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Blockade of Nicotine Self-Administration with Nicotinic Antagonists in Rats

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WATKINS, S. S., M. P. EPPING-JORDAN, G. F. KOOB AND A. MARKOU. *Blockade of nicotine self-administration with nicotine antagonists in rats.* PHARMACOL BIOCHEM BEHAV **62**(4) 743–751, 1999.—The reinforcing properties of a variety of drugs abused by humans have been investigated using the technique of intravenous self-administration in the rat. To examine the effect of nicotine dose on nicotine self-administration, Wistar rats were allowed to self-administer various doses of nicotine using a within-subjects Latin square design. An inverted U-shaped dose–response curve was obtained, with the highest rates of responding at the 0.03 mg/kg/inf dose. With 1-h daily nicotine self-administration sessions, rats did not appear dependent on nicotine 24 h later, as indicated by the absence of somatic signs of withdrawal after subcutaneous injection of a nicotinic acetylcholine receptor antagonist, mecamylamine (0.57 mg/kg). In another set of studies, pretreatment with subcutaneous mecamylamine or dihydro-β-erythroidine, two nicotinic acetylcholine receptor antagonists, resulted in significant dose-dependent reductions in nicotine self-administration, at two nicotine doses (0.03 and 0.06 mg/kg/inf). These results indicate that nicotine is an effective reinforcer in Wistar rats under the present parameters, and that these reinforcing effects are mediated by activation of nicotinic acetylcholine receptors. © 1999 Elsevier Science Inc.

Nicotine Self-administration Reinforcement Mecamylamine Dihydro-β-erythroidine Rat

APPROXIMATELY 25% of the United States population smokes cigarettes, with the majority meeting the DSM-IV diagnostic criteria for substance dependence (28). Although cigarette smoke contains over a thousand chemicals, overwhelming evidence points to nicotine as the sole component leading to dependence (2,49). Similar to other drugs of abuse, such as other psychomotor stimulants, opiates, and ethanol, nicotine produces tolerance, dependence, and has reinforcing actions (49). Nicotine has been shown to serve as a reinforcer in several species including rodents, dogs, nonhuman primates, and humans using the intravenous self-administration paradigm (5,7,16,17,21,22,23,27,40,43,45,46,50,51). Abstinence after chronic nicotine exposure results in a withdrawal syndrome in both humans (28,42) and rats (19,30), which has both somatic and affective components.

Both noncompetitive and competitive nicotinic antagonists have been shown to antagonize several of the behavioral effects of nicotine. For instance, mecamylamine, a noncompetitive nicotinic acetylcholine receptor (nAChR) antagonist acting at the ion channel (1), has been shown to decrease nicotine-induced hyperlocomotion (6), as well as oral (20) and intravenous nicotine self-administration (7,22,23,40,46). Dihydro-β-erythroidine (DHβE), a competitive nAChR antagonist with high affinity for the neuronal α4 receptor subunit (25,26), antagonized nicotine-induced hypothermia and hyperlocomotion in mice (14). In rats, DHβE has been shown to block the nicotine-induced increases in locomotor activity and the discriminative stimulus effects of nicotine (13,47), and to decrease nicotine self-administration after microinjections directly into the ventral tegmental area (11).

The purpose of the present study was to determine the role of nAChRs in nicotine reinforcement by examining the effects of a noncompetitive nAChR antagonist, mecamylamine, and the effects of a competitive nAChR antagonist, DHβE,

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on nicotine self-administration. The current studies used a fixed-ratio 1, timeout 20 s (FR1TO20s) schedule of reinforcement under limited access conditions to prevent the development of dependence. The results show that reliable intravenous self-administration of nicotine was obtained under these conditions in Wistar rats, and that systemic injection of both noncompetitive and competitive nAChR antagonists blocked nicotine self-administration.

METHOD

Subjects

Male Wistar rats from Charles River, Kingston, NY, were used in Experiments 1 and 2. In Experiment 3, male Wistar rats bred at the Beckman Laboratories of The Scripps Research Institute from a Wistar stock originally obtained from Charles River, NY, were used. At Beckman Laboratories, rats are bred using a circular-pair random system of breeding to maintain genetic heterogeneity. New breeders are obtained from Charles River as determined by our internal Genetics Advisory Board. Animals were group housed in a temperature-controlled vivarium on a reverse light–dark cycle (lights on 2200–1000 h) with ad libitum access to food and water prior to the beginning of experimental procedures. The mean range of rat weights through the duration of the experiments was 350–400 g. All behavioral testing occurred during the dark phase of the light–dark cycle. After training to lever press for food, subjects in Experiments 1 and 2 were returned to ad libitum access to food and water for the duration of the experiments. The subjects in Experiment 3 from the Beckman Laboratories were given ad libitum access to food, but when a larger number of these subjects compared to Charles River rats failed to meet the criterion for nicotine self-administration (at least five infusions/h by day 10 of nicotine self-administration; for further details see the Experimental Procedure section), these subjects were removed from ad libitum access to food and fed one 20-g meal per day immediately after the self-administration session. This meal restriction in Experiment 3 led to rats self-administering nicotine in amounts similar to those self-administered in Experiments 1 and 2. All subjects were treated in accordance with the National Institutes of Health guidelines regarding the principles of animal care. Animal facilities and experimental protocols were in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Apparatus

Food training took place in Coulbourn operant chambers (Model E10.10, Coulbourn Instruments, Lehigh Valley, PA) housed inside ventilated, sound-attenuating chambers. Operant chambers were equipped with a single lever mounted on one wall 2 cm above the floor with a cue light mounted 3 cm above the lever. A food hopper was located 5 cm to the left of the lever in the center of the same wall. Experimental parameters and data collection were controlled by IBM compatible PCs using in-house-designed software.

All nicotine self-administration training and testing took place in in-house-constructed Plexiglas operant chambers with stainless steel mesh floors housed inside ventilated, sound-attenuating chambers. A retractable lever (Model RRL-005, BRS/LVE, Laurel, MD) was mounted on one wall, 2 cm above the floor, with a cue light mounted 3 cm above the lever. Experimental parameters and data collection were controlled by an IBM compatible PC using in-house-designed software. Intravenous infusions were delivered by an infusion pump (Model A, Razel Scientific Instruments, Stamford, CT) through Tygon tubing (0.020″ i.d. × 0.060″ o.d., 0.20″ wall, Fisher Scientific, Pittsburgh, PA) housed inside a spring leash that was connected on one end to a stainless steel swivel to allow free movement of the animal and on the other end to the catheter base mounted in the midscapular region of the rat.

Surgery

Two days after returning to ad libitum food after food training (see procedure below), rats were anesthetized with an oxygen–halothane vapor mixture (1–3% halothane) and prepared with chronic intravenous jugular catheters as described previously (4). Catheters consisted of Silastic tubing (Baxter Scientific, McGaw Park, IL) attached to a stainless steel guide cannula (Item #C3136, Plastics One, Roanoke, VA) bent at a right angle and encased in dental cement anchored with a 2.3-cm square of durable plastic mesh (Small Parts, Miami Lakes, FL). The tubing was passed subcutaneously from the animals' midscapular region to the right external jugular vein, where it was inserted and secured with suture thread. Animals were allowed a minimum of 4 days recovery from surgery before being given access to nicotine. Animals also were injected intravenously with an antibiotic (Timentin, 100 mg/kg/day) for 5 days after surgery to help prevent postoperative infection. To ensure patency, catheters were flushed daily with approximately 0.1–0.3 ml saline containing 0.02% heparin. When not in use, catheters were capped with a short length of Tygon tubing (Fisher Scientific, Pittsburgh, PA) plugged with monofilament and covered with a stainless steel cap. Catheter patency was tested with Brevital Sodium (1% methohexital sodium, Eli Lilly, Indianapolis, IN) whenever an animal not receiving drug pretreatment displayed self-administration behavior outside baseline performance. Animals with patent catheters exhibit pronounced loss of muscle tone within 2 s of an intravenous injection of Brevital.

Drugs

(−)-Nicotine hydrogen tartrate salt ([−]-1-methyl-2-[3-pyridyl] pyrrolidine) and mecamylamine hydrochloride were obtained from Sigma, St. Louis, MO; dihydro-β-erythroidine hydrobromide was obtained from Research Biochemicals International, Natick, MA. All drugs were dissolved in physiological saline (0.9% sodium chloride). Mecamylamine and DHβE were administered subcutaneously in a volume of 1 ml/kg. Nicotine doses refer to free base, while all other drug doses refer to the salt form.

Experimental Procedures

Prior to intravenous catheterization, animals were trained to lever press for food reinforcement. Rats were initially food deprived (5 g lab chow/day) for 48 h. On subsequent food training days, rats were fed 20 g of standard rat chow per day immediately after each daily food training session. Food training began on a continuous reinforcement schedule with a timeout (TO) duration of 0 s. Then, the timeout duration was gradually increased in increments of 5 s. At each stage of training, animals had to achieve the criterion of 100 pellets earned during a daily 1-h session before training at the next level began. Food training continued until the subjects earned 100 reinforcers in a daily 1-h session on an FR1TO20s sched-

ule of reinforcement. Food training typically required 5–7 days. At the completion of food training, all rats were returned to ad libitum food prior to catheterization. Subjects in Experiments 1 and 2 remained on ad libitum food access for the duration of the experiments. In Experiment 3, after 10 days of exposure to the training dose of nicotine (0.03 mg/kg/inf), only 6 of the 17 rats had met criterion (minimum of five infusions during the 1-h session). At this point, all rats in Experiment 3 were removed from ad libitum food and fed one 20-g meal per day immediately after the self-administration session. Under food restriction, 14 of the 17 rats met criterion within approximately 8 days, and these rats were sustained on 20 g of rat chow per day for the remainder of the study. Given that the rats used in all three experiments were of the Wistar strain, it is unclear why rats from a local supplier were slow to acquire nicotine self-administration and required food restriction. It is possible that the stress associated with crosscountry transportation (e.g., food and water deprivation, changes in temperature and altitude, exposure to unpredictable noises and movements) contributed to the rats in Experiments 1 and 2 maintaining higher levels of nicotine self-administration than the subjects in Experiment 3.

Experiment 1: Nicotine Dose–Response Curve

Rats ($n = 7$) were given access to nicotine (approximately 0.03 mg/kg/infusion; 0.01 mg/infusion based on mean rat weights over the duration of the experiment, in a volume of 0.1 ml delivered over 4 s) under an FR1TO20s schedule during daily 3-h sessions. Each nicotine self-administration session began with two noncontingent injections, followed by extension of the lever into the chamber, which signaled the onset of the 3-h self-administration session. A signal light mounted above the lever indicated onset of injection and remained lit for 20 s, during which time lever presses were recorded, but had no scheduled consequences. Animals that did not earn an injection at least once per hour during the initial training period were given one or two noncontingent injections every 60 min for a maximum of 7 days. The presentation of noncontingent injections for animals that were not responding occurred only during the training period and not at any time during the experiments. After acquisition of stable responding for the training dose of nicotine (criterion of less than 20% deviation from the mean of the total number of reinforcers earned in three consecutive sessions for each rat), self-administration of various doses of nicotine (approximately 0.003, 0.01, 0.03, or 0.06 mg/kg/inf; 0.001, 0.003, 0.01, or 0.02 mg/inf, all doses refer to the base) was tested using a between-session, within-subjects Latin square design. Unpublished observations from our laboratory indicate that most rats will not acquire self-administration of a high dose of nicotine (0.06 mg/kg/inf) unless subjects have the opportunity to self-administer a lower nicotine dose (0.03 mg/kg/inf) for several days previously, thus, potentially allowing tolerance to develop to the aversive effects of nicotine. Rats were allowed to self-administer each dose of nicotine for 3 consecutive days. After completion of the Latin square, rats were returned to the 0.03 mg/kg/inf dose for an additional 3 days to assess the stability of nicotine self-administration at this dose. Next, saline was substituted for nicotine, with rats receiving two noncontingent saline injections before the lever was extended. After self-administration behavior was extinguished (defined as less than 30% of the responses for the training dose within each rat), reacquisition of stable nicotine self-administration at the training dose (0.03 mg/kg/inf) was examined.

Experiment 2: Effects of Mecamylamine Hydrochloride on Nicotine Self-Administration

Naive rats ($n = 13$) were prepared with catheters and allowed to self-administer nicotine at the 0.03 mg/kg/inf dose. Rats were then divided into two groups and allowed access to either 0.03 ($n = 6$) or 0.06 ($n = 7$) mg/kg/inf nicotine during daily 3-h sessions. After acquisition of stable responding at these doses, each rat received a single subcutaneous (SC) injection of saline to habituate rats to the injection procedure. The effects of injections of the noncompetitive nAChR antagonist, mecamylamine hydrochloride (0, 1.0, 2.0, or 4.0 mg/kg, SC, 20 min pretreatment prior to the beginning of the self-administration session; all doses refer to salt form), on self-administration of nicotine were examined in a between-session within-subjects Latin square design, with at least 48 h between each SC drug pretreatment. These mecamylamine doses were selected because they were shown previously to block the effects of nicotine on operant responding and locomotor activity [e.g., (6,7)]. After each mecamylamine or vehicle injection, rats were placed into the operant chambers and allowed to self-administer nicotine during a typical 3-h session.

Experiment 3: Effects of Dihydro-β-Erythroidine Hydrobromide on Nicotine Self-Administration

Naive rats ($n = 14$) were prepared with catheters and allowed to self-administer nicotine at the 0.03 mg/kg/inf dose during daily 1-h sessions. Based on the results of Experiment 2 indicating that mecamylamine produced decreases in nicotine self-administration primarily during the first hour of the self-administration session, 1-h sessions were used in Experiment 3. After acquisition of stable responding, each rat received a single SC injection of saline. Rats received injections of DHβE (0, 2.0, 4.0, 8.0 mg/kg, SC, 10 min pretreatment; all doses refer to salt form) in a between-session within-subjects Latin square design, with at least 48 h between each SC drug pretreatment. These doses were selected based on results of previous studies, indicating that similar doses blocked nicotine-induced hyperlocomotion and drug discrimination [e.g., (47)]. After each DHβE or vehicle injection, rats were placed into the operant chambers and allowed to self-administer nicotine during a 1-h session. After completion of the Latin square all rats received a single injection of 16.0 mg/kg DHβE (SC, 10 min pretreatment).

A subset of the same subjects ($n = 11$, plus one additional subject without prior DHβE exposure) were then allowed access to 0.06 mg/kg/inf nicotine during daily 1-h sessions. After acquisition of stable responding at this dose (defined as a minimum of five earned infusions during the 1-h session, and less than 20% variation during 3 consecutive baseline days), each rat received a single SC saline injection. Rats then received injections of DHβE (same doses and pretreatment times as above) in a between-session within-subjects Latin square design, with at least 48 h between each SC drug pretreatment. After completion of the Latin square, all rats received a single injection of 16.0 mg/kg DHβE (SC, 10 min pretreatment).

Finally, to test whether chronic 1-h daily exposure to intravenous nicotine self-administration leads to the development of dependence, rats were administered mecamylamine 24 h after the last nicotine self-administration session. Rats that had completed both DHβE dose-effect functions ($n = 5$) and control drug-naive rats with no prior nicotine experience ($n = 5$)

were given mecamylamine 24 h after the last nicotine self-administration session and observed for somatic signs of withdrawal. The dose of mecamylamine was 0.57 mg/kg, administered subcutaneously 10 min before the observation of somatic signs of withdrawal. This dose was selected based on preliminary data indicating mecamylamine precipitation of nicotine withdrawal with this dose. For assessment of somatic withdrawal signs, each rat was placed in a cylindrical plastic observation chamber, and the frequency of abstinence symptoms was recorded for 10 min using an opiate-abstinence scale modified to score nicotine abstinence (19,30). The somatic signs recorded were eyeblinks, body shakes, chews, cheek tremor, escape attempts, foot licks, gasps, genital licks, hops, head shakes, ptosis, scratches, teeth chattering, writhes, and yawns. Multiple successive counts of any sign required a distinct pause between episodes and ptosis was counted a maximum of once per minute. The total number of somatic signs per 10 min observation period was defined as the sum of the number of occurrences of all the above signs.

Data Analyses

All analyses were performed using the Biomedical Computer Programs for Personal Computers Statistical Package (BMDP, Los Angeles, CA). Criterion for significance was set at the 0.05 level.

The effects of nicotine dose on the mean number of nicotine injections earned during 3-h sessions were examined using a one-factor within-subjects ANOVA. The analysis was performed on the mean of the second and third day of self-administration at each nicotine dose including the 0 mg/kg/inf dose (i.e., saline extinction condition). After observation of a significant main effect of dose, differences among individual means were examined using post hoc comparisons. Two additional within-subjects ANOVAs were performed; one ANOVA examined the stability of responding for the training dose of nicotine (0.03 mg/kg/inf) during three different phases of testing (baseline, dose–response determination, rebaseline), and the other tested for effects of order of nicotine dose presented according to the Latin square design.

The effects of mecamylamine or DHβE pretreatment on the mean percent of baseline nicotine injections during the first 10 min of the session (mecamylamine and DHβE), the first hour of a 3-h session and entire 3-h session (mecamylamine), and 1-h session (DHβE) were examined using overall one-factor within-subjects ANOVAs with antagonist dose as the within-subject factor. Analyses on the first 10 min and first hour of the session were performed to evaluate the effects of mecamylamine and DHβE on the initial phase of nicotine self-administration. Separate ANOVAs were performed on each dose of nicotine self-administered (0.03 and 0.06 mg/kg/inf). After a significant main effect of mecamylamine or DHβE dose, differences among individual dose means were examined using post hoc comparisons. Additional ANOVAs were conducted to test for effects of mecamylamine or DHβE dose order on responding for nicotine. Paired *t*-tests were conducted to examine the effects of SC saline injection prior to each Latin square compared to the effects of SC saline injection within each Latin square on the mean number of nicotine injections earned in the first hour of a 3-h nicotine self-administration session (mecamylamine) or during the 1-h session (DHβE). Finally, a one-way ANOVA was conducted on the total number of somatic withdrawal signs observed after subcutaneous mecamylamine injections.

RESULTS

Experiment 1: Nicotine Dose–Response Curve

Acquisition of stable nicotine self-administration at the training dose (0.03 mg/kg/inf) required approximately 10 days with 82% of subjects (i.e., 18 of 22 subjects) meeting criterion for acquisition of the behavior (less than 20% deviation from the mean number of injections earned in three consecutive sessions with a minimum criterion of five infusions per hour). An ANOVA ($n = 7$) revealed a significant main effect of nicotine dose on the number of nicotine injections earned during 3-h sessions of nicotine or saline self-administration, $F(4, 24) = 11.76$, $p < 0.001$ (see Fig. 1). Post hoc comparisons revealed that all nicotine doses [0.003 mg/kg/inf, $F(1, 12) = 9.39$, $p < 0.01$; 0.01 mg/kg/inf, $F(1, 12) = 29.31$, $p < 0.001$; 0.03 mg/kg/inf, $F(1, 12) = 38.29$, $p < 0.001$; and 0.06 mg/kg/inf, $F(1, 12) = 9.39$, $p < 0.01$) maintained significantly higher numbers of responses compared to saline. Further, the 0.03 mg/kg/inf nicotine dose maintained significantly higher numbers of responses than either the 0.003 mg/kg/inf, $F(1, 12) = 9.76$, $p < 0.05$, or the 0.06 mg/kg/inf, $F(1, 12) = 9.76$, $p < 0.05$, doses. Another ANOVA revealed no significant order effect of nicotine dose on the mean number of nicotine injections earned during the 3-h self-administration sessions, $F(6, 18) = 0.739$, NS.

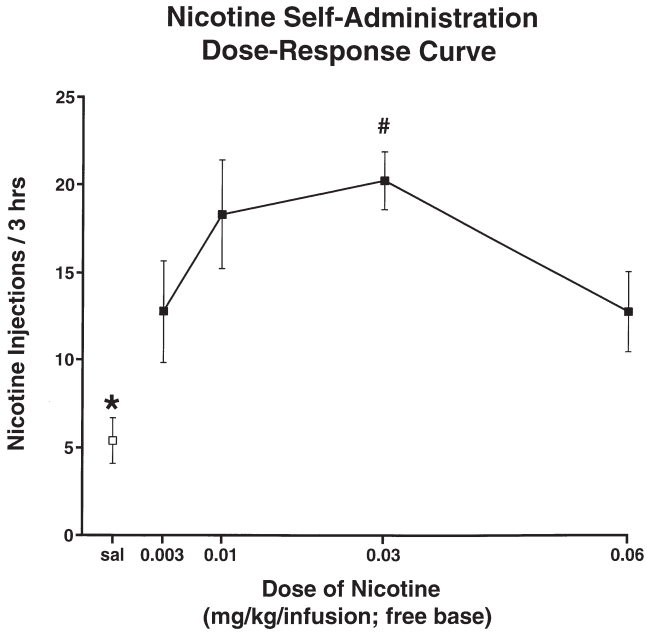

FIG. 1 Mean number of nicotine infusions earned during 3-h sessions of intravenous nicotine self-administration in male Wistar rats ($n = 7$). Closed squares represent the mean (±SEM) of the second and third days of 3 days of self-administration at each nicotine dose (0, 0.003, 0.01, 0.03, and 0.06 mg/kg/infusion; IV). The open square represents the mean (±SEM) of the second and third day after substitution of saline for nicotine. All rats were initially trained on 0.03 mg/kg/infusion nicotine. Asterisk (*) indicates that responding for saline was significantly different from responding for all nicotine doses ($p < 0.05$) by post hoc comparisons after a significant main effect in an ANOVA. Number sign (#) indicates that responding for the 0.03 dose was significantly higher than responding for the 0.003 and 0.06 mg/kg/infusion nicotine doses ($p < 0.05$). All nicotine doses listed refer to free base.

Further, the number of nicotine injections earned at the training dose of nicotine (0.03 mg/kg/inf) did not vary across three different phases of testing (mean of 2 days of self-administration at training dose under baseline conditions during three different phases of testing: baseline, during the dose/response determination, and rebaseline after the dose/response determination) (mean ± SEM = 17.42 ± 2.22, 19.25 ± 1.56, 21.83 ± 1.61, respectively), as revealed by the ANOVA, $F(2, 10) = 2.25$, $p > 0.05$. One rat was excluded from this analysis because it was initially trained on the 0.06-mg/kg/inf nicotine dose instead of the 0.03 mg/kg/inf nicotine dose.

Experiment 2: Effects of Mecamylamine Hydrochloride on Nicotine Self-Administration

An ANOVA on the effects of mecamylamine pretreatments on self-administration at the 0.03 mg/kg/inf nicotine dose revealed a significant main effect of mecamylamine dose during the first hour of a 3-h session, $F(3, 15) = 32.84$, $p < 0.001$. Post hoc comparisons indicated that the 2.0 mg/kg and 4.0 mg/kg mecamylamine pretreatments significantly reduced the number of nicotine injections earned during the first hour of the 3-h nicotine self-administration session compared to pretreatment with either 0 or 1.0 mg/kg mecamylamine ($p < 0.01$) (see Fig. 2A).

Similarly, an ANOVA on the effects of mecamylamine pretreatments on nicotine self-administration at the 0.06-mg/kg/inf nicotine dose revealed a significant main effect of mecamylamine dose during the first hour of a 3-h nicotine self-administration session, $F(3, 18) = 3.57$, $p < 0.05$. Post hoc comparisons indicated that the 1.0 mg/kg, $F(1, 12) = 4.89$, $p < 0.05$, 2.0 mg/kg, $F(1, 12) = 9.88$, $p < 0.05$, and 4.0 mg/kg, $F(1, 12) = 5.014$, $p < 0.05$, mecamylamine pretreatments significantly reduced the number of nicotine injections earned during the first hour of a 3-h session compared to pretreatment with saline (see Fig. 2B). Overall, ANOVAs conducted to examine the effects of mecamylamine pretreatments on the first 10 min of the session, one each at the 0.03- and 0.06-mg/kg/inf nicotine doses revealed no significant effects of mecamylamine pretreatments on the number of nicotine injections earned, Fs < 1, NS. ANOVAs conducted to test the effects of mecamylamine dose order on responding for nicotine injections during the first hour of a 3-h session revealed no significant effects of dose order at the 0.03 mg/kg/inf, $F(3, 15) = 1.53$, NS, and 0.06 mg/kg/inf, $F(3, 18) = 1.10$, NS nicotine doses.

An ANOVA on the effects of mecamylamine pretreatments on nicotine self-administration at the 0.03 mg/kg/inf nicotine dose revealed a significant main effect of mecamylamine for the entire 3-h session, $F(3, 15) = 7.85$, $p < 0.01$. Post hoc means comparisons indicated that the 4.0 mg/kg mecamylamine (mean = 6.0; SEM = 1.1) pretreatment significantly reduced the number of injections earned during the entire 3-h session when compared to injections earned after either the 0 mg/kg [mean ± SEM = 17.5 ± 3.11, $F(1, 11) = 15.72$, $p < 0.01$] or the 1.0 mg/kg [mean ± SEM = 18.4 ± 4.95, $F(1, 11) = 18.57$, $p < 0.01$] mecamylamine doses. A similar ANOVA on the effects of mecamylamine pretreatments on nicotine self-administration at the 0.06-mg/kg/inf nicotine dose revealed no significant effects of mecamylamine for the entire 3-h session, $F(3, 18) = 0.870$, NS.

Finally, a t-test conducted to examine the effects of SC saline injection prior to the Latin square (mean ± SEM = 8.0 ± 0.95) compared to the effects of SC saline injection within the Latin square (mean ± SEM = 8.4 ± 0.66) on the mean number of nicotine injections earned in the first hour of a 3-h self-administration session revealed no significant differences between responding for nicotine after SC saline injections at the two different time points, $t(12) = 0.37$, NS.

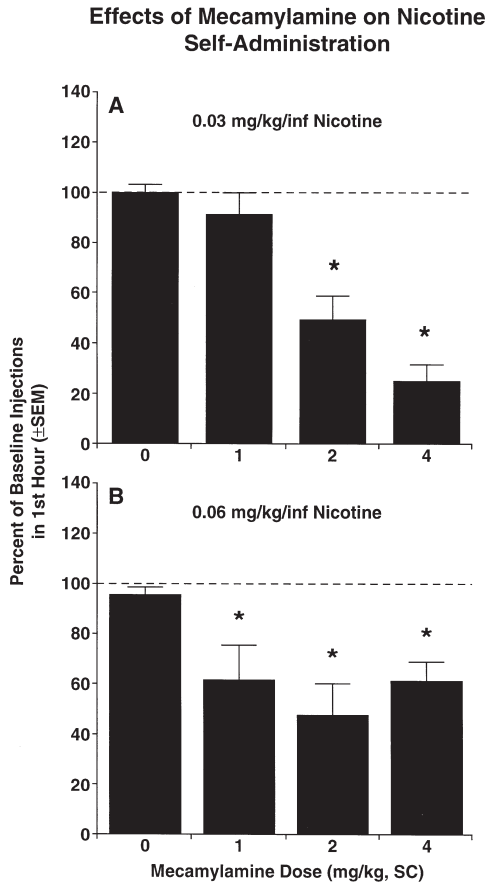

FIG. 2 Effects of mecamylamine hydrochloride (0, 1.0, 2.0, 4.0 mg/kg, SC, administered 20 min prior to session; doses refer to the salt form) on nicotine self-administration (A: 0.03 mg/kg/inf nicotine, $n = 6$; B: 0.06 mg/kg/inf nicotine, $n = 7$) during the first hour of a 3-h session. Values represent the mean percent (±SEM) of baseline nicotine self-administration rates during the first hour of daily 3-h sessions. Asterisks (*) indicate statistically significant differences from the saline (0 mg/kg) and the 1 mg/kg mecamylamine condition in Fig. 2A, and from the saline (0 mg/kg) condition in Fig. 2B, ($p < 0.05$) by post-hoc comparisons after a significant main effect in an ANOVA.

Experiment 3: Effects of DHβE on Nicotine Self-Administration

An ANOVA on the effects of DHβE pretreatments on self-administration at the 0.03 mg/kg/inf nicotine dose revealed a significant main effect of DHβE dose on number of injections, $F(4, 52) = 13.74$, $p < 0.001$. Post hoc comparisons indicated that pretreatments with all doses of DHβE significantly reduced the number of infusions earned during the session compared to pretreatment with 0 mg/kg DHβE (2.0 mg/kg = $p < 0.05$, all others $p < 0.01$). Further, a dose-dependent effect of DHβE pretreatment on self-administration was indicated by the observation that 16.0-mg/kg DHβE pretreatment

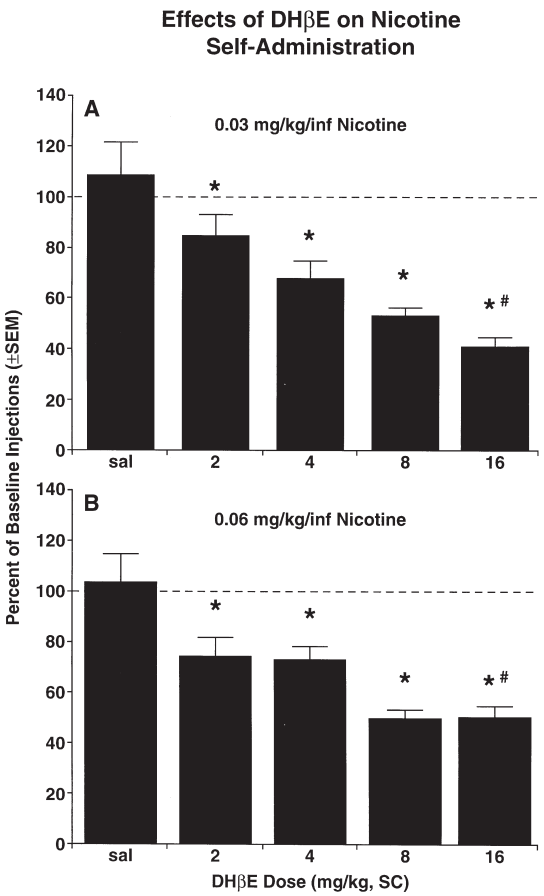

FIG. 3 Effects of dihydro-β-erythroidine (0, 2.0, 4.0, 8.0, 16.0 mg/kg, SC, administered 10 min prior to session; doses refer to the salt form) on nicotine self-administration (A: 0.03 mg/kg/inf nicotine, $n = 14$; B: 0.06 mg/kg/inf nicotine, $n = 12$). Values represent the mean percent (±SEM) of baseline nicotine self-administration rates during 1-h sessions. Asterisks (*) indicate statistically significant differences from the saline vehicle condition ($p < 0.05$) by post hoc comparisons after a significant main effect in an ANOVA. Number signs (#) indicate statistically significant differences from 2 and 4 mg/kg DHβE ($p < 0.05$).

significantly reduced the number of nicotine infusions earned compared to pretreatment with 2.0 or 4.0 mg/kg DHβE ($p < 0.01$ and $p < 0.05$, respectively) (see Fig. 3A).

Similarly, an ANOVA on the effects of DHβE pretreatments on self-administration at the 0.06-mg/kg/inf nicotine dose revealed a significant main effect of DHβE dose, $F(4, 44) = 15.08$, $p < 0.001$. Post hoc comparisons indicated that pretreatment with all doses of DHβE significantly reduced the number of nicotine infusions earned during the session compared to pretreatment with 0 mg/kg DHβE, ($p < 0.01$). Further, post hoc comparisons indicated a dose-dependent effect of DHβE dose with 8.0 and 16.0 mg/kg pretreatment with DHβE significantly reducing the number of nicotine infusions earned compared to pretreatment with 2.0 or 4.0 mg/kg DHβE ($p < 0.05$), (see Fig. 3B).

Overall ANOVAs conducted to examine the effects of DHβE pretreatments on the initial 10 min of nicotine self-administration, one each at the 0.03 mg/kg/inf and 0.06 mg/kg/inf nicotine doses, revealed no significant effects of DHβE pretreatment on the number of infusions earned (Fs < 1, NS). Two separate overall ANOVAs conducted to test the effects of DHβE dose order on responding for nicotine at the 0.03 and 0.06 mg/kg/inf nicotine doses revealed no significant effects of dose order on self-administration behavior (Fs < 1, NS).

Two separate t-tests conducted to compare the effects of SC saline infusions prior to each Latin square (0.03 mg/kg/inf: mean ± SEM = 11.29 ± 0.62; 0.06 mg/kg/inf: mean ± SEM = 8.58 ± 0.38) with the effects of SC saline injection within each Latin square (0.03 mg/kg/inf: mean ± SEM = 12.64 ± 1.03; 0.06 mg/kg/inf: mean ± SEM = 8.08 ± 0.54) on the mean number of nicotine infusions earned revealed no significant differences [0.03 mg/kg/inf: $t(14) = 0.55$, NS; 0.06 mg/kg/inf: $t(12) = 0.97$, NS]. Finally, a one-way ANOVA on the number of somatic signs observed after subcutaneous administration of mecamylamine revealed no differences between the rats self-administering nicotine (mean ± SEM = 4.57 ± 0.57) and controls (mean ± SEM = 4.71 ± 1.49), $F(1, 12) = 0.30$, NS.

DISCUSSION

Development of animal models of intravenous nicotine self-administration is critical to the continued investigation of the neurobiological substrates of nicotine reinforcement. The first experiment demonstrated that nicotine is self-administered by rats across a range of doses, with the highest number of infusions earned at the 0.03 mg/kg/inf dose. All doses of nicotine tested maintained significantly higher levels of responding than did saline. Further, while the nicotine unit doses were not adjusted for each individual rat, the training dose of nicotine (0.03 mg/kg/inf) maintained stable responding across three different phases of self-administration testing over a period of approximately 4 weeks, indicating that the slight fluctuations in body weight over the duration of the experiment did not influence nicotine intake. Moreover, there was no effect of order of presentation of the various nicotine doses. Finally, animals were not dependent on nicotine, as indicated by the absence of somatic withdrawal symptoms after injection of mecamylamine at a dose that precipitated somatic signs in nicotine-dependent rats (31). In summary, the results indicate that intravenous nicotine is consistently self-administered across a range of doses by rats and that this behavior is not maintained by a drug-dependent state when 1-h daily self-administration sessions are used.

The model of nicotine self-administration described here confirms and extends the results of previous demonstrations of intravenous nicotine self-administration in rats. The total number of infusions per session, dose range, and shape of the dose–response curve are similar to those reported previously (7,16,17). There are, however, a number of differences in the present study from previous investigations of intravenous nicotine self-administration in rats that indicate a greater generality of the reinforcing effects of nicotine than has been posited by others (18). The present study used the Wistar rat strain that has been used in other successful studies of nicotine self-administration (5,12). Thus far, nicotine self-administration in drug-naive rats has been obtained in Long–Evans (7), Sprague–Dawley (16,17,43,44), Holtzmann (51), and Wistar [present study; (5,12)] rat strains, while others have been unable to obtain intravenous nicotine self-administration in the Fisher F-344 rat (18). Nicotine self-administration also has been obtained in Sprague–Dawley rats that had been previously trained to self-administer cocaine (5). In addition, the present experiments used a 20 s timeout period after the drug

infusion, during which time infusions were unavailable, while other studies in rats that have not been previously trained on cocaine have used 60 s (7–11,16,17) or 30 s (5) timeout periods. Using a short timeout period (20 s) indicated that the decreases in self-administration behavior observed after the antagonist administration were not due to the schedule contingencies preventing the subjects from self-administering nicotine in sufficiently rapid succession to compensate for the effects of the antagonists [(3), see below]. Finally, substitution of saline for nicotine resulted in more rapid extinction in the present study than that reported by others [3–6 days for the present study vs. 4–6 days (16), 5–10 days (7), and 4–13 days (5)]. This difference in the rate of extinction is most likely due to the more rapid extinction typically observed with the use of an FR1 schedule of reinforcement, as in the present study, compared to either an FR5 (7) or an FR3 (5) schedule of reinforcement.

The results also demonstrated that subcutaneous pretreatment with the noncompetitive nAChR antagonist mecamylamine hydrochloride or the competitive nAChR antagonist DHβE hydrobromide dose-dependently reduced self-administration of both 0.03 and 0.06 mg/kg/inf nicotine doses. These results are similar to those obtained in other rat and primate studies using mecamylamine (7,21–24,46). These decreases in responding are not likely attributable to nonspecific effects of mecamylamine or DHβE infusions because the effects of these two antagonists on other measures of motor performance provided by an intracranial self-stimulation paradigm (29,32,33) indicated no behaviorally depressant effects at these antagonist doses (unpublished observations). Thus, the attenuating effects of the nAChR antagonists on responding for nicotine likely reflect a selective alteration of the properties of nicotine induced through blockade of nAChRs.

The decrease in nicotine self-administration after pretreatment with the antagonists demonstrated no compensatory increases in responding. Similar findings have been reported in studies using Long–Evans rats (7) and monkeys (24) with mecamylamine. Nevertheless, in laboratory tests with humans, mecamylamine has been shown to increase cigarette smoking up to 30%, and this finding is thought to reflect compensatory, self-titration behavior (37,39,48). The discrepancy between the human data and the animal data may reflect a difference in the dependence state of the organism. Rats in the present studies were exposed to daily nicotine for short periods of time, and hence, these subjects were not dependent on nicotine, as indicated by the lack of somatic withdrawal signs after subcutaneous administration of mecamylamine. A dependent state may be necessary to observe increases in nicotine intake after nAChR blockade, such that in humans, increasing smoking after mecamylamine administration reflects a stronger motivation for nicotine, presumably due to dependence. In addition, the self-administration dose-response function for nicotine is different than that for cocaine (3) or heroin (36), with most self-administered nicotine doses being on the ascending limb of the function. One hypothesis may be that antagonism of the nAChR blocks the reinforcing effects of intravenous nicotine and that at high doses, nicotine has both reinforcing and aversive properties that prevent the animals from increasing responding in an attempt to overcome nAChR blockade. Further investigation of the aversive component to nicotine is warranted.

DHβE has a high affinity for α4β2 and α4β4 receptor subtypes (25,26). Nicotine has the highest affinity for the α4β2 nAChR subtype, and thus, antagonists selective for this receptor subtype appear to be particularly effective in blocking the actions of nicotine (52). Autoradiography and in situ hybridization studies have indicated that various nAChR α and β subunit combinations are present throughout the mesolimbic dopamine pathway, including the ventral tegmental area, prefrontal cortex, amygdala, septal area, and nucleus accumbens, with strong α4 subunit expression in the ventral tegmental area and the substantia nigra (15,34,41). It has been hypothesized that nicotinic receptors located on cell bodies in the ventral tegmental area play a more important role in nicotine reinforcement than those in the nucleus accumbens based on the effects of nicotinic receptor activation in these two brain sites on dopamine release (38). That is, continuous infusion of nicotine into the ventral tegmental area has been shown to produce a longer lasting increase in extracellular dopamine levels in the nucleus accumbens than nicotine infused into the nucleus accumbens (38). Therefore, antagonists that block activity in the nAChR located on cell bodies in the ventral tegmental area region may block the reinforcing actions of nicotine. Infusions of DHβE into the ventral tegmental area produced a significant decrease in nicotine self-administration behavior (11), supporting the hypothesis of the involvement of local nAChRs in the ventral tegmental area in nicotine reinforcement. The current results indicate that systemic administration of DHβE also produces a decrease in nicotine self-administration. Because both DHβE (14) and mecamylamine (35) readily cross the blood–brain barrier, it is speculated that the attenuation of nicotine self-administration behavior is due to antagonism of nAChRs located on cell bodies in the ventral tegmental area, thus blocking nicotine-induced dopamine release in the nucleus accumbens and decreasing the reinforcing properties of nicotine.

In conclusion, the model of nicotine self-administration reported here expands the number of conditions under which intravenous nicotine has been shown to act as a reinforcer in rats. In addition, the results indicate that exposure to nicotine during daily 1-h sessions does not induce a state of drug dependence. Furthermore, these studies have shown that systemic administration of both the noncompetitive nAChR antagonist mecamylamine hydrochloride and the competitive nAChR antagonist dihydro-β-erythroidine hydrobromide blocked responding for intravenous nicotine, indicating that activation of nAChRs is involved in the reinforcing actions of nicotine. In summary, results suggest that intravenous self-administration of nicotine can provide a reliable model for the reinforcing effects of nicotine in nondependent rats, and that pharmacological antagonism of nicotinic acetylcholine receptors alters this reinforcing action.

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