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ORIGINAL INVESTIGATION

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Nornicotine is self-administered intravenously by rats

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Abstract *Rationale:* Nicotine is a tobacco alkaloid known to be important in the acquisition and maintenance of tobacco smoking. However, other constituents in tobacco may contribute to the dependence liability. *Objective:* The present report sought to determine whether nornicotine, a tobacco alkaloid and metabolite of nicotine, has a reinforcing effect. *Methods:* Rats were prepared with a jugular catheter, then were allowed to self-administer intravenously either S(–)-nicotine (0.03 mg/kg/infusion), RS(±)-nornicotine (0.3 mg/kg/infusion) or saline using a two-lever operant procedure. The response requirement for each infusion was incremented gradually from a fixed ratio 1 (FR1) to FR5. When responding stabilized on the FR5, other doses of nicotine (0.01 mg/kg/infusion and 0.06 mg/kg/infusion) and nornicotine (0.075, 0.15, and 0.6 mg/kg/infusion) were tested for their ability to control responding. *Results:* Similar to nicotine, rats self-administered nornicotine significantly above saline control levels. Within the dose ranges tested, both nicotine and nornicotine yielded relatively flat dose–response functions. Extinction of responding was evident when saline was substituted for nornicotine, and responding was reinstated when nornicotine again was available. The rate of nornicotine self-administration was similar between rats tested with either 24-h or 48-h inter-session intervals. *Conclusion:* These results indicate that nornicotine contributes to the dependence liability associated with tobacco use.

Key words Nornicotine · Nicotine · Self-administration · Drug reward · Tobacco use · Nicotine metabolites

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Introduction

It is now widely recognized that tobacco smoking behavior is maintained due to the presence of the psychostimulant nicotine in the inhalant smoke (Stolerman and Jarvis 1995). The reinforcing effect of nicotine has been demonstrated in laboratory animals using the intravenous self-administration paradigm. Reliable rates of nicotine self-administration have been observed under fixed ratio (FR) schedules of reinforcement in a variety of species, including monkeys (Goldberg et al. 1981), dogs (Risner and Goldberg 1983), and rats (Corrigall and Coen 1989; Donny et al. 1995, 1998; Shoaib et al. 1997). Nicotine self-administration in rats is described by a relatively flat inverted U-shaped dose–response curve, with maximal responding occurring at a training dose of about 0.03 mg/kg/infusion (Corrigall and Coen 1989; Donny et al. 1995). Nicotine self-administration is decreased by the nicotinic receptor antagonist mecamylamine (Corrigall and Coen 1989; Shoaib et al. 1997), indicating that nicotinic receptors are involved. Moreover, the reward-relevant nicotinic receptors appear to be localized, at least in part, on the mesolimbic dopamine system (Corrigall et al. 1992).

In addition to nicotine, mounting evidence suggests that other tobacco alkaloids may contribute to the dopamine-related behavioral effects of tobacco use. In particular, nornicotine is an alkaloid found in tobacco and structurally related to nicotine that has recently received attention as a psychoactive agent (Crooks and Dwoskin 1997). Nornicotine is present in *Nicotiana tobaccum* (Kisaki and Tamaki 1961) and is also known to be a minor peripheral N-demethylated metabolite of nicotine in various animal species, including humans and rats (Bowman et al. 1959; McKennis et al. 1962; Cundy and Crooks 1984). Even though only about 8% of nicotine is metabolized to nornicotine in the periphery (Curvall and Kazeni 1993), the plasma half-life in humans is substantially longer for nornicotine (8 h) than for nicotine (1 h; Kyerematen et al. 1990). In addition, since substantial biotransformation of nicotine to nornicotine

occurs locally in brain (Crooks et al. 1995, 1997), nornicotine may accumulate in brain with repeated tobacco use.

There is now convincing evidence that nornicotine, similar to nicotine, activates brain dopaminergic systems. Nornicotine evokes a concentration-dependent and calcium-dependent release of dopamine from rat and mouse striatal slices and synaptosomes (Grady et al. 1992; Dwoskin et al. 1993). Nicotinic receptor antagonists inhibit dopamine release evoked by low concentrations of nornicotine (<100 μM; Teng et al. 1997), indicating the involvement of a nicotinic receptor-mediated mechanism.

To the extent that nornicotine and nicotine share a common ability to evoke dopamine release via nicotinic receptors, it can be hypothesized that nornicotine will activate dopamine-mediated behaviors, such as locomotor activity and reward, in a manner similar to nicotine. Consistent with this hypothesis, acute nornicotine produces nicotine-like effects on locomotor activity in mice and rats (Mattila 1963; Stolerman et al. 1995). In rats, chronic pretreatment with nornicotine also produces cross-sensitization to the locomotor stimulant effects of a nicotine challenge (Dwoskin et al. 1999), suggesting that these drugs have a common mechanism of action. However, there have been no reports assessing the potential rewarding effects of nornicotine. Thus, the present study examined whether nornicotine functions as a reinforcer using a procedure shown previously to support reliable nicotine self-administration in rats (Corrigall and Coen 1989).

Materials and methods

Animals

Adult male Sprague-Dawley rats (200–225 g body weight) were obtained from Harlan Industries (Indianapolis, Ind.) and were caged individually with free access to food and water in the home cage. The colony room was controlled for temperature (24°C) and relative humidity (45%), with lights on from 0700 hours to 1900 hours. Prior to the start of each experiment, animals were acclimated to the colony room for at least 1 week and were handled for 2 days. Behavioral testing was conducted during the light phase of the cycle. All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee and they conformed to the *Guide for the Care and Use of Laboratory Animals* (1996 edition).

Surgery

Animals were anesthetized (100 mg/kg ketamine, 5 mg/kg diazepam, i.p.) and implanted with a catheter into the jugular vein. A silastic tube was inserted into the vein and exited out through the top of a head mount that was affixed to the top of the skull with dental acrylic and metal screws (Peltier and Schenk 1993). Daily infusions of heparinized saline and streptokinase (Pharmacia, Columbus Ohio; 250,000 IU, 2 mg/ml heparinized saline, 0.1 ml/rat/day) were used to maintain patency of the silastic catheter. At the end of each experiment, each animal was injected through the catheter with morphine (15 mg/kg, i.v.) and the presence of a rapid cataleptic response was used to confirm catheter patency.

Apparatus

For nicotine and nornicotine self-administration, operant chambers (ENV-001, Med Associates, St Albans, Vt.) enclosed in a sound attenuating environment were used. Located in the bottom center of the front panel in each chamber was a 5×4.2-cm opening to a recessed food tray. Two metal response levers were located on the front panel, one on each side of the food tray. The center of each lever was mounted 7.3 cm from the grid floor. A 28-V white cue light, 3 cm in diameter, was centered 6 cm above each lever. Drug infusions were delivered using a syringe pump (Med Associates, PHM-100) and a water-tight swivel that allowed a catheter to be attached from the syringe (10 ml) to the head mount of the animal in the operant chamber. A personal computer, using Med Associates interface, controlled the experimental sessions and collected data.

Self-administration procedure

The general procedure described previously by Corrigall and Coen (1989) was used to assess nicotine and nornicotine self-administration. Rats were first reduced to 85% of normal body weight using a restricted food access regimen and shaped to lever press for a sucrose pellet in the operant chamber. Only one lever (counterbalanced for left or right) was available during this phase of pretraining. Across each session (15 min/session), the schedule of reinforcement was increased incrementally from a FR1 to FR5; each increment required that rats receive a minimum of 20 reinforcers during the session. After reaching criterion on the FR5, rats were maintained undisturbed in the home cage with food and water available continuously for a minimum of 1 week. This period of free feed was sufficient to restore rats to normal body weights. Rats were then surgically implanted with a chronic jugular catheter and allowed to recover for 7 days.

Nicotine and nornicotine self-administration were assessed during 1-h daily sessions. Drug was infused (0.1 ml, 2.5 s) following depression of one lever (active lever); responding on the second lever (inactive lever) was recorded, but was not reinforced. Each drug infusion was followed by a 20-s signaled (cue lights) time-out interval, during which responding was not reinforced on either lever. Across sessions, the schedule of reinforcement was increased from a FR1 (five sessions), to a FR2 (three sessions), then maintained at FR5 until responding stabilized. Acquisition of stable responding was operationally defined by the following criteria: (1) less than a 20% difference in number of infusions across three consecutive sessions; (2) greater than a 2:1 ratio of active to inactive lever presses; and (3) five or more infusions per session. During the entire phase of drug self-administration, food access was restricted to 20 g/day in the home cage, given at the end of each operant session. This restricted food regimen has been shown to be sufficient for rats to gain weight across sessions (Corrigall and Coen 1989; Donny et al. 1995).

Experiments

In the first experiment, rats were assigned randomly to one of two groups. One group (n=14) was assessed for nicotine self-administration (0.03 mg/kg/infusion) using the two-lever procedure described previously. The second group (n=7) was treated similarly, except that saline infusions were administered rather than nicotine infusions across the acquisition sessions. In order to ascertain the dose–response curve for nicotine self-administration, rats that reached stable responding for nicotine on the FR5 within ten sessions (n=13 out of 14 total) were tested subsequently on a minimum of two consecutive sessions with additional doses of nicotine given in the following order: 0.01 mg/kg/infusion and 0.06 mg/kg/infusion.

In the second experiment, rats (n=18) were trained to self-administer nornicotine. Since available behavioral data suggests an approximately tenfold shift in relative potencies between nicotine and nornicotine (Risner et al. 1988), we chose a training dose of nornicotine (0.3 mg/kg/infusion) tenfold greater than that used to

establish nicotine self-administration. Saline control rats were assessed concomitantly with the nornicotine self-administration group. In order to ascertain the dose–response curve for nornicotine self-administration, rats that reached stable responding on the FR5 schedule (n=13 out of 18 total) were subsequently tested on a minimum of two consecutive sessions with additional doses of nornicotine given in the following order: 0.15, 0.6 and 0.075, mg/kg/infusion.

In a third experiment, rats (n=8) were trained to self-administer nornicotine as described previously, except that the interval between self-administration sessions was 48 h rather than 24 h. A separate saline control group (n=4) was also assessed using the 48-h inter-session interval. This experiment was conducted because the plasma half-life of nornicotine is eightfold longer than for nicotine (Kyerematen et al. 1990). Taking into account its pharmacokinetic characteristics, it is probable that significant brain levels of nornicotine may be present 24 h after the self-administration session. Since session-to-session residual levels of nornicotine might be expected to reduce responding using a 24-h inter-session interval, this possibility was minimized by increasing the inter-session interval to 48 h.

To further evaluate whether nornicotine was able to control operant responding, rats from experiments 2 and 3 that reached stable responding for nornicotine on the FR5 were tested subsequently for saline substitution. Saline was substituted for the nornicotine training dose across five consecutive sessions. Nornicotine infusions were then reinstated for four additional sessions.

Drugs

S(–)-Nicotine ditartrate was purchased from Research Biochemicals Inc. (Natick, Mass.) and (±)-nornicotine was purchased from either ICN Biochemicals (Costa Mesa, Calif.) or Sigma (St. Louis, Mo.). Drugs were prepared in 0.9% NaCl and the pH was adjusted to 7.0 prior to injection. In all experiments, drug dosages are expressed as free base weight.

Statistics

Data were analyzed using an analysis of variance (ANOVA). Subsequent analyses were performed using an F test for simple main effects or an LSD test for pairwise comparisons. In all cases, significance was declared at P<0.05.

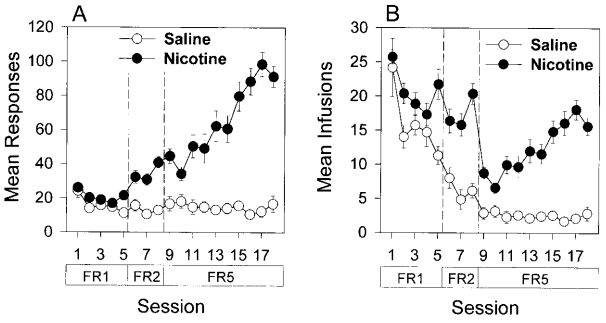

Fig. 1A, B Acquisition of nicotine self-administration using a training dose of 0.03 mg/kg/infusion. **A** Mean (±SEM) number of responses on the active lever across incremental fixed ratio (*FR*) sessions in rats responding for either nicotine or saline. **B** Mean (±SEM) number of infusions earned across incremental FR sessions in rats responding for either nicotine or saline. In both panels, the nicotine data were obtained from 13 rats and the saline data were obtained from 7 rats

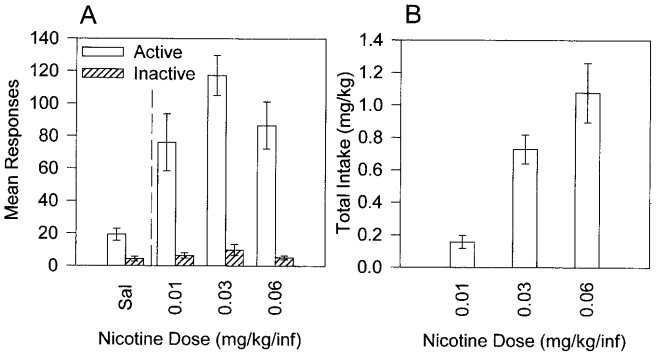

Fig. 2A, B Dose–response curve for nicotine self-administration. **A** Mean number of active and inactive lever presses in rats responding for different doses of nicotine. The curve was obtained from five rats; for comparison, the data from the saline control group (n=7) is illustrated in the left portion of the figure. **B** Mean total intake for different doses of nornicotine using the same five rats as those used to generate the results in **A**

Results

Nicotine self-administration

As expected, reliable nicotine self-administration was established across repeated acquisition sessions (Fig. 1A). An overall ANOVA revealed a significant interaction between infusion drug (nicotine vs saline) and session ($F_{17,272}$=20.83, P<0.001). Responding on the active lever increased across the incremental FR sessions in the nicotine group ($F_{17,170}$=33.14, P<0.001), but not in the saline group. Responding on the inactive lever (no infusion) in the nicotine group was negligible (less than 10 per session) at the end of FR5 training, and there was no significant difference in inactive lever pressing between the nicotine and saline groups on any session (results not shown).

The number of nicotine infusions earned also varied as a function of session (Fig. 1B). On the FR1 schedule, rats earned approximately 20 nicotine infusions per session. When the schedule was incremented to an FR2, there was a transient decrease in the number of nicotine infusions earned, but the infusion rate eventually returned to approximately 20 per session. Similarly, when the schedule was incremented to an FR5, there was a transient decrease in infusions earned, followed by a return to nearly 20 infusions per session. These results suggest that rats adjusted the number of responses across incrementing FR sessions in order to maintain a relatively constant number of nicotine infusions.

Analysis of the within-subject dose–response results indicated that the number of responses on the active lever differed significantly as a function of nicotine dose ($F_{2,8}$=7.35, P<0.05; Fig. 2A); in this analysis, the saline control group was not included. Pairwise comparisons among nicotine doses revealed that responding was significantly higher for 0.03 mg/kg/infusion than for both 0.01 mg/kg/infusion and 0.06 mg/kg/infusion. There was no significant difference in responding between 0.01 mg/kg/infusion and 0.06 mg/kg/infusion. When the re-

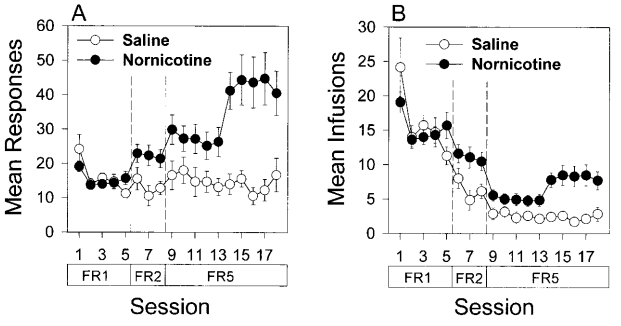

Fig. 3A, B Acquisition of nornicotine self-administration using a training dose of 0.3 mg/kg/infusion. **A** Mean (±SEM) number of responses on the active lever across incremental fixed ratio (FR) sessions in rats responding for either nornicotine or saline. **B** Mean (±SEM) number of infusions earned across incremental FR sessions in rats responding for either nornicotine or saline. In both panels, the nornicotine data were obtained from 13 rats and the saline data were obtained from 7 rats

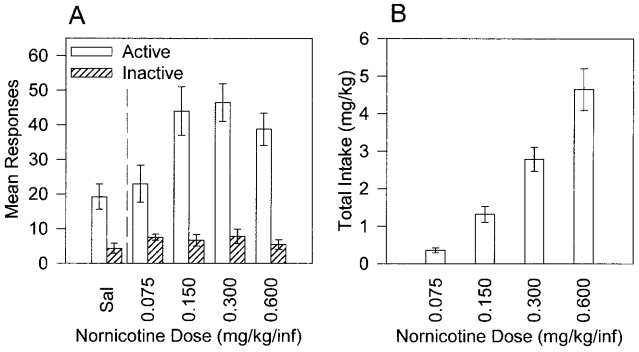

Fig. 4A, B Dose–response curve for nornicotine self-administration. **A** Mean number of active and inactive lever presses in rats responding for different doses of nornicotine. The curve was obtained from six rats; for comparison, the data from the saline control group (n=7) is illustrated in the *left portion* of the figure. **B** Mean total intake for different doses of nornicotine using the same six rats as those used to generate the results in **A**

sults were plotted as total nicotine intake within the session, there was a clear dose-dependent increase in intake ($F_{2,8}$=27.12, P<0.001; Fig. 2B). Pairwise comparisons among doses revealed that total intake for each nicotine dose differed significantly from both other doses.

Nornicotine self-administration

Similar to nicotine self-administration, reliable nornicotine self-administration was established across repeated acquisition sessions (Fig. 3A). An overall ANOVA revealed a significant interaction between infusion drug (nornicotine vs saline) and session ($F_{17,306}$=5.12, P<0.001). Responding on the active lever increased across the incremental FR sessions in the nornicotine group ($F_{17,204}$=8.28, P<0.001) but not in the saline group. However, the number of responses on the FR5 in the nornicotine group was below that obtained with the nicotine group (*cf.* Fig. 1A and Fig. 3A). There was no significant difference in inactive lever pressing between the nornicotine and saline groups on any session (results not shown).

The number of infusions was also greater in the nornicotine group than the saline group across sessions (Fig. 3B). On the FR1 schedule, rats earned approximately 15 nornicotine infusions per session. When the schedule was incremented to an FR2, there was a decrease in the number of nornicotine infusions earned to approximately ten per session. When the schedule was incremented to an FR5, there was a transient decrease in nornicotine infusions earned, but the infusion rate increased across FR5 sessions to a level near that observed on the FR2. This latter effect was not evident in the saline group across sessions. Thus, similar to nicotine, rats showed an adjustment in responding in order to maintain a relatively constant number of nornicotine infusions, although the number of infusions was below that obtained with nicotine.

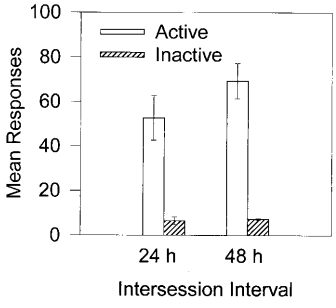

Fig. 5 Effect of varying inter-session interval on the number of active and inactive lever presses in rats responding for nornicotine at stable criterion on the FR5. The 24-h data were obtained from eight rats and the 48-h data were obtained from four rats

Analysis of the within-subject dose–response results indicated that the number of responses on the active lever differed significantly as a function of nornicotine dose ($F_{3,15}$=8.44, P<0.05; Fig. 4A); in this analysis, the saline control group was not included. Pairwise comparisons among nornicotine doses revealed that responding was significantly higher for 0.15, 0.3 and 0.6 mg/kg/infusion than for 0.075 mg/kg/infusion. There were no significant differences in responding among the 0.15, 0.3 and 0.6 mg/kg/infusion doses. When the results were plotted as total nornicotine intake within the session, there was a clear dose-dependent increase in intake ($F_{3,15}$=48.18, P<0.001; Fig. 4B). Pairwise comparisons among doses revealed that total intake for each nornicotine dose differed significantly from all other doses.

The number of responses on the active and inactive levers was similar between rats self-administering nornicotine during either 24-h or 48-h inter-session intervals (Fig. 5). An overall analysis of variance of these data revealed a significant main effect of lever ($F_{1,10}$=48.19, P<0.001), but there was no significant effect of inter-session interval, nor was there a significant interaction between lever and inter-session interval.

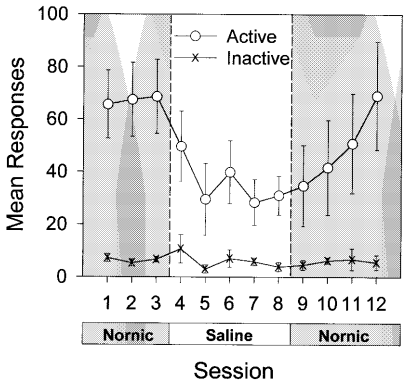

Fig. 6 Effect of substituting saline for nornicotine on the number of active and inactive lever presses. These data were obtained from six rats that reached stable criterion on the nornicotine training dose

Results from the saline substitution procedure are depicted in Fig. 6. Substitution of saline for nornicotine (0.3 mg/kg/infusion) produced a decline in responding on the active lever, whereas reinstatement of contingent nornicotine infusions increased responding to levels similar to those maintained prior to the saline substitution. To analyze these data, the number of responses on the active lever were collapsed across the five saline sessions and compared with the number of responses on the active lever collapsed across the three baseline nornicotine sessions. This analysis revealed that responding was significantly lower on saline sessions than on nornicotine sessions ($F_{1,5}$=9.25, P<0.05). When nornicotine was reinstated following saline substitution, there was a significant increase in responding across sessions relative to the last session with saline ($F_{4,20}$=3.31, P<0.05).

Discussion

Using a procedure shown previously to establish reliable nicotine self-administration in rats (Corrigall and Coen 1989), the present study compared the ability of intravenous nicotine or nornicotine to serve as reinforcers. The number of responses engendered by contingent infusions of either nicotine (0.03 mg/kg/infusion) or nornicotine (0.3 mg/kg/infusion) was greater than the number of responses obtained on either an inactive lever (no infusion) or a saline infusion lever. Changing the test dose yielded a relatively flat dose–response function for both nicotine and nornicotine self-administration rates. Within the dose ranges tested (0.01–0.06 mg/kg/infusion for nicotine; 0.075–0.6 mg/kg/infusion for nornicotine), responding was more avid for nicotine than for nornicotine. The rate of nornicotine self-administration was not significantly different between rats tested with an inter-session interval of either 24 h or 48 h. Importantly, responding declined towards extinction when saline was substituted for nornicotine, indicating that nornicotine controlled responding. These results provide the first direct evidence suggesting that nornicotine contributes to the acquisition and maintenance of tobacco use.

While reliable self-administration of nicotine and nornicotine was clearly obtained using the general method of Corrigall and Coen (1989), it should be noted that this method also yielded a low but continuous rate of responding in rats that self-infused only saline throughout acquisition training. There may be at least two important reasons why we did not observe complete extinction of the lever press response in saline controls across sessions in the present report. First, rats were initially pretrained to lever press on an FR5 schedule for a highly palatable sucrose reinforcer and were maintained on a restricted food regimen throughout the study. Extinction of responding in the saline control group may have been enhanced if we had used a less palatable reinforcer (e.g., standard food pellets) across fewer pretraining sessions or eliminated the restricted food regimen. Second, extinction of responding in the saline control group may have been enhanced if the pretraining phase with sucrose and acquisition phase with contingent infusions occurred in different apparatuses, as described by others (Watkins et al. 1999). This minimizes the possibility that acquisition of drug self-administration is established during the time when the sucrose reinforcement is being extinguished. In any case, the results from the present report illustrate the importance of a saline control group when examining drug reinforcers that do not engender avid self-administration, such as nornicotine.

The dose–response curves for both nicotine and nornicotine self-administration rates were relatively flat when compared with other stimulant drugs such as amphetamine or cocaine (Hemby et al. 1996; Carroll and Lac 1997; Bardo et al. 1999). This likely reflects an inherent property of the drugs tested, rather than to the procedure, since self-administration dose–response curves with nicotine are typically flat relative to cocaine across a variety of species, including rats (Corrigall and Coen 1989; Donny et al. 1995; Tessari et al. 1995; present results), mice (Rasmussen and Swedberg 1998), dogs (Risner and Goldberg 1983) and humans (Rose and Corrigall 1997). However, since only FR5 responding was examined in the present study, it is possible that sharper dose–response functions for nicotine and nornicotine self-administration may be obtained using other FR schedules or a progressive ratio schedule.

While these preliminary results indicate that nornicotine functions as a positive reinforcer, it did not maintain avid self-administration compared with nicotine. One interpretation of the low rate of nornicotine self-administration may be related to its pharmacokinetics. Since the elimination half-life for nornicotine is approximately eight times longer than that for nicotine (Kyerematen et al. 1990), each infusion may produce a rewarding effect that is more protracted than that produced by nicotine. Alternatively, the low rate of nornicotine self-administration may reflect a lower efficacy or a lower ability to cross the blood–brain barrier than nicotine. Regardless of the mechanism, however, the fact that nornicotine is weakly self-administered suggests that it may be a potential substitution pharmacotherapy for tobacco smoking cessation which engenders low addiction liability.

Some caution is warranted when cross-comparing the dose–response functions for nicotine and nornicotine self-administration in the present report. Since nicotine is biotransformed into nornicotine in both the periphery and brain (Curvall and Kazeni 1993; Crooks et al. 1995, 1997), rats self-administering nicotine also received cumulative exposure to nornicotine across the operant session. It is not possible to directly determine the absolute contribution of nicotine on responding independent of nornicotine, as there is no method presently available to specifically inhibit the biotransformation of nicotine to nornicotine. This problem is not apparent in rats self-administering nornicotine, as nornicotine is not biotransformed into nicotine, but rather into the inactive metabolite norcotinine (Crooks and Dwoskin 1997). Further work is needed to rule out any contribution of norcotinine to the reinforcing effect of nornicotine observed in the present report.

In addition to this caveat, nicotine self-administration was determined using the pure S(–)-enantiomer, whereas nornicotine self-administration was determined using the commercially available RS(±)-racemate. Previous work has shown that S(–)-nornicotine is more potent than R(+)-nornicotine in evoking dopamine release in brain (Teng et al. 1997) and in altering schedule-controlled operant responding (Risner et al. 1988). These previous results suggest that more avid nornicotine self-administration may occur using the pure S(–)-enantiomer. Regardless of this possibility, however, it is important to note that nornicotine is present in *Nicotiana tobaccum* in both S(–) and R(+) enantiomeric forms (Kisaki and Tamaki 1961), whereas the major alkaloid nicotine is always present in this plant as the enantiomerically pure S(–) form. Thus, the present data collected with RS(±)-nornicotine are relevant to our understanding of the pharmacological effects of tobacco use.

Finally, although the present results do not directly address the neural mechanism, there is reason to suggest that nornicotine self-administration, similar to nicotine self-administration, may involve the mesolimbic dopamine reward system. Nicotine reinforcement is reduced by pretreatment with dopamine antagonists (Corrigall and Coen 1991; Dawe et al. 1995) or by lesioning the nucleus accumbens with the neurotoxin 6-hydroxydopamine (Corrigall et al. 1992). Both behavioral and neurochemical evidence indicate that the reward-relevant nicotinic receptors are located directly on mesolimbic dopamine neurons (Wise 1998). Nornicotine and nicotine may share a common ability to activate these reward-relevant nicotinic receptors. In support of this, the present results indicate that nornicotine has a lower potency than nicotine to support optimal self-administration. This is in agreement with in vitro studies showing that nornicotine is less potent than nicotine in displacing [^{3}H]nicotine binding from rat brain membranes (Reavill et al. 1988; Copeland et al. 1991; Zhang and Norberg 1993) and in releasing [^{3}H]dopamine in rat striatal slices (Dwoskin et al. 1993, 1995).

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