Pharmacology Biochemistry and Behavior, Vol. 60, No. 2, pp. 567–573, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(98)00003-3

Reinforcing Effects of Nicotinic Compounds: Intravenous Self-administration in Drug-Naive Mice

THØGER RASMUSSEN AND MICHAEL D. B. SWEDBERG

Novo Nordisk A/S, Behavioral Pharmacology, Novo Nordisk Park, DK-2760 Måløv, Denmark

Received 11 April 1997; Revised 19 December 1997; Accepted 19 December 1997

RASMUSSEN, T. AND M. D. B. SWEDBERG. *Reinforcing effects of nicotinic compounds: Intravenous self-administration in drug-naive mice.* PHARMACOL BIOCHEM BEHAV **60**(2) 567–573, 1998.—The nicotinic compounds (−)-cytisine, (−)-lobeline, (±)-epibatidine, *(S)*-3methyl-5-(1-methyl-2-pyrrolidinyl)isoaxzole (ABT-418), (−)-nicotine, and cocaine were compared in an acute self-administration model using drug-naive mice that could self-administer intravenous infusions contingent on nose poking (fixed ratio 1 with no time out). Although the nose pokes of yoked control mice were unaffected by unit dose, inverted U-shaped unit dose–response curves were seen with cocaine (up to 0.26 mg/kg/infusion), nicotine (up to 0.175 mg/kg/infusion), cytisine (up to 0.125 mg/kg/infusion), and lobeline (up to 1.25 mg/kg/infusion) in mice receiving infusions contingent upon nose poke responses. Epibatidine (up to 1.25 μg/kg/infusion) and ABT-418 (up to 0.125 mg/kg/infusion) failed to exhibit inverted U-shaped unit dose–response curves. The present studies demonstrate that cytisine and lobeline, but not ABT-418 or epibatidine, were self-administered by drug-naive mice in a manner similar to cocaine and nicotine. These findings are discussed in terms of potency and selectivity at the $\alpha_4\beta_2$ nicotinic acetylcholine receptor subunit combination. © 1998 Elsevier Science Inc.

(−)-Nicotine (−)-Cytisine (−)-Lobeline (±)-Epibatidine (*S*)-3-methyl-5-(1-methyl-2pyrrolidinyl)isoaxzole ABT-418 Cocaine Mouse Drug-naive Self-administration

DRUGS of abuse have been shown to maintain operant responding in several species under a variety of schedules of reinforcement (18,21,33). Traditionally, drug self-administration studies have been conducted using chronic preparations (18,21,33). Typically, in a chronic procedure animals are used as their own controls so that full unit dose–response curves are obtained from each animal, thus requiring a lengthy period of time to fully assess a compound. Recently, studies have appeared utilizing an acute mouse model, enabling rapid assessment of the reinforcing properties of a compound. For example, cocaine (22) and morphine (6,22,23,28) were dose dependently self-administered intravenously in drug-naive mice. Nicotine, long known for its abuse liability and dependence potential (30,33), has recently been shown to possess similar self-administration properties in mice (24). Even though this acute model is assumed to assess the initiation but not chronic maintenance of self-administration behavior, the data available so far indicate a high degree of correspondence to results from chronic models utilizing, for example, monkeys or rats (see below).

Cocaine and morphine have been shown to activate brain dopaminergic systems (13). Dopamine release in the nucleus accumbens has been suggested to serve as the basis for the reinforcing effects of self-administered cocaine and opiates (21). Nicotine activates heterogenously distributed subtypes of nicotinic acetylcholine receptors (nAChR) (12), some of which have been demonstrated to cause dopamine release in the nucleus accumbens (26). Hence, nicotine's reinforcing effects may also be attributed to activation of a common dopaminergic pathway (32).

In recent years, an interest in nicotine and nicotinic compounds for treatment of Alzheimer's disease has developed after several demonstrations of cognition enhancing effects of these drugs (9,12,27). These findings have formed the basis for the synthesis of nicotinic analogues, such as, for example, the newly synthesized (*S*)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoaxzole (ABT-418), aimed at the treatment of Alzheimer's disease (1,10,11). Nicotine's known abuse potential necessitates careful attention to the abuse liability issue in the search for novel AChR ligands with therapeutic efficacy.

Requests for reprints should be addressed to Thøger Rasmussen, Novo Nordisk A/S, Behavioral Pharmacology, Novo Nordisk Park, DK-2760 Måløv, Denmark.

In the present studies, we used an acute intravenous self-administration procedure in drug-naive mice to assess potential reinforcing properties of the nicotinic compounds cytisine, lobeline, epibatidine, and ABT-418. Nicotine, lobeline, and cytisine have previously been shown to have different receptor binding and pharmacological profiles (25,29). Epibatidine is a newly synthesized alkaloid from the poison frog *Epipedobates tricolor* with nicotinic binding and pharmacology (2,7, 15). ABT-418 is a recently synthesized cholinergic channel activator with nicotinic pharmacology (8,10). Although certain behavioral and receptor binding properties of these compounds differ from those of nicotine (see below), they have been shown to share with nicotine the ability to increase acetylcholine activity via heterogenously distributed subtypes of nAChR (1,10,12). Cocaine (22) and nicotine (24), previously demonstrated to maintain self-administration in drug-naive mice, were used as reference compounds.

METHOD

Animals

Male Bom:NMRI mice (n = 764 pairs (1528 mice)) (Bomholtegaard, Ry, Denmark), weighing 20–22 g, were used. Upon arrival, mice were housed 20 per cage for 1–3 days in a 12 L:12 D cycle with lights on at 0600 h, 40–70% relative humidity, and free access to food (Altromin® 1324, Brogården, Copenhagen, Denmark) and water (1% citric acid, pH 2–3). Twenty-four hours before testing, mice were grouped four per cage, from which they were transferred to the test boxes immediately prior to the onset of the experiment. The present studies were conducted in accordance with the Declaration of Helsinki and approved by the Danish Committee for Animal Research.

Drugs

Cocaine HCl, (−)-nicotine tartrate, (−)-cytisine HCl, and (−)-lobeline HCl were obtained from Sigma, St. Louis, MO. (±)-Epibatidine HBr and ABT-418 HBr were synthesized at Novo Nordisk A/S according to previously published protocols (5,16). All drugs were freshly prepared and dissolved in 0.9% saline. Doses refer to the forms indicated.

Apparatus

Boxes were designed as previously described (6) and modified as follows. Each box was made of transparent 3-mm plastic (8 × 8 × 8 cm inner size) with one centered frontal hole (12 mm diameter) 1 cm above the box floor and one centered posterior vertical chink (5 mm wide). Dual photo cells (WE6-N136, SICK optik elektronik, Frankfurt AM, Germany) positioned at the lateral sides of each test box projected an infrared beam 1 mm in front of the anterior wall at the level of the center of the nose poke hole. Test boxes were placed side by side with interposed shields to prevent the mice from seeing each other. Dual fluorescent tubes (Philips TLD 36W/83) supplied diffuse top lighting 110 cm above the box floors.

Technique

Mice were placed 40 cm below a 250-Watt infrared light bulb for 2–3 min to provide vasodilitation of the tail to ease insertion of the infusion needle in the right lateral tail vein. In the test box, the mouse tail extended through the chink such that the entire length of the tail remained outside the box. Immediately outside the chink, the tail was fixed using double adhesive tape (tesa®, Beiersdorf AG, Hamburg, Germany). A nose poke through the anterior hole of the box interrupted the infrared beam, activating the photocell connected to an interface (SG 502, Med Associates Inc., East Fairfield, VT). Each nose poke of the contingent mouse activated a syringe pump (PHM-100A, Med Associates Inc.) holding a 20-ml syringe (OMNIFIX®, B. Braun Melsungen AG, Melsungen, Germany) to which a 75-cm PVC tubing (inner diameter 0.9 mm; Original-Perfusor® Leitung MR, B. Braun Melsungen AG) was connected. Between the tube and the infusion needle (0.45 × 12 mm, Terumo Europe N.V., Leuven, Belgium), a backcheck valve (Safsite BC-1000, Burron Medical Inc., Bethlehem, PA) prevented reflux of blood. Experiments were controlled and behavior was recorded using a PC and MED-PC 2.09 software (Med Associates Inc.).

Procedure

Testing was performed between 0800 and 1600 h, and conducted such that high and low doses were tested in the morning and in the afternoon in a counterbalanced fashion. Testing of each drug covered 2 days, and the vehicle control mice were tested on both days (approximately half in the morning and half in the afternoon) with data subsequently pooled. A between-subject design was used with each mouse tested once only, on vehicle (0.9% saline) or drug. Each mouse was assigned to either a contingent or a yoked control group. Contingent and yoked control mice were paired with one another such that both mice received an intravenous infusion of either vehicle or drug upon each nose poke of the contingent mouse. A fixed ratio 1 (FR1) schedule with no time out was used, and an unlimited number of infusions were available during the session. Infusions were of 0.3-s duration. Any nose pokes of the yoked control mice were recorded, but did not produce infusions. The use of yoked controls has been suggested for the purpose of evaluating potential drug-induced psychomotoric side effects such as, for example, behavioral stereotypy (6). It is assumed that drug self-administration in the contingent mouse results from reinforcing drug properties only to the extent that the paired yoked control mouse makes a significantly lower number of nose pokes compared to the contingent mouse. At the beginning of the experiment, one 1.4 μl priming infusion of the respective test condition (vehicle or drug) was made by the experimenter after insertion of the needle. After the priming infusion followed a 10-min habituation period with no infusions. Following the habituation period the self-administration session started, and both the contingent and the yoked control mice received an intravenous infusion of 1.4 μl of either vehicle or drug upon each nose poke of the contingent mouse. Sessions terminated after 30 min (habituation period excluded). Any pair of mice (contingent and yoked control) were removed from further analysis if one or more of the following exclusion criteria were met in at least one mouse of a pair: 1) the contingent mouse produced less than one nose poke (based on the assumption that any associative learning about reinforcing properties of a self-administered drug necessarily must be based upon at least one such pairing of events); 2) a mouse was found dead at the end of the trial; 3) the hind leg of a mouse extended through the chink of the test box at the end of the trial; 4) proper intravenous insertion of the needle could not be verified after the trial by observing free flow of approximately 20–30 μl of experimenter-administered infusion medium. Proper insertion was defined as: (a) the tail retaining its natural color, and (b)

the vein immediately above the needle tip loosing its dark bluish appearance due to the influx of the experimenter-administered infusion medium. Improper insertion was defined as observing blanching of the tail. As a final exclusion criterion; 5) a pair of mice was excluded if, when disconnecting a mouse from the IV catheter, an occlusion of the needle was observed such that no infusion medium passed through the needle when the syringe pump was manually activated.

Data Analysis

A total of 152 pairs of mice were excluded from data analysis according to the criteria mentioned above. Unit dose of drug including vehicle were used as the independent variable, and the mean numbers of nose pokes (±SEM), and the cumulative amounts of self-administered drug (±SEM) were used as the dependent variables. A two-way ANOVA was used to evaluate effects of group (contingent and yoked control), unit dose (including vehicle), and interactions between group and unit dose. A one-way ANOVA was used to evaluate the effect of single unit dose on cumulative amounts of self-administered drug. For post hoc testing, Scheffé's *F* procedure was used to evaluate single comparisons between groups of contingent drug mice and respective contingent vehicle mice, and Fisher's PLSD (Protected Least Significance Difference) procedure was used to evaluate single comparisons between cumulative amounts of drug. The rationale for comparing single groups of contingent drug mice with respective group of contingent vehicle mice was to identify unit doses of respective drug maintaining self-administration behavior, i.e., to determine that the infusion itself does not produce self-administration. The rationale for comparing contingent mice with yoked control mice was to control for nose poking behavior being maintained by contingent infusions and not by, for example, drug-induced psychomotoric side effects possibly produced by noncontingent infusions. The rationale for comparing cumulative amounts of self-administered drug as a function of unit dose was to identify doses at which titration may occur. The minimum effective unit dose (MED) was defined as the lowest unit dose significantly increasing the number of nose pokes above the level of contingent vehicle. Doses were expressed as mg/kg or μg/kg per infusion. Statistical tests were calculated using StatView® v4.0 for Macintosh. Significance levels of 0.05 were chosen for statistical tests.

RESULTS

Cocaine, nicotine, cytisine, and lobeline all maintained self-administration in contingent mice, yielding inverted U-shaped dose–response curves significantly different from yoked control mice. The dose–response curve of ABT-418 appeared flat with no significant difference between contingent mice and yoked control mice. Epibatidine induced higher nose-poke rates in yoked control mice relative to contingent mice.

Cocaine (Fig. 1) showed significant main effects on nose poking rates of group, $F(1, 118) = 14.06$, $p < 0.001$, unit dose, $F(4, 118) = 2.87$, $p < 0.05$, and group by unit dose interaction, $F(4, 118) = 2.47$, $p < 0.05$. In the contingent cocaine mice, the MED and the unit dose at which the number of nose pokes peaked was 0.19 mg/kg/infusion ($p < 0.01$ relative to contingent vehicle control mice). At 0.26 mg/kg/infusion the number of nose pokes maintained by cocaine decreased to yield a nonsignificant difference from contingent vehicle mice. A significant main effect of unit dose on cumulative amounts of self-administered drug, $F(4, 47) = 9.02$, $p < 0.001$, was observed,

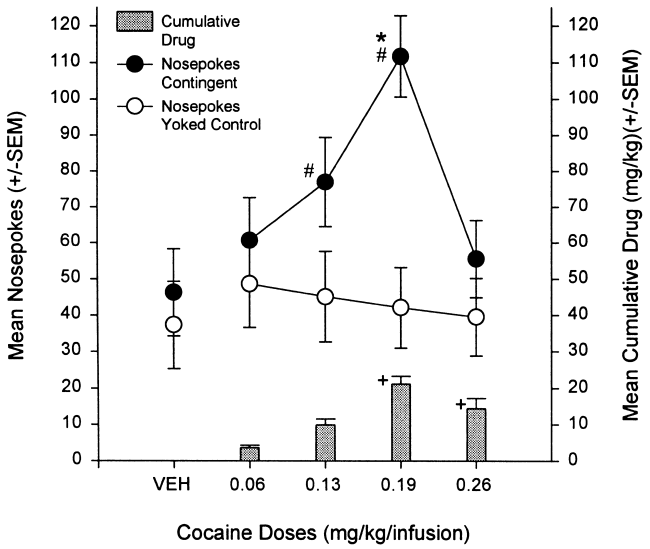

FIG. 1. Cocaine HCl self-administered in drug-naive mice. Shown are mean numbers of nose pokes (±SEM) in contingent mice and yoked control mice, and cumulative amounts of self-administered drug. *$p < 0.05$ compared to respective contingent vehicle control, #$p < 0.05$ compared to respective yoked control, +$p < 0.05$ compared to cumulative amounts of self-administered drug at 0.06 mg/kg/infusion. Numbers of pairs (*n*) (contingent and yoked control mouse) are: vehicle $n = 12$; cocaine 0.06 $n = 12$; cocaine 0.13 $n = 11$; cocaine 0.19 $n = 14$; and cocaine 0.26 $n = 15$.

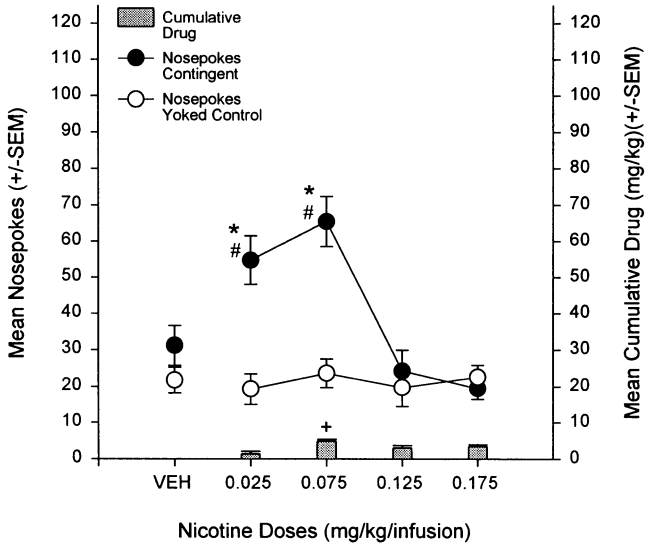

FIG. 2. (−)-Nicotine tartrate self-administered in drug-naive mice. Shown are mean numbers of nose pokes (±SEM) in contingent mice and yoked control mice, and cumulative amounts of self-administered drug. *$p < 0.05$ compared to respective contingent vehicle control, #$p < 0.05$ compared to respective yoked control, +$p < 0.05$ compared to cumulative amounts of self-administered drug at 0.025 mg/kg/infusion. Numbers of pairs (*n*) (contingent and yoked control mouse) are: vehicle $n = 18$; nicotine 0.025 $n = 21$; nicotine 0.075 $n = 25$; nicotine 0.125 $n = 16$; and nicotine 0.175 $n = 21$.

with 0.19 and 0.26 mg/kg/infusion being significantly different ($p < 0.05$) relative to lowest drug unit dose (0.06 mg/kg/infusion).

Nicotine (Fig. 2) showed significant main effects on nose-poking rates of group, $F(1, 224) = 32.05$, $p < 0.01$, unit dose, $F(4, 224) = 7.56$, $p < 0.01$, and group by unit dose interaction, $F(4, 224) = 7.07$, $p < 0.01$. In the contingent nicotine mice, the MED was 0.025 mg/kg/infusion ($p < 0.01$ relative to contingent vehicle control mice) and the unit dose at which the number of nose pokes peaked was 0.075 mg/kg/infusion ($p < 0.01$ relative to contingent vehicle control mice). At 0.125 mg/kg/infusion the number of nose pokes maintained by nicotine decreased to yield a nonsignificant difference from contingent vehicle mice. A significant main effect of unit dose on cumulative amounts of self-administered drug, $F(4, 78) = 4.74$, $p < 0.05$, was observed with 0.075 mg/kg/infusion being significantly different ($p < 0.05$) relative to lowest drug unit dose (0.025 mg/kg/infusion).

Cytisine (Fig. 3) showed significant main effects on nose-poking rates of group, $F(1, 218) = 17.36$, $p < 0.001$, unit dose, $F(5, 218) = 5.52$, $p < 0.001$, and a nonsignificant group by unit dose interaction. In the contingent cytisine mice, the MED and the unit dose at which the number of nose pokes peaked was 0.025 mg/kg/infusion ($p < 0.05$ relative to contingent vehicle mice). At 0.05 mg/kg/infusion the number of nose pokes maintained by cytisine decreased to yield a nonsignificant difference from contingent vehicle mice. A significant main effect of unit dose on cumulative amounts of self-administered drug, $F(4, 92) = 4.71$, $p < 0.05$, was observed with 0.075 mg/kg/infusion being significantly different ($p < 0.05$) from lowest drug unit dose (0.025 mg/kg/infusion).

Lobeline (Fig. 4) showed significant main effects on nose poking rates of group, $F(1, 238) = 12.68$, $p < 0.001$, unit dose, $F(5, 238) = 3.08$, $p < 0.01$, and group by unit dose interaction, $F(5, 238) = 4.41$, $p < 0.001$. In the contingent lobeline mice, the unit dose at which the number of nose pokes peaked was 0.5 mg/kg/infusion (nonsignificant relative to contingent vehicle mice). A significant main effect of unit dose on cumulative amounts of self-administered drug, $F(4, 99) = 4.86$, $p < 0.05$, was observed with unit doses of 0.5, 0.75, and 1 mg/kg/infusion being significantly different ($p < 0.05$) relative to lowest drug unit dose (0.25 mg/kg/infusion).

ABT-418 (Fig. 5) showed nonsignificant main effects on nose poking of group, unit dose, and group by unit dose interaction. In contingent ABT-418 mice, the unit dose at which the number of nose pokes peaked was 0.125 mg/kg/infusion (nonsignificant relative to contingent saline control mice). A significant main effect of unit dose on cumulative amounts of self-administered drug, $F(4, 96) = 11.48$, $p < 0.001$, was observed with 0.1 and 0.125 mg/kg/infusion being significantly different ($p < 0.05$) from lowest drug unit dose (0.025 mg/kg/infusion).

Epibatidine (Fig. 6) showed a significant main effect on nose-poking rates of group, $F(1, 212) = 7.46$, $p < 0.01$, a nonsignificant main effect of unit dose, and a nonsignificant group by unit dose interaction. The main effect of group was due to a higher number of nose pokes of yoked control (mean = 45.65, SEM = 4.89) as opposed to contingent (mean = 30.36, SEM = 2.52) mice. A nonsignificant main effect of unit dose on cumulative amounts of self-administered drug was observed.

DISCUSSION

The present data confirm previous findings that drug-naive mice dose dependently will self-administer intravenous infusions of nicotine (24) and cocaine (22). Similar dose–response

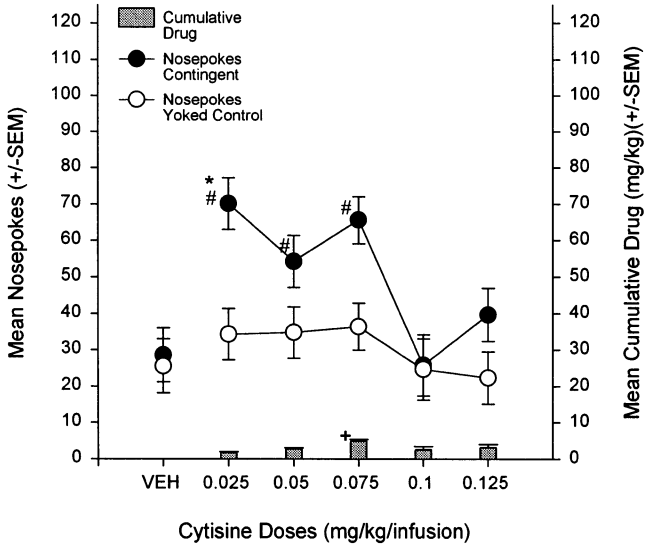

FIG. 3. (−)-Cytisine HCl self-administered in drug-naive mice. Shown are mean numbers of nose pokes (±SEM) in contingent mice and yoked control mice, and cumulative amounts of self-administered drug. *$p < 0.05$ compared to respective contingent vehicle control, #$p < 0.05$ compared to respective yoked control, +$p < 0.05$ compared to cumulative amounts of self-administered drug at 0.025 mg/kg/infusion. Numbers of pairs (*n*) (contingent and yoked control mouse) are: vehicle $n = 18$; cytisine 0.025 $n = 20$; cytisine 0.050 $n = 20$; cytisine 0.075 $n = 24$; cytisine 0.100 $n = 14$; and cytisine 0.125 $n = 19$.

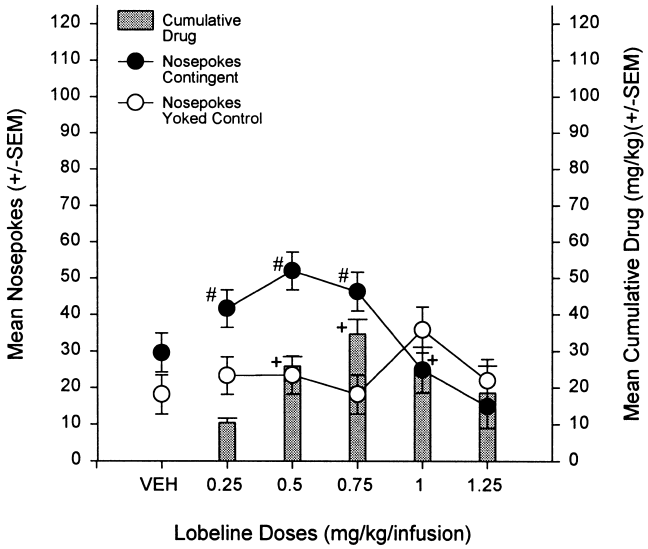

FIG. 4. (−)-Lobeline HCl self-administered in drug-naive mice. Shown are mean numbers of nose pokes (±SEM) in contingent mice and yoked control mice, and cumulative amounts of self-administered drug. #$p < 0.05$ compared to respective yoked control, +$p < 0.05$ compared to cumulative amounts of self-administered drug at 0.25 mg/kg/infusion. Numbers of pairs (*n*) (contingent and yoked control mouse) are: vehicle $n = 22$; lobeline 0.25 $n = 24$; lobeline 0.50 $n = 23$; lobeline 0.75 $n = 23$; lobeline 1.00 $n = 16$; and lobeline 1.25 $n = 18$.

effects were found with cytisine and, although short of being statistically significant, lobeline. The separation between the contingent and the yoked control groups show that nose poking was not due to drug-induced psychomotoric side effects. Although no significant group by unit dose interaction was observed for cytisine, post hoc evaluations showed a significant increase in the number of nose pokes in contingent mice self-administering cytisine, relative to contingent saline control mice, and no significant dose-dependent increases in nose pokes of yoked control mice.

Although not significantly different from vehicle control, self-administered unit doses of lobeline were 10-fold higher than those of nicotine. A similar difference in potency has also been found with regard to memory-enhancing effects in a passive avoidance task and in suppressing locomotor activity (9). It appears that lobeline has mixed pharmacological properties such that, apart from its agonistic effects, it may under some circumstances serve as a functional antagonist. For example, lobeline has been reported to fully reverse nicotine induced [^{3}H]dopamine release in rat striatal slices (12), suggesting that the reinforcing properties may depend on other than dopaminergic actions. This is particularly interesting because dopamine-releasing effects in the nucleus accumbens (26,31) has been suggested as the mechanism for the reinforcing effects of cocaine and morphine (21) and of nicotine (32). Also, differential effects of lobeline and nicotine have been reported in that lobeline failed to substitute (25) or partially substituted (34) for nicotine in nicotine drug discrimination studies. These apparent dissimilarities between lobeline and nicotine are not consistent with the present findings in that there was significant main effect of unit dose on self-administration of both lobeline and nicotine.

Cytisine was self-administered at dose levels similar to nicotine with similar unit dose–response curves. However, results from nicotine drug discrimination experiments have shown partial generalization with cytisine (4,34), whereas in rats trained to discriminate cytisine, full generalization occurred to nicotine (4). Thus, in contrast to this asymmetric generalization pattern, the reinforcing properties appear to be similar. It has been suggested that nicotine's discriminative effect is less specifically mediated than those of cytisine (4). Thus, it appears that the reinforcing effects of nicotine and cytisine are mediated by the same mechanism(s), whereas the discriminative effects of cytisine may be more specifically mediated.

The dose–response effect of epibatidine in contingent mice was unlike the pattern seen with nicotine in that epibatidine produced higher levels of nose poking in the yoked control mice than in the contingent mice. It has been shown that epibatidine binds at the neuromuscular junction nAChR (K_i 2.7 nM), at least 7000-fold more potently than nicotine (12). The increase in nose pokes of epibatidine yoked control mice may have been caused by potent muscular activation resulting in psychomotoric disturbances that affected the contingent mice and the yoked control mice differently, possibly due to the different circumstances of administration (see below).

ABT-418 appeared not to have reinforcing effects in contingent mice at any unit dose investigated, although one cannot exclude the possibility that higher unit doses may have maintained self-administration. It should be noted, however, that the presently used unit dose range of ABT-418 HBr (0.025– 0.125 mg/kg/infusion) was well above the ABT-418 unit dose of 0.015 mg/kg (~0.062 (μmol/kg) that was demonstrated to improve learning and memory in mouse passive avoidance (10). Our findings are consistent with previous data indicating that ABT-418 is less efficacious than nicotine in producing discriminative effects and less potent than nicotine in activating dopaminergic neurons in vitro in the VTA (3),

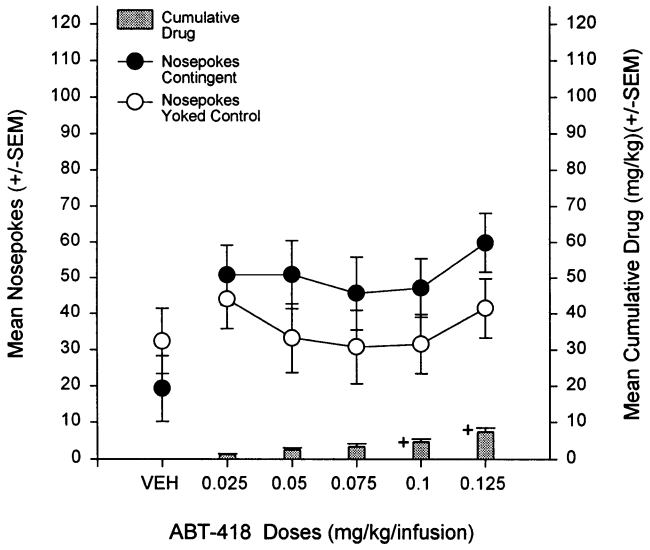

FIG. 5. ABT-418 HBr self-administered in drug-naive mice. Shown are mean numbers of nose pokes (±SEM) in contingent mice and yoked control mice, and cumulative amounts of self-administered drug. $+p < 0.05$ compared to cumulative amounts of self-administered drug at 0.025 mg/kg/infusion. Numbers of pairs (*n*) (contingent and yoked control mouse) are: vehicle $n = 19$; ABT-418 0.025 $n = 23$; ABT-418 0.050 $n = 17$; ABT-418 0.075 $n = 15$; ABT-418 0.100 $n = 23$; and ABT-418 0.125 $n = 23$.

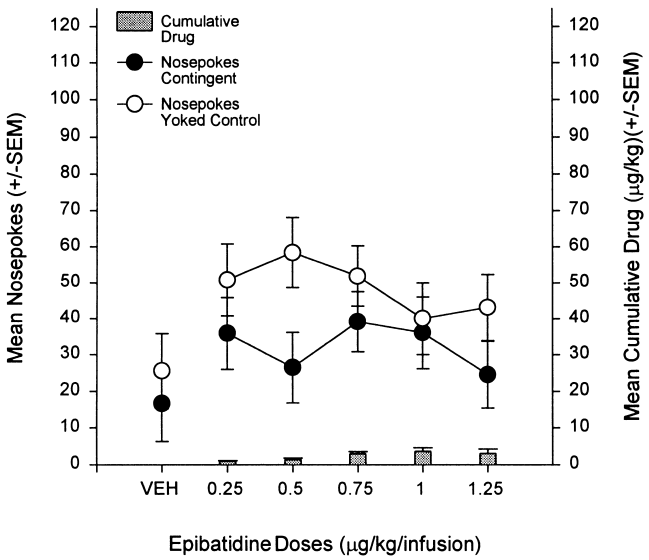

FIG. 6. (±)-Epibatidine HBr self-administered in drug-naive mice. Shown are mean numbers of nose pokes (±SEM) in contingent mice and yoked control mice, and cumulative amounts of self-administered drug. Numbers of pairs (*n*) (contingent and yoked control mouse) are: vehicle $n = 16$; epibatidine 0.25 $n = 17$; epibatidine 0.50 $n = 18$; epibatidine 0.75 $n = 24$; epibatidine 1.00 $n = 17$; and epibatidine 1.25 $n = 20$.

suggesting decreased abuse liability of ABT-418 compared to nicotine. The lack of separation between contingent and yoked control ABT-418 mice might be attributed to a slightly elevated number of nose pokes in ABT-418 yoked control mice compared to, for example, yoked control nicotine mice. The absence of dose-dependent self-administration with ABT-418 and the elevated yoked control nose poking warrant further investigation.

The present findings are consistent with receptor binding assays expressing the human $\alpha_4\beta_2$ subunit combination. Nicotine, cytisine, and lobeline had K_is (nM) of 1.05, 0.43, and 1.92, respectively, whereas epibatidine had a K_i of 0.07, while ABT-418 was considerably less potent with a K_i of 7.89 nM (17). In contrast to cytisine and nicotine, (^{3}H)epibatidine showed affinity for two binding sites, and its binding was greater in several brain regions as compared to cytisine (20). In our hands, antinociceptive doses of epibatidine in the mouse grid shock test (35) are equal to or only slightly below toxic doses (unpublished observations). It appears that epibatidines lower selectivity coupled with its greater potency on the $\alpha_4\beta_2$ subunit combination compared to nicotine, and its neuromuscular activity (see above) may account for epibatidines lack of reinforcing effects. ABT-418's lower potency at the $\alpha_4\beta_2$ subunit combination (17), its lower potency in the nicotine drug discrimination assay and in activating dopaminergic VTA neurons (3), may explain the lack of reinforcing effects with the self-administered unit doses presently used. It cannot, however, be ruled out that further increments in unit dose would have induced reinforcing effects.

The present studies employed an acute operant drug self-administration model, evaluating the initiation of self-administration behavior. This model has been suggested to evaluate drug abuse potential (6,22). Whereas acute reinforcing properties of a drug can be assessed in this model, chronic reinforcing properties, i.e., chronic maintenance of operant behavior by a drug, are not directly measured. However, there appears to be a good correspondence between the acute self-administration properties of nicotine (24), cocaine (22), and morphine (6,22) and chronic self-administration properties of these drugs (21,33). Therefore, one advantage of the present model in comparison to chronic preparations is the lack of the need to take into account the potential development of drug withdrawal as a factor in the maintenance of self-administration. These observations suggest that this model may be useful for initial screening for abuse liability in mice with minimal compound usage.

The present studies raise the question of whether the lack of significant dose-dependent decreases in mean cumulative amounts of self-administered cocaine, beyond the peak unit doses, is due to titration. This interpretation predicts that the mouse would self-administer until a certain concentration of drug is reached, i.e., fewer nose pokes would be required at high unit doses of the drug to reach the desired level. A decrease in cumulative amounts of self-administered drug may indicate aversive effects caused by the cumulative drug concentration or by the increased unit dose self-administered. These issues do apply to the data seen with ABT-418 in that increasing cumulative amounts of self-administered drug were seen up to the maximum unit dose of 0.125 mg/kg/infusion. Therefore, it cannot be determined whether further increments in unit dose would produce a further increase, decrease, or no change in cumulative amounts of self-administered ABT-418.

At high unit doses, death occurred 22 times—in each case in the yoked controls. This preponderance is even more noteworthy given that in several of these cases, the contingent mouse self-administered an even larger amount of drug than the yoked control mouse had received before it died. This observation is in accordance with previous data showing increased lethality of cocaine in the yoked control as opposed to contingent rats (14). As these dissociations of lethality are probably based on the different behavioral circumstances of the animals (14), it is worth considering whether similar dissociations would apply to the sensitivity to potential psychomotoric side effects in drug-naive contingent vs. yoked control mice. With reference to the present data, one could speculate that this might partly account for the unexpected high number of nose pokes of yoked control epibatidine mice. When using noncontingent yoked control mice in self-administration studies such perspectives need to be taken into account. It seems that the circumstances of administration, and, in particular, the fact that the drug is received response contingently, are of importance. The possible mechanisms behind these differences in sensitivity between yoked control mice and contingent mice need further investigation. An improvement of the present self-administration model might be to furnish each test box with two nose poke holes (19), nose pokes in one of which provide contingent infusions and nose pokes in the second having no programmed consequences. By having each mouse serving as its own control, the above-mentioned inherent constraints of using yoked control mice might be eliminated.

In conclusion, the present studies support prior demonstrations (22–24) of intravenous self-adminstration in drug-naive mice as a rapid model to assess drug abuse potential with minimal amounts of compound. The nicotinic compounds cytisine, and to a certain extent lobeline, but not epibatidine or ABT-418, were self-administered by drug-naive mice in a manner similar to cocaine and nicotine. The findings demonstrate the importance of assessing drug abuse potential with novel nicotinic compounds targeted for the clinic and shows that nicotinic compounds may well differ in this regard.

ACKNOWLEDGEMENTS

Thanks are due to Preben H. Olesen and Ulrich Ehrbar for synthesizing ABT-418 and (±)-epibatidine.

REFERENCES

1. Arneric, S. P.; Sullivan, J. P.; Briggs, C. A.; Donelly Roberts, D.; Anderson, D. J.; Raszkiewicz, J. L.; Hughes, M. L.; Cadman, E. D.; Adams, P.; Garvey, D. S.; Wasicak, J. T.; Williams, M.: (*S*)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoaxzole (ABT-418): A novel cholinergic ligand with cognition-enhancing and anxiolytic activities: I. In vitro characterization. J. Pharmacol. Exp. Ther. 270: 310–318; 1994.
2. Badio, B.; Daly, J. W.: Epibatidine, a potent analgetic and nicotinic agonist. Mol. Pharmacol. 45:563–569; 1994.
3. Brioni, J. D.; Kim, D. J. B.; Brodie, M. S.; Decker, M. W.; Arneric, S. P.: ABT-418: Discriminative stimulus properties and effect on ventral tegmental cell activity. Psychopharmacology (Berlin) 119:368–375; 1995.
4. Chandler, C. J.; Stolerman, I. P.: Discriminative stimulus properties of the nicotinic agonist cytisine. Psychopharmacology (Berlin) 129:257–264; 1997.
5. Clayton, S. C.; Reagan, A. C.: A total synthesis of (±)-epibatidine. Tetrahedron Lett. 34:7493–7496; 1993.

6. Criswell, H. E.; Ridings, A.: Intravenous self-administration of morphine by naive mice. Pharmacol. Biochem. Behav. 18:467–470; 1983.
7. Damaj, M. I.; Creasy, K. R.; Grove, A. D.; Rosecrans, J. A.; Martin, B. R.: Pharmacological effects of epibatidine optical enantiomers. Brain Res. 664:34–40; 1994.
8. Damaj, M. I.; Creasy, K. R.; Welch, S. P.; Rosecrans, J. A.; Aceto, M. D.; Martin, B. R.: Comparative pharmacology of nicotine and ABT-418, a new nicotinic agonist. Psychopharmacology (Berlin) 120:483–490; 1995.
9. Decker, M. W.; Majchrzak, M. J.; Arneric, S. P.: Effects of lobeline, a nicotinic receptor agonist, on learning and memory. Pharmacol. Biochem. Behav. 45:571–576; 1993.
10. Decker, M. W.; Brioni, J. W.; Sullivan, J. P.; Buckley, M. J.; Radek, R. J.; Raszkiewicz, J. L.; Kang, C. H.; Kim, D. J. B.; Giardina, W. J.; Wasicak, J. T., Garvey, D. S.; Williams, M.; Arneric, S. P.: (*S)*-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoaxzole (ABT-418): A novel cholinergic ligand with cognition-enhancing and anxiolytic activities: II. In vivo characterization. J. Pharmacol. Exp. Ther. 270:319–328; 1994.
11. Decker, M. W.; Curzon, P.; Brioni, J. D.; Arneric, S. P.: Effects of ABT-418 a novel cholinergic ligand on place learning in septal-lesioned rats. Eur. J. Pharmacol. 261:217–222; 1994.
12. Decker, M. W.; Brioni, J. D.; Bannon, A. W.; Arneric, S. P.: Diversity of neuronal nicotinic acetylcholine receptors: Lessons from behavior and implications for CNS therapeutics. Life Sci. 56:545–570; 1995.
13. Di Chiara, G.; Imperato, A.: Drugs abused by humans preferentially increase synaptic dopamine concentration in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA 85:5274–5278; 1988.
14. Dworkin, S. I.; Mirkis, S.; Smith, J. E.: Response-dependent vs. response-independent presentation of cocaine: Differences in the lethal effects of the drug. Psychopharmacology (Berlin) 117:262–266; 1995.
15. Fisher, M.; Huangfu, D.; Shen, T. Y.; Guynet, T. P. G.: Epibatidine, an alkaloid from the poison frog *Epipedobates tricolor*, is a powerful ganglionic depolarizing agent. J. Pharmacol. Exp. Ther. 270:702–707; 1994.
16. Garvey, D. S.; Wasicak, J.; Decker, M. W.; Brioni, J. D.; Sullivan, J. P.; Carrera, G. M.; Holladay, M. H.; Arneric, S. P.; Williams, M.: Novel isoxazoles which interact with brain cholinergic channel receptors that have intrinsic cognitive enhancing and anxiolytic activities. J. Med. Chem. 37:1055–1059; 1994.
17. Gopalakrishan, M.; Monteggia, L. M.; Anderson, D. J.; Molinari, E. J.; Piattoni-Kaplan, M.; Donelly Roberts, D.; Arneric, S. P.; Sullivan, J. P.: Stable expression, pharmacological properties and regulation of the human neuronal nicotinic acetylcholine alpha 4 beta 2 receptor. J. Pharmacol. Exp. Ther. 276:289–297, 1996.
18. Goldberg, S. R.; Spealman, R. D.; Goldberg, D. M.: Persistent behavior at high rates maintained by intravenous self-administration of nicotine. Science 214:573–575; 1981.
19. Grahame, N. J.; Cunningham, C. L.: Genetic differences in intravenous cocaine self-administration between C57BL/6J and DBA/2J mice. Psychopharmacology (Berlin) 122:281–291; 1995.
20. Khan, I. M.; Yaksh, T. L.; Taylor, P.: Epibatidine binding sites and activity in the spinal cord. Brain Res. 753:269–282, 1997.
21. Koob, G. F.: Drugs of abuse: Anatomy, pharmacology and function of reward pathways. Trends Pharmacol. Sci. 13:177–184; 1992.
22. Kuzmin, A.; Zvartau, E.; Gessa, L. G.; Martellotta, M. C.; Fratta, W.: Calcium antagonists isradipine and nimodipine suppress cocaine and morphine intravenous self-administration in drug-naive mice. Pharmacol. Biochem. Behav. 41:497–500; 1992.
23. Kuzmin, A.; Patkina, N. A.; Zvartau, E.: Analgesic and reinforcing effects of morphine in mice. Influence of Bay K-8644 and nimodipine. Brain Res. 652:1–8; 1994.
24. Martellotta, M. C.; Kuzmin, A.; Zvartau, E.; Cossu, G.; Gessa, G. L.; Fratta, W.: Isradipine inhibits nicotine intravenous self-administration in drug-naive mice. Pharmacol. Biochem. Behav. 52:271–274; 1995.
25. Reavill, C.; Walther, B.; Stolerman, I. P.; Testa, B.: Behavioral and pharmacokinetic studies on nicotine, cytisine and lobeline. Neuropharmacology 29:619–624; 1990.
26. Rowell, P. P.; Carr, L. A.; Garner, A. C.: Stimulation of [^{3}H]dopamine release by nicotine in rat nucleus accumbens. J. Neurochem. 49:1449–1454; 1987.
27. Sahakian, B.; Jones, G.; Levy, R.; Gray, J.; Warburton, D.: The effects of nicotine on attention, information processing and short-term memory in patients with dementia of the Alzheimer type. Br. J. Psychiatry 154:797–800; 1989.
28. Semenova, S.; Kuzmin, A.; Zvartau, E.: Strain differences in the action of morphine in mice. Pharmacol. Biochem. Behav. 50:17–21; 1995.
29. Sloan, J. W.; Martin, W. R.; Bostwick, M.; Hook, R.; Wala, E.: The comparative binding characteristics of nicotinic ligands and their pharmacology. Pharmacol. Biochem. Behav. 30:255–267; 1988.
30. Stolerman, I. P.: Basic mechanisms of nicotine addiction. ECNP 2:282–283; 1992.
31. Stolerman, I. P.; Reavill, C.: Primary cholinergic and indirect dopaminergic mediation of behavioral effects of nicotine. Prog. Brain Res. 79:227–237; 1989.
32. Stolerman, I. P.; Shoaib, M.: The neurobiology of tobacco addiction. Trends Pharmacol. Sci. 12:467–473; 1991.
33. Swedberg, M. D. B.; Henningfield, J. E.; Goldberg, S. R.: Nicotine dependency: Animal studies. In: Wonnacott, S.; Russell, M. A. H.; Stolerman, I. P., eds. Nicotine psychopharmacology. Oxford: Oxford University Press; 1990:39–76.
34. Swedberg, M. D. B.; Rimvall, K.; Suzdak, P. D.: Lobeline and cytisine: Evidence for partial agonism in the drug discrimination procedure. Soc. Neurosci. Abstr. 20:1136; 1994.
35. Swedberg, M. D. B.: The mouse grid-shock analgesia test: Pharmacological characterization of latency to vocalization threshold as an index of antinociception. J. Pharmacol. Exp. Ther. 269: 1021–1028; 1994.