Rats were trained to respond for sucrose pellets (45 mg; P. J. Nues, Inc., Lancaster, NH). Following lever-press training, rats were placed on a fixed ratio 1 schedule of reinforcement for one 15-min session. If rats received 20 pellets, the schedule was increased to a fixed ratio 2 for 1 session, and subsequently to a fixed ratio 5 for 3 sessions. After the third lever-press training session on a fixed ratio 5 schedule, rats were allowed free access to food, and surgery was performed approximately 7 days later as described previously. Following recovery from surgery, rats were reintroduced to the operant conditioning chamber. However, responses on a fixed ratio 1 schedule resulted in i.v.
infusions of nicotine (0.02 mg/kg/infusion). At the beginning of each infusion, the cue lights were illuminated for 20 s to signal a time-out period, during which responses were recorded but not reinforced. The rats remained on the fixed ratio 1 schedule for approximately 7 days. During the next 14 to 21 days, the schedule of reinforcement was increased gradually to a fixed ratio 5. Before administration of a pretreatment drug, the following criteria for stable responding on the fixed ratio 5 schedule were required: 1) at least five infusions per session, 2) less than 20% variability across two consecutive sessions, and 3) a 2:1 (active/inactive lever) response ratio. The nicotine self-administration session duration was 60 min. Upon completion of the experiment, catheter patency was verified by observing a rapid cataleptic response following an i.v. infusion of morphine (15 mg/kg).

Sucrose-Maintained Responding. The procedures in these experiments were similar to those in the nicotine self-administration experiments, except that rats did not undergo surgery and the session duration was 15 min. Rats were trained to lever press for sucrose pellets until they reached the fixed ratio 5 criteria for stable responding. The criteria for drug pretreatment were similar to those for the nicotine self-administration experiments (see above), except that the rats were required to earn at least 10 pellets for two consecutive sessions.

Experimental Procedures

Experiment 1: Effect of Acute Pretreatment with Reboxetine, (+)-SS-Reboxetine, or Mecamylamine on Nicotine Self-Administration. Groups of rats (n = 7–8/group) were pretreated with either vehicle, reboxetine, (+)-SS-reboxetine, or mecamylamine 15 to 20 min before placement in the operant conditioning chamber for nicotine self-administration. Doses of reboxetine and (+)-SS-reboxetine administered were 0.3, 1, 3, 5.6, 10, or 17 mg/kg. Doses of mecamylamine administered were 0.5, 1, 2, or 4 mg/kg. Drug doses were administered according to a Latin-square design, with each rat being randomly assigned to a different order of dose presentation. Furthermore, different groups of rats were used to assess each pretreatment drug. Following each session in which rats were pretreated with drug, two maintenance sessions occurred in which rats were allowed to self-administer nicotine in the absence of pretreatment. These intervening maintenance sessions allowed for elimination of the pretreatment drug and recovery of operant baseline responding.

Experiment 2: Effect of Acute Pretreatment with Reboxetine or (+)-SS-Reboxetine on Sucrose-Maintained Responding. Groups of rats (n = 6/group) were pretreated with either vehicle, reboxetine, or (+)-SS-reboxetine 15 min before placement in the operant conditioning chamber for sucrose pellet reinforcement. Doses of reboxetine and (+)-SS-reboxetine were 1, 3, 5.6, 10, 17, or 30 mg/kg. Doses of drug were administered according to a Latin-square design.

Experiment 3: Effect of Repeated Pretreatment with Reboxetine on Nicotine Self-Administration. Since experiments 1 and 2 revealed no significant differences between the effect of reboxetine and (+)-SS-reboxetine, only the effect of reboxetine was determined in the subsequent experiments. For 14 consecutive sessions, groups of rats were injected with either reboxetine (5.6 mg/kg, n = 8) or vehicle (n = 6) 15 min before the daily nicotine self-administration session. The dose of reboxetine was chosen based on the results of experiment 1, which demonstrated that the 5.6 mg/kg dose decreased nicotine self-administration by ~50%. To determine whether responding for nicotine returned to baseline levels 24 h after termination of reboxetine pretreatment, both groups of rats were injected with vehicle prior to the nicotine self-administration on session 15.

Experiment 4: Effect of Repeated Pretreatment with Reboxetine on Sucrose-Maintained Responding. For 14 consecutive sessions, groups of rats (n = 7/group) were pretreated with either reboxetine (5.6 mg/kg) or vehicle 15 min before the daily sucrose-maintained responding session. Subsequently, all rats received vehicle pretreatment on session 15.

Experiment 5: Effect of Repeated Pretreatment with Reboxetine and/or Nicotine on Locomotor Activity. Since reboxetine decreased responding for nicotine and sucrose, the ability of reboxetine to inhibit nicotine-induced hyperactivity also was assessed. Rats were assigned to one of four treatment groups (n = 3–4/group) that were injected with either reboxetine (5.6 mg/kg) or vehicle 15 min before the session and subsequently with nicotine (0.8 mg/kg) or saline immediately before the session. At the beginning of the experiment, each rat was injected with saline and placed in the locomotor chamber for 60 min on two occasions to acclimate them to the procedure and reduce novelty-induced exploration of the chamber. Subsequently, rats received their respective drug pretreatments prior to 14 consecutive, daily 60-min sessions (sessions 1–14). On session 15, rats previously administered reboxetine were injected with vehicle; otherwise, treatment proceeded as on sessions 1 to 14. Results from session 15 determined whether reboxetine attenuated the development of nicotine-induced sensitization. On session 16, all rats were injected with vehicle followed by saline, and then were placed in the activity chamber. Results from session 16 determined whether context conditioning to nicotine occurred and whether conditioning was attenuated by reboxetine.

Data Analysis

For the dose-response experiments (experiments 1 and 2), a two-way mixed-design analysis of variance (ANOVA) was conducted on the data, expressed as percentage change scores, in which reboxetine form ([+] versus (+)-SS) was a between-groups factor and reboxetine dose was a within-subjects factor. To make direct comparisons between reboxetine forms, percentage change scores were used to control for differences in baseline rates of responding between groups (see Results). In experiment 1, a separate one-way ANOVA was conducted on the mecamylamine data expressed as percentage change scores. The percentage change scores were calculated according to the following equation: baseline number of responses – number of responses on a drug-pretreatment session/baseline number of responses × 100. The baseline number of responses was defined as the average number of responses of the two maintenance sessions preceding a drug pretreatment session. For the chronic pretreatment experiments (experiments 3 and 4), a two-way mixed-design ANOVA was conducted on the number of nicotine infusions with treatment group (reboxetine versus vehicle) as a between-subjects factor and session as a within-subject factor. A two-way between-groups design ANOVA was conducted on the locomotor activity counts emitted following the first injection (reboxetine or vehicle) and following the second injection (nicotine or saline) (experiment 5). Contrasts of interest were analyzed by correlated and independent t tests for within-group and between-group comparisons, respectively. Comparisons were considered significant at α < 0.05 (two-tailed).

Results

Effect of Acute Pretreatment with Reboxetine, (+)-SS-Reboxetine, or Mecamylamine on Nicotine Self-Administration (Experiment 1). To determine whether responding during the maintenance sessions was stable across the course of the experiment, a two-way ANOVA was performed on the average number of responses during the two maintenance sessions prior to each drug pretreatment session. A significant main effect of reboxetine form [F(1,13) = 5.1, p < 0.05] was obtained, indicating that average baseline responding was higher for the group tested with reboxetine (101 ± 7 responses; mean ± S.E.M.) compared with the group administered (+)-SS-reboxetine (78 ± 6.5). Importantly, the main effect of dose and the reboxetine form × dose interaction were not significant, demonstrating that within-group baseline responding was stable over the course of the experiment. When collapsed across
groups, the overall mean baseline number of responses on the active lever was $88 \pm 2.0$ responses during the 60-min session, which is consistent with response rates previously reported for nicotine self-administration during 60-min sessions (Corrigall and Coen, 1989).

To compare directly the effects of reboxetine on nicotine self-administration (60-min session) and sucrose-maintained responding (15-min session), an analysis of responding during the first 15 min of the nicotine self-administration session was performed. When collapsed across reboxetine form and dose, the overall baseline rate of responding on the active lever on a fixed ratio 5 schedule was $34 \pm 0.6$ (mean $\pm$ S.E.M.) responses during the first 15 min of the session. Both forms of reboxetine were found to dose dependently decrease nicotine self-administration [main effect of dose $F_{(6,78)} = 13.1, p < 0.001$], but the two reboxetine forms did not differ in this respect (i.e., neither a significant main effect of reboxetine form nor reboxetine form $\times$ dose interaction; Fig. 1, top panel). Moreover, all doses of reboxetine (collapsed across reboxetine form) decreased nicotine self-administration compared with vehicle pretreatment. The maximal effect for reboxetine to decrease responding for nicotine was $\sim 60\%$ relative to vehicle control. Individual variability was noted in the acute dose-response experiment. The group data were representative of a majority of the rats; however, two of the eight rats in the group showed little or no decrease in nicotine self-administration until doses of 10 or 17 mg/kg reboxetine were administered. With respect to the group data, similar results were obtained regardless of whether the responses were analyzed across the first 15 min of the session or across the entire 60-min session; however, the maximal decrease produced by reboxetine across the 60-min session was only $\sim 40\%$ compared with control (data not shown).

A two-way ANOVA followed by correlated $t$ tests conducted on the baseline number of responses emitted on the inactive lever ($\sim 8$ responses in a 60-min session) revealed that none of the doses of reboxetine (collapsed across reboxetine forms) decreased responding significantly on the inactive lever (data not shown), indicating that reboxetine specifically decreased operant responding for nicotine.

With respect to the group administered mecamylamine, the overall baseline rate of fixed ratio 5 responding on the active lever was $91 \pm 2.4$ (mean $\pm$ S.E.M.) responses during the 60-min session and $33 \pm 0.7$ (mean $\pm$ S.E.M.) responses during the first 15 min of the session. Baseline responding was stable over the course of the experiment, as demonstrated by a nonsignificant effect of dose $F_{(4,28)} = 0.68, p > 0.05$. A one-way ANOVA conducted on responding for nicotine following administration of mecamylamine revealed a significant main effect of dose $F_{(4,28)} = 5.1, p < 0.01$. The highest dose of mecamylamine tested ($4$ mg/kg) produced an $\sim 65\%$ decrease in nicotine self-administration relative to control during the first 15 min of the session (Fig. 1, bottom panel). When the data were analyzed across the entire 60-min session, the $4$ mg/kg dose of mecamylamine decreased nicotine self-administration by $\sim 60\%$ (data not shown).

The number of baseline responses emitted on the inactive lever was $\sim 6$ responses during the 60-min session. One-way ANOVA revealed that none of the mecamylamine doses decreased responding on the inactive lever (data not shown), indicating that mecamylamine specifically decreased operant responding for nicotine.
Similar to the results from the self-administration experiment, analysis of sucrose-maintained responding by two-way ANOVA revealed that responses on the inactive lever were not altered after administration of either reboxetine form, and the interaction of reboxetine form × dose also was not significant. These results suggest that doses of reboxetine specifically decreased operant responding for sucrose. However, since the baseline number of responses on the inactive lever was extremely low (<1 response in a 15-min session) compared with the baseline number of responses on the active lever (~480 responses in a 15-min session), it may be that reboxetine failed to alter responding on the inactive lever due to a floor effect.

**Effect of Repeated Pretreatment with Reboxetine on Nicotine Self-Administration (Experiment 3).** A reboxetine dose of 5.6 mg/kg was found to decrease responding for nicotine by ~50% relative to control, and this dose did not alter responding on the inactive lever (experiment 1). As such, this reboxetine dose was chosen to examine the ability of repeated reboxetine (given once daily for 14 sessions) to alter nicotine self-administration (Fig. 3). Analysis of the data from the first 15 min of the session was similar to that for the entire 60-min session; however, as previously observed, the reboxetine-induced decrease in nicotine self-administration was less robust when the entire session was included in the analysis. Furthermore, the data from the first 15 min of the session were analyzed to compare the results from the self-administration experiments with those in experiments determining its effect on sucrose-maintained responding.

Two-way ANOVA revealed a significant main effect of treatment group [$F_{(1,12)} = 7.2, p < 0.05$]; however, neither the main effect of session nor the group × session interaction was significant. Point-by-point comparisons (i.e., group comparisons at each treatment session) revealed that reboxetine (5.6 mg/kg) decreased responding for nicotine during 8 of the 14 pretreatment sessions. Moreover, when the vehicle was injected on session 15, the number of nicotine infusions earned by rats previously pretreated with reboxetine was not different from the control group, indicating that reboxetine administration was required to maintain the decrease in responding for nicotine. Within-subject comparisons across sessions in the reboxetine pretreatment group revealed no differences in the number of infusions earned. A comparison of the number of infusions across sessions in the control group also revealed no significant differences.

Similar to the acute reboxetine dose-response experiment, individual variability was noted following repeated reboxetine administration (data not shown). Three rats in the group showed a reboxetine-induced decrease in nicotine self-administration for 14 consecutive pretreatments, suggesting that tolerance did not develop. Two rats showed an initial reboxetine-induced decrease in nicotine self-administration on session 1, but tolerance occurred across subsequent sessions. Two additional rats showed no decrease in responding for nicotine on sessions 1 to 3 following reboxetine pretreatment but showed a dramatic decrease in responding on subsequent sessions. Another rat in the group showed a decrease in responding for nicotine during the first session but increased responding across sessions 2 to 13. Collectively, these results demonstrate that tolerance to reboxetine did not develop reliably across repeated administrations, although a considerable amount of variability was apparent among individual rats.

**The Effect of Repeated Pretreatment with Reboxetine on Sucrose-Maintained Responding (Experiment 4).** The effect of repeated administration of reboxetine (5.6 mg/kg)
mg/kg) on sucrose-maintained responding was determined also across 14 daily sessions. Two-way ANOVA revealed a significant main effect of session \( F_{(15,180)} = 11.0, p < 0.001 \) and group \( F_{(1,12)} = 7.2, p < 0.05 \), and a significant group \( \times \) session interaction \( F_{(15,180)} = 2.9, p < 0.001 \). Follow-up between-group point-by-point comparisons revealed an initial ~30% decrease in responding relative to the control group on session 1 (Fig. 4). This decrease in responding was maintained across the 14 sessions, except for sessions 3 to 6 (Fig. 4). When the vehicle was injected on session 15, the number of sucrose pellets earned by rats previously pretreated with reboxetine was not different from that of the control group, indicating that reboxetine was required to maintain the decrease in responding for sucrose. It should be noted, however, that responding for sucrose did not differ on days 14 and 15 for the reboxetine pretreatment group, suggesting some carryover effect of reboxetine on sucrose-maintained responding. Furthermore, the lack of difference between the control and reboxetine groups on session 15 was due partially to an unexpected decrease in sucrose-maintained responding. This apparent increase in responding in the reboxetine pretreatment group does not indicate tolerance to the effect of reboxetine, because the same relative increase in responding was observed in the control group. Thus, both between-group and within-subject analyses indicate that tolerance did not develop to the reboxetine-induced decrease in sucrose-maintained responding.

**Effect of Repeated Pretreatment with Reboxetine and/or Nicotine on Locomotor Activity (Experiment 5).** Because reboxetine (5.6 mg/kg) decreased responding when given acutely, and the decrease was maintained following repeated administration for both nicotine (experiment 3) and sucrose (experiment 4) reinforcement, it may be that reboxetine nonselectively decreased operant responding by producing general sedation or motor impairment. Also, it was of interest to determine whether reboxetine attenuated nicotine-induced hyperactivity. To determine whether reboxetine (5.6 mg/kg) altered activity, it was administered once daily for 14 sessions 15 min before placement in the activity chambers, and nicotine or saline was administered immediately before each session. A three-way ANOVA conducted on the activity data revealed that rats pretreated with nicotine increased locomotor activity relative to saline-pretreated rats across sessions \( F_{(13,130)} = 13.6, p < 0.001 \), indicative of sensitization. This dose of reboxetine did not alter basal activity and did not alter nicotine-induced hyperactivity across sessions (Fig. 5).

A two-way ANOVA conducted on the activity data from the sensitization test (session 15) revealed that rats given repeated nicotine were more active than rats given repeated saline \( F_{(1,10)} = 81.3, p < 0.001 \). Moreover, there was no difference in activity between the groups that previously received repeated nicotine, with or without repeated reboxetine pretreatment, indicating that this dose of reboxetine did not inhibit the development of nicotine sensitization (Fig. 6, top panel). Additionally, a two-way ANOVA conducted on the data from the conditioning test (session 16, in which only vehicle and saline injections were administered) revealed that rats previously treated with nicotine were more active...
The present study demonstrates that acute pretreatment with either reboxetine or (+)-(S,S)-reboxetine dose dependently decreases both nicotine self-administration and sucrose-maintained responding. Although a wide range of reboxetine doses was tested in the current study, the dose-response curves were relatively flat, and complete inhibition of responding for nicotine or sucrose was not obtained. Furthermore, both reboxetine and (+)-(S,S)-reboxetine decreased (~60%) nicotine self-administration to a greater extent in the first 15 min of the session compared with that during the entire 60-min session (~40%). Reboxetine has been reported to be rapidly absorbed ($t_{max} = ~30\text{ min}$) and then eliminated (plasma half-life = ~55 min) in rats (Dostert et al., 1997). In the present study, reboxetine was administered 15 min before the 60-min session. As such, pharmacokinetics of reboxetine may in part explain the larger effect during the first 15 min of the session as compared with the entire 60-min session. Moreover, the magnitude of the reboxetine-induced decrease in nicotine self-administration (~60%) was greater than that observed for sucrose-maintained responding (~20%) during comparable periods of time. However, baseline responding for sucrose reinforcement tended to be greater than baseline responding for nicotine reinforcement. As such, the more robust response rate with sucrose may have been more resistant to inhibition by reboxetine. In general, however, drugs more readily decrease high rates of responding compared with low rates (Phillips et al., 1991), and thus, differences in response rate do not likely explain the differential sensitivity of the reboxetine-induced decrease in nicotine- and sucrose-maintained responding. Therefore, the results of this study suggest some selectivity of reboxetine to decrease nicotine self-administration. Alternatively, the difference in the magnitude of the reboxetine-induced decrease between responding for nicotine and for sucrose may reflect an inherent difference in the role of norepinephrine and/or the interaction of norepinephrine and dopamine in nicotine and food reinforcement (Di Chiara et al., 1998).

After repeated administration, the ability of reboxetine (5.6 mg/kg) to decrease nicotine self-administration was maintained when the data were analyzed by comparing the reboxetine pretreatment group with the control group. These results suggest that tolerance did not develop to the reboxetine-induced decrease in nicotine self-administration. However, large individual differences were observed with respect to the effect of repeated reboxetine pretreatment on nicotine self-administration. Reboxetine also decreased responding for sucrose across 14 consecutive sessions, when the reboxetine pretreatment group was compared with the control group, indicating that tolerance also did not develop to this effect. In contrast to the decrease in responding for nicotine across repeated reboxetine pretreatments, however, there was less variability among individual rats in the reboxetine-induced decrease in responding for sucrose. Furthermore, the relative magnitude of the reboxetine-induced decrease in responding for nicotine and for sucrose was maintained following repeated administration.

Importantly, a dose of reboxetine (5.6 mg/kg), which de-
increased responding for nicotine by ~50%, did not decrease locomotor activity. Additionally, repeated administration of reboxetine did not inhibit the development of locomotor sensitization induced by repeated injection of nicotine, nor did it inhibit the conditioned hyperactivity induced by repeated nicotine. These latter results indicate that the reboxetine-induced decrease in nicotine self-administration was not due to general sedation or motor impairment.

The reinforcing effect of nicotine is generally accepted as being mediated by the stimulation of nAChRs located on the cell bodies and terminals of the mesolimbic dopamine system. Nicotine self-administration is decreased by selective dopamine antagonists and by lesioning the mesolimbic dopamine system, both of which result in robust decreases in nicotine self-administration (Corrigall and Coen, 1991; Corrigall et al., 1992). Thus, it seems likely that the reboxetine-induced decrease in nicotine self-administration may be due to an alteration in the activity of the mesolimbic dopamine system. However, it is unlikely that reboxetine modulates mesolimbic dopamine activity via direct inhibition of dopamine transporter function, since reboxetine has a low affinity (IC50 = 89 μM) for dopamine transporters (Miller et al., 2002b). Moreover, after 14 days of once daily administration of reboxetine, the ability of reboxetine to inhibit dopamine transporters is not altered (IC50 = 100 μM; Miller et al., 2002b), further suggesting that inhibition of dopamine transporters was not involved in the reboxetine-induced decrease in nicotine self-administration across 14 sessions in the present study.

Noradrenergic neurons of the locus coeruleus send projections directly to the ventral tegmental area (Phillipson, 1979) and to the shell region of the nucleus accumbens (Delfs et al., 1998). Additionally, the locus coeruleus sends indirect projections to the ventral tegmental area via the hippocampus (Lindvall and Bjorklund, 1983). Stimulation of locus coeruleus neurons modulates the activity of ventral tegmental area dopamine neurons (Grenhoff et al., 1993), suggesting that the noradrenergic system interacts with the mesolimbic dopamine system, potentially contributing to reward. Systemic administration of reboxetine has also been reported to increase norepinephrine release in the hippocampus and frontal cortex (Sacchetti et al., 1999). In a recent report, reboxetine was shown to increase the burst firing pattern, but not the average firing frequency, of ventral tegmental area dopamine neurons; however, systemic administration of reboxetine increased dopamine release in the prefrontal cortex but, paradoxically, not in nucleus accumbens (Linner et al., 2001). Since increased dopamine release in the nucleus accumbens is generally considered critical for reward, these observations suggest that reboxetine would not serve as a reinforcer. However, if reboxetine exerts an inhibitory influence on accumbal dopamine release via a modulatory noradrenergic system, then this could be an indirect mechanism to explain the decrease in nicotine self-administration observed in the present study.

Alternatively, the reboxetine-induced decrease in nicotine self-administration may be due to a direct noradrenergic mechanism. Recently, it has been suggested that norepinephrine may contribute to nicotine reinforcement (Picciotto and Corrigall, 2002). Whereas the present study found that a norepinephrine reuptake inhibitor decreased nicotine self-administration, other studies have shown that noradrenergic antagonists (Yo- kel and Wise, 1976), reuptake inhibitors (Tella, 1995), and lesions of the noradrenergic system (Roberts et al., 1977) do not alter self-administration of other stimulant drugs such as amphetamine and cocaine. Collectively, these results suggest that the noradrenergic system may uniquely contribute to nicotine reinforcement.

The present results taken together with the results of others, however, suggest that the interaction of reboxetine at the nor- ephrine transporter may not be responsible for the decrease in nicotine self-administration. (+)-(S,S)- and (−)-(R,R)-Reboxetine have been reported to differ by ~20-fold in potency for inhibition of norepinephrine uptake (IC50 values of 3.6 nM and 85 nM, respectively; Dostert et al., 1997). Furthermore, the effects of reboxetine have been reported to result more from the actions of (+)-(S,S)- compared with the (−)-(R,R)-enantiomer (Benedetti et al., 1995). In contrast, in the current study, no differences were observed between the racemic and (+)-(S,S)-forms of reboxetine with respect to the observed decrease in nicotine self-administration. Thus, the reported difference in affinity at the norepinephrine transporter, but lack of observed difference between (+)-(S,S)- and racemic reboxetine to decrease nicotine self-administration, suggests that the norepi- nephrine transporter may not be responsible for the decrease in nicotine self-administration.

Another mechanism by which reboxetine may decrease nicotine self-administration is by acting as an antagonist at nAChRs. Reboxetine has been shown to inhibit nicotine-evoked 86Rb+ efflux (IC50 = 650 nM), indicating functional antagonism of the α4β2+ nAChR subtype (Miller et al., 2002b). Several gene-knockout studies have implicated the β2-subunit in nicotine self-administration (Picciotto et al., 1998; Epping-Jordan et al., 1999; Cordero-Erausquinm et al., 2000). Pharmacological studies have also implicated the α4β2+ subtype in mediating nicotine reinforcement (Stolerman et al., 1997; Watkins et al., 1999; Grottick et al., 2000). Specifically, the competitive nico- tinic receptor antagonist, dihydro-β-erythroididine (DHβE), which has greater selectivity (100-fold) for the α4β2+ than for the α7+ nAChR subtype (Chavez-Noriega et al., 1997), attenuates nicotine self-administration in rats (Stolerman et al., 1997; Watkins et al., 1999; Grottick et al., 2000). Additionally, the present study showed that the noncompetitive antagonist mecamylamine decreased nicotine self-administration, consistent with previous work (Corrigall and Coen, 1989; Watkins et al., 1999). Thus, the ability of reboxetine to decrease nicotine self-administration is consistent with inhibition of α4β2+ nAChR function.

Reboxetine failed to attenuate the development of nicotine-induced locomotor sensitization. The latter finding was surprising because both competitive and noncompetitive inhibitors of nAChRs (DHβE and mecamylamine, respectively) have been reported to attenuate the development of nicotine-induced sensitization (Stolerman et al., 1997; Miller et al., 2001). However, the failure of reboxetine to attenuate the development of nicotine-induced locomotor sensitization may have been due to the use of an insufficient dose of reboxetine (5.6 mg/kg) in the current study. Also, methodological differences between the present study and those investigating the effects of DHβE and mecamylamine (Stolerman et al., 1997; Miller et al., 2001) limit direct comparisons.

In addition to the inhibitory activity of reboxetine at α4β2+ receptors, reboxetine also potently (IC50 value = 7.3 nM) inhibited nictinic-evoked [3H]norepinephrine release from superfused rat hippocampal slices, consistent with functional antagonism of the α3β4+ nAChR subtype (Miller et al.,
2002b). Thus, reboxetine may also decrease nicotine self-administration via inhibition of α3β4* nAChRs. Furthermore, the 90-fold greater potency of reboxetine to inhibit α3β4* receptors relative to α4β2* receptors, taken together with the current findings that reboxetine decreases nicotine self-administration, but not locomotor activity, suggests that α3β4* nAChRs may be specifically involved in nicotine reinforcement. Thus, α3β4* nAChRs may be a novel target for development of new medications for smoking cessation.

Interestingly, affinities for reboxetine enantiomers at different nAChR subtypes have not been reported. The lack of observed differences between (+)-S,S- and racemic reboxetine to decrease nicotine self-administration in the current study suggests that (+)-(S,S) and (−)-(R,R)-reboxetine will have similar affinity for the specific nAChR subtype mediating this effect. Although the specific neurochemical mechanism(s) by which reboxetine decreases nicotine self-administration have not been elucidated, the current findings, taken together with the results of a recent report on the neurochemical effects of reboxetine (Miller et al., 2002b), suggest that reboxetine inhibition of α3β4* nAChRs, α4β2* nAChRs, norepinephrine transporters, and/or a combination of interactions at these sites may play an important role.

References

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