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Anatabine Significantly Decreases Nicotine Self-Administration

Nancy K. Mello, Peter A. Fivel, Stephen J. Kohut, and S. Barak Caine
McLean Hospital/Harvard Medical School

Nicotine addiction is associated with many lethal disorders (cancer, cardiovascular and pulmonary disease), and more effective medications to aid smoking cessation are urgently needed. Anatabine is 1 of the most abundant minor tobacco alkaloids, but relatively little is known about its interactions with the abuse-related effects of nicotine. The acute effects of anatabine or saline on nicotine- and food-maintained responding were examined in 7 rhesus monkeys (*Macaca mulatta*). Nicotine (0.01 mg/kg/inj, base) and banana-flavored food pellets (1 g) were available under a second-order schedule (FR 2 [VR 16:S]). Anatabine or saline injections were administered 15 min before the 11:00 a.m. food self-administration session began. Anatabine (0.18–3.2 mg/kg, IM) dose-dependently reduced nicotine self-administration (0.01 mg/kg/inj) ($p = .036 – 0.0003$). Food-maintained responding was decreased only at the highest dose of anatabine (3.2 mg/kg; $p = .003$). Each monkey returned to baseline levels of nicotine self-administration after anatabine treatment, and there was no evidence of catheter malfunction. Next, the effects of anatabine and saline on the nicotine dose-effect curve (0.001-0.1 mg/kg/inj) were evaluated. Anatabine (0.32 and 1.0 mg/kg, IM) decreased the peak of the nicotine dose-effect curve ($p < .001 – p < .0001$), with no significant effect on food-maintained responding. The abuse liability of anatabine also was examined, and monkeys did not self-administer anatabine (0.0032–0.32 mg/kg/inj) above saline levels. These findings are consistent with anatabine's effects on nicotine self-administration in rats (Caine et al., 2014). These data suggest that anatabine could be an effective agonist medication for treatment of nicotine addiction.

Keywords: agonist medications, anatabine, anatabine self-administration, nicotine addiction, nicotine self-administration, tobacco alkaloids

The mortality and morbidity associated with nicotine addiction is well documented (Jha et al., 2013), and the estimated economic costs exceed $158 billion (CDC, 2002, 2005). Cigarette smoking relapse rates after treatment are very high (92–95%) (Messer, Trinidad, Al-Delaimy, & Pierce, 2008), and more effective medications to aid smoking cessation attempts are urgently needed (Henningfield, Shiffman, Ferguson, & Gritz, 2009; Pollock, Koustova, Hoffman, Shurtleff, & Volkow, 2009). An ideal pharmacotherapy would be an agonist medication that shared some positive effects with nicotine but had minimal abuse liability or toxic side effects.

Tobacco contains a number of minor alkaloids in addition to nicotine, the primary psychoactive ingredient. Anatabine is one of the most abundant of the minor tobacco alkaloids and, like nicotine, is an agonist at nicotinic acetylcholine receptors (nAChRs) (Benowitz, 2010; Clark, Rand, & Vanov, 1965). The structure of anatabine in comparison with nicotine is shown in Figure 1. Anatabine and nicotine share several structural similarities consistent with the notion that anatabine could be an effective substitute for nicotine. However, there are also differences that could affect binding affinities at relevant receptor sites (Armstrong, Wang, Lee, & Liu, 1999)

Relatively little is known about the behavioral effects of anatabine or its interactions with the abuse-related properties of nicotine. In rats, a combination of five minor tobacco alkaloids, including anatabine, enhanced the reinforcing and locomotor effects of nicotine, but did not maintain self-administration above saline levels (Clemens, Caillé, Stinus, & Cador, 2009). Our behavioral studies in rats showed that anatabine shared discriminative stimulus effects with nicotine, and had a longer duration of action, but was not self-administered (Caine et al., 2014). These findings encouraged us to examine the effects of anatabine on nicotine self-administration by rhesus monkeys. Rhesus monkeys and humans share many similarities, including their anatomy, physiology, and neurochemistry of brain transmitter systems (Weerts, Fantegrossi, & Goodwin, 2007). Rhesus monkeys and humans also have similar profiles of nicotine metabolism and pharmacokinetics (Seaton, Kyerematen, Morgan, Jeszenka, & Vesell, 1991). In addition, medication evaluations in our nonhuman primate drug

Nancy K. Mello, Peter A. Fivel, Stephen J. Kohut, and S. Barak Caine, McLean Hospital/Harvard Medical School, Alcohol And Drug Abuse Research Center.

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Correspondence concerning this article should be addressed to Nancy K. Mello, McLean Hospital/Harvard Medical School Alcohol And Drug Abuse Research Center, 115 Mill Street, Belmont, MA 02478. E-mail: nmello@mclean.harvard.edu

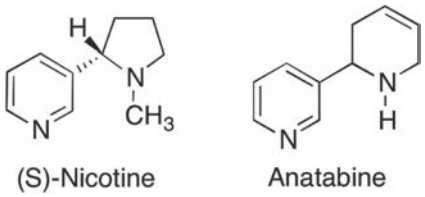

Figure 1. Structure of (S)-nicotine and anatabine: Tobacco contains only the (S)- enantiomer of nicotine, whereas anatabine is present as mixtures of the respective optical isomers (Armstrong et al., 1999). Nicotine and anatabine both have a 2-substituted pyridine ring. However, nicotine has a 5-membered pyrrolidine ring that contains tertiary nitrogen, whereas anatabine has a 6-membered tetrahydropyridine ring with secondary nitrogen.

self-administration model of addiction have shown good concordance with clinical trials (Mello, 2005).

In the present study, we examined the acute effects of anatabine on IV nicotine- and food-maintained responding. In the first study, the effects of ascending doses of anatabine (0.18–3.2 mg/kg, IM) on a reinforcing dose of nicotine and food-maintained responding were examined. In the second study, dose-effect curves for IV nicotine (0.001–0.1 mg/kg, inj) were determined under saline treatment conditions. Then the acute effects of three doses of anatabine (0.032, 0.32, and 1.0 mg/kg, IM) on the nicotine dose-effect curve and food-maintained responding were examined. Finally, the abuse liability of anatabine (0.0032–0.32 mg/kg/inj) was studied by substituting it for nicotine. This is the first study to examine the interactions between anatabine and the abuse-related effects of nicotine in rhesus monkeys.

Method

Subjects

Seven adult rhesus monkeys (*Macaca mulatta*) (5 females, 2 males) that weighed between 6 and 10 kg were studied. Females were gonadally intact, but menstrual cycles were unstable, and both amenorrhea and short cycles were observed. Similar disruptions of the menstrual cycle have also been reported in rhesus females during chronic cocaine self- administration (Mello, Mendelson, Kelly, Diaz-Migoyo, & Sholar, 1997). All monkeys had a history of cocaine and nicotine self-administration. Each day, monkeys received multiple vitamins, fresh fruit and vegetables, and Lab Diet Jumbo Monkey Biscuits (PMI Feeds Inc., St. Louis, MO) to supplement response-contingent banana-flavored pellets, fortified with vitamin C (Formula 4TUR banana flavor, grain-based pellet, Purina Mills Test Diet, Richmond, IN). Food supplements were given twice a day between 9:00 and 9:30 a.m. and between 1:00 and 1:30 p.m. Water was continuously available from an automatic watering system. A 12-hr light–dark cycle was in effect (lights on 7 a.m. – 7 p.m.), and the experimental chamber was dark during food and drug self-administration sessions.

Animal maintenance and research were conducted in accordance with the guidelines provided by the National Institutes of Health (NIH) Office of Laboratory Animal Welfare (OLAW), The Committee on the Care and Use of Laboratory Animals, and the U.S. Department of Agriculture (USDA). The facility is licensed by the USDA, and protocols were approved by the Institutional Animal Care and Use Committee (IACUC). Monkeys were observed at least twice every day, and any changes in general activity were noted. The health of the monkeys was periodically monitored by consultant veterinarians trained in primate medicine. Operant food and drug acquisition procedures provided an opportunity for enrichment and for monkeys to manipulate their environment (Line, 1987). Monkeys had visual, auditory, and olfactory contact with other monkeys throughout the study.

IV Catheter Implantation

Nicotine and saline solutions were administered through surgically implanted venous catheters. Double lumen Silicone rubber catheters (I.D. 0.028 in, O.D. 0.088 in; Saint Gobain Performance Plastics, Beaverton, MI) were placed in the internal or external jugular or femoral vein. All surgical procedures were performed under aseptic conditions. Monkeys were initially sedated with ketamine (5–10 mg/kg, IM). Atropine (0.05 mg/kg) SC or IM was administered to reduce salivation. After insertion of an endotracheal tube, anesthesia was maintained with isofluorane (1%–2% mixed with oxygen). After surgery, monkeys were given procaine penicillin G at 20,000 units/kg, IM twice daily for 5 days, or cephalexin 20 mg/kg, PO twice daily for 5 days. An analgesic dose of buprenorphine (0.032 mg/kg, IM) and Metacam (meloxicam; 0.1 mg/kg, SC) was administered twice daily for 3 days.

The intravenous catheter exited in the midscapular region and was protected by a tether system consisting of a custom-fitted nylon vest connected to a flexible stainless steel cable and fluid swivel (Lomir Biomedical, Inc., Malone, NY). This flexible tether system permits monkeys to move freely. Catheter patency was evaluated periodically by administration of a short-acting barbiturate, methohexital sodium (4 mg/kg) through the catheter lumen. If muscle tone decreased within 10 sec after drug administration, the catheter was considered patent.

Apparatus and Operant Procedures

Apparatus. Monkeys lived in stainless steel chambers (64 × 64 × 79 cm) equipped with a custom-designed operant response panel (28 × 28 cm), a pellet dispenser (Gerbrands Model G5210, Arlington, MA), and two syringe pumps (Model 981210, Harvard Apparatus, Inc., South Natick, MA), one for each lumen of the double-lumen catheter. Room lights were off during all experimental sessions. Schedules of reinforcement were programmed with custom-designed software and IBM-compatible computers and interface systems (Med Associates, St. Albans, VT). Additional details of this apparatus have been described previously (Mello et al., 1995). Drug concentrations were varied by computer-controlled changes in pump infusion duration (Fivel, 2011).

Nicotine self-administration. Monkeys were trained to self-administer IV nicotine (0.01 mg/kg/inj) and food pellets (1*g*) on the same second-order schedule of reinforcement (FR 2 [VR 16:S]). During food self-administration sessions, the response key (6.4 × 6.4 cm) on the operant panel was illuminated with a red light. Completion of the response requirement under a second-order Fixed Ratio 2, Variable Ratio 16 (FR 2, [VR 16:S]) schedule resulted in presentation of a 1-s red light beneath the response key. Completion of a second VR16 resulted in delivery of a 1-g banana-flavored pellet. During control or nicotine self-administration ses-

sions, the response key was illuminated with a green light, and completion of the response requirement resulted in delivery of nicotine over 1 sec through one lumen of the double-lumen catheter. A 10-s time-out followed delivery of each drug or saline injection or food pellet, during which stimulus lights remained off and responding had no scheduled consequences. After 25 food pellets were delivered, all stimulus lights were turned off, and responding had no scheduled consequences for the remainder of that session. Thus, a monkey could earn a maximum of 100 food pellets/day in four daily food self-administration sessions that began at 11:00 a.m., 3:00 p.m., 7:00 p.m., and 6:00 a.m. the next morning.

Testing procedures. The sequence of food and nicotine sessions is shown in Figure 2. Test sessions began at 11:00 a.m. and consisted of a 30-min food self-administration session and a 90-min nicotine self-administration session. A second 60-min nicotine session began at 4:00 p.m. immediately after the 3:00 p.m. food session. A time-out period during which responding had no scheduled consequences followed each food and nicotine self-administration session. Nicotine injections were not limited during the 90-min test session, but were limited to 20 during the 1-hr session at 4:00 p.m. When these studies began, monkeys were nicotine-experienced and had at least five months of nicotine exposure.

During test sessions, a single dose of anatabine (0.18–3.2 mg/kg, IM) or saline control treatment was given 15 min. before the midday food self-administration session began. After each dose of anatabine or saline, monkeys returned to stable baseline levels of nicotine- and food-maintained responding before the next treatment dose was administered. This control is essential to avoid confounding effects of the previous medication dose and to establish that catheter malfunction did not account for any decreases in nicotine self-administration. Systematic assessments to monitor any changes in behavior (e.g., sedation or agitation) were conducted after each saline or anatabine test session (Kato & Yanagita, 1981).

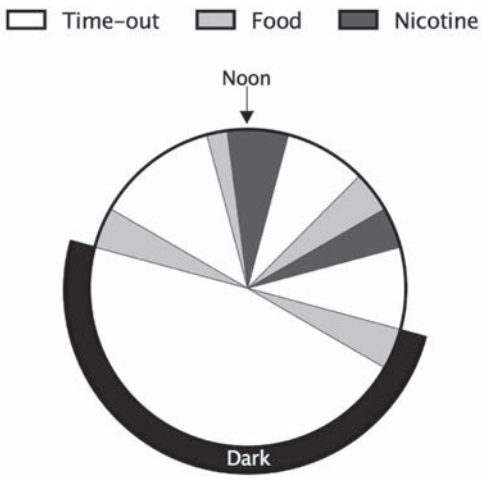

Figure 2. Schematic diagram of daily sequence of operant sessions: Food self- administration sessions (gray wedges) that began at 7:00 a.m., 3:00 p.m., and 7:00 p.m. lasted for 1 hour and the session that began at 11:00 a.m. lasted for 30 min. Nicotine or saline self- administration sessions (black wedges) that began at 11:30 a.m. lasted for 90 min, and the session that began at 4:00 p.m. lasted for 1 hr. Time-out periods when responding that had no scheduled consequences are shown as white. A 12-hr light–dark cycle was in effect, and the experimental chamber was dark during food and drug self-administration sessions.

Anatabine self-administration. Anatabine was substituted for nicotine to determine whether it maintained responding leading to its self-administration. Increasing doses of anatabine (0.0032–0.32 mg/kg/inj) and saline were available for 3 consecutive days on the same second-order schedule as nicotine. Unlimited injections of anatabine and saline were available during the 90-min test session to permit comparison with nicotine self-administration. If there was no upward trend after 3 days, the anatabine dose was increased by [1/2] log unit. The anatabine dose range overlapped the dose range that maintained nicotine self-administration as well as doses that reduced nicotine self-administration.

Data analysis. The primary dependent variables were the total number of drug or saline injections and food pellets earned during the 11 a.m. session. The number of injections self-administered after each treatment condition were averaged for statistical analysis. One-way ANOVA was used to determine anatabine's effects on self-administration of nicotine (0.01 mg/kg) and food-maintained responding. Repeated measures analysis of variance (ANOVA) with factors of anatabine treatment and dose of nicotine was used to determine anatabine's effect on drug self-administration, and food-maintained responding. One-way ANOVA for repeated measures for saline and anatabine treatment was also used to determine which doses of nicotine maintained significantly more self-administration than saline levels. A significant ANOVA ($p < .05$) was followed by Dunnett post hoc tests.

Drugs. (−)-Nicotine hydrogen tartrate was obtained commercially (Sigma-Aldrich, St. Louis, MO) and solubilized in sterile water buffered with NaOH to achieve a pH of 6–7. Anatabine was donated by Rock Creek Pharmaceuticals (Gloucester, MA) and prepared in sterile water. All IV drugs were sterile-filtered with a .22-micron syringe-driven filter.

Results

Effects of Anatabine on Nicotine and Food-Maintained Responding

Anatabine dose-ranging studies were conducted initially to determine what doses effectively altered nicotine self-administration in comparison to saline control treatment. Monkeys earned an average of 20.37 ± 2.45 nicotine injections (0.01 mg/kg/inj) and 25 food pellets during the test sessions conducted after vehicle control treatment. Administration of single doses of anatabine (0.18 −3.2 mg/kg, IM) dose-dependently decreased nicotine self-administration of a reinforcing dose of nicotine (0.01 mg/kg/inj; see Figure 3). Decreases in nicotine-maintained responding were significantly different from the saline treatment baseline at anatabine doses of 1.8 and 3.2 mg/kg, IM ($p = .036–0.0003$). Food-maintained responding also decreased as a function of increasing doses of anatabine and these changes were statistically significant at the highest doses of anatabine ($p = .0003$). Anatabine was significantly (i.e., 2.4-fold) more potent at decreasing responding for nicotine- over food-maintained responding as determined by ED_{50} analysis (see Table 1).

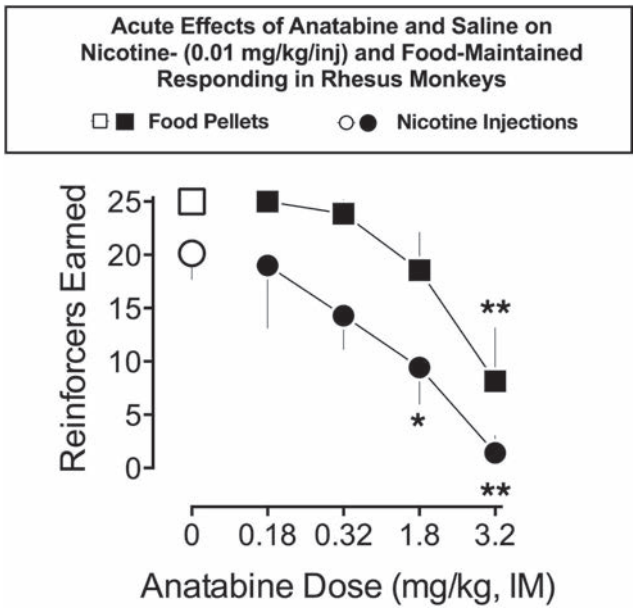

Figure 3. Acute effects of anatabine and saline on nicotine- and food-maintained responding. Abscissa: saline or ascending doses of anatabine (0.18–3.2 mg/kg, IM). Points above 0 indicate reinforcers earned during saline control treatment. Ordinate: number of 1-g food pellets (open and closed squares) and nicotine injections (0.01 mg/kg/inj, IV; open and closed circles). One-way ANOVA for repeated measures found a significant effect of anatabine on nicotine (0.01 mg/kg/inj) maintained responding ($F(4, 24) = 7.591$; $p = .0004$). Dunnet post hoc tests showed that 1.8 and 3.2 mg/kg anatabine significantly reduced nicotine self-administration ($p = .036$ and 0.0003, respectively). Anatabine also had a significant effect on food-maintained responding, $F(4, 24) = 8.18$; $p = .0003$. Dunnet post hoc tests showed that 3.2 mg/kg anatabine significantly reduced food self-administration ($p = .0003$). Each data point is the mean ± *SE* of seven monkeys. * $p < .05$, ** $p < .001$.

Subsequently, we determined the nicotine dose-effect curve (0.001–0.1 mg/kg/inj) under the same conditions in 4 monkeys (see Figure 4). During vehicle treatment, the nicotine dose-effect curve peaked at 0.0032 mg/kg/inj. Over the dose-range studied, nicotine did not change food-maintained responding. The lowest dose of anatabine (0.032 mg/kg, IM) did not alter the nicotine dose-effect curve appreciably from control conditions. A higher dose of anatabine (0.32 mg/kg, IM) decreased the peak of the nicotine dose-effect curve significantly ($p = .001$). The highest dose of anatabine (1.0 mg/kg, IM) also decreased the peak of the nicotine dose-effect curve significantly ($p < .0001$).

Food-maintained responding did not change significantly during treatment with 0.032 and 0.32 mg/kg, IM anatabine. Food-maintained responding decreased slightly during treatment with the highest dose of anatabine, but there was considerable variability between animals and these decreases were not significant.

Abuse Liability of Anatabine

Anatabine (0.0032–0.32 mg/kg/inj) was substituted for nicotine to determine whether it maintained responding leading to its self-administration. Figure 5 shows that anatabine did not maintain self-administration at levels above saline self-administration. These data suggest that anatabine has relatively low abuse liability in rhesus monkeys under these conditions.

Discussion

This is the first evaluation of the effects of anatabine, a minor tobacco alkaloid, on nicotine and food self-administration by nonhuman primates. Our major findings were as follows: (1) Anatabine (0.18–3.2 mg/kg, IM) significantly decreased self-administration of a highly reinforcing dose of nicotine. There were minimal effects on food-maintained responding except at the highest anatabine dose. (2) In studies of the effects of anatabine on the nicotine dose-effect curve (0.0032–0.32 mg/kg/inj), anatabine (0.32 and 1.0 mg/kg) significantly decreased the peak of the nicotine dose-effect curve (0.003 mg/kg/inj; $p < .001$–0.0001). Food- maintained responding did not decrease significantly, indicating that anatabine's effects were selective for nicotine. (3) Finally, anatabine (0.0032–0.32 mg/kg/inj) did not maintain self-administration significantly above saline levels, suggesting that it has low abuse liability. These findings in rhesus monkey are consistent with our studies in rodents in which anatabine (1.8–5.6 mg/kg, IP) significantly decreased nicotine self-administration (0.003–0.01 mg/kg/inj; Caine et al., 2014). Anatabine also did not maintain responding significantly above saline levels in rodents (Caine et al., 2014; Clemens et al., 2009). Taken together, these data suggest that anatabine could be an effective agonist medication for treatment of nicotine addiction.

Relatively little is known about the mechanisms underlying anatabine's interactions with nicotine. Like nicotine, anatabine has agonist activity at the nicotinic acetylcholine receptors (nAChRs) (Benowitz, 2010). Although anatabine shares some structural similarities with nicotine as shown in Figure 1, there are also some notable differences. For example, anatabine has a secondary nitrogen that can be oxidized more easily than a tertiary nitrogen in the nicotine molecule. Thus, the position of nitrogen in each molecule would differ slightly (Armstrong et al., 1999). These differences in combination with differences in the degree of substitution may affect binding affinities at nicotinic receptors.

Our in vitro studies showed that anatabine was a full agonist relative to nicotine and acetylcholine in HEK293 cells transfected with α4β2 ion channel cDNA (Caine et al., 2014). The α4β2 subunits appear to be critically important for nicotine's reinforcing

Table 1
ED_{50} Values (=95% Confidence Limits) for Anatabine to Decrease Nicotine (0.01 Mg/kg/inj)- and Food-Maintained Responding

Reinforcer	ED_{50}
Nicotine (0.01 mg/kg/inj)	0.854 (0.565 – 1.292)*
Food	2.085 (1.319 – 3.294)

Note. ED_{50} values, defined as the dose of anatabine that reduced nicotine- and food-maintained responding to 50% of baseline levels, were determined using log-linear interpolation with individual subject dose-effect curves. Log ED_{50} values were converted to linear values for data presentation. In two subjects, responding did not decrease to below 50% for food-maintained responding. Thus, a conservative estimate of ED_{50} value was determined by assuming the next ¼-log dose (i.e., 5.6 mg/kg) would eliminate responding. * Non-Overlapping ED_{50} values.

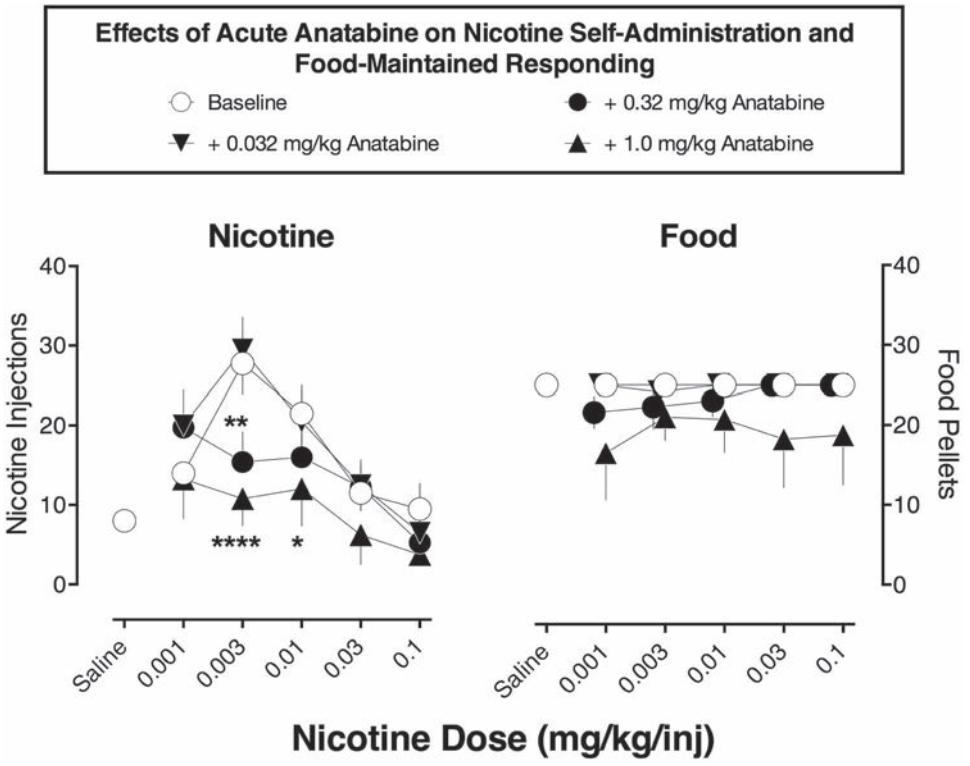

Figure 4. Acute effects of anatabine on nicotine and food dose-effect curves. Abscissa: Unit doses of nicotine available for self-administration (0.001–0.1 mg/kg/inj, IV). Points above saline show data from saline treatment sessions when saline was available for self- administration. Ordinates: number of nicotine injections self-administered (left) and number of food pellets earned (right) during each treatment condition. Nicotine and food self-administration during saline treatment is shown as open circles, and during anatabine treatment as closed downward pointing triangles (▼; 0.032 mg/kg, IM), closed circles (●; 0.32 mg/kg, IM), and closed upward pointing triangles (▲; 1.0 mg/kg, IM). Each data point is the mean ± *SE* of four monkeys. Two-way repeated measures analysis of variance (ANOVA) on nicotine self- administration with factors of Nicotine Dose and Anatabine Treatment found a main effect of Nicotine Dose, $F(4, 12) = 15.12$; $p = .0001$, with a trend for significance of Anatabine Treatment, $F(3, 9) = 3.511$; $p = .062$. The ANOVA also found a significant Anatabine Treatment × Nicotine Dose Interaction, $F(12, 36) = 2.89$; $p = .0067$. Dunnett post hoc tests found that 0.32 mg/kg anatabine significantly decreased 0.0032 mg/kg/inj nicotine self-administration ($p = .001$), whereas 1.0 mg/kg anatabine significantly decreased nicotine self-administration at doses of 0.0032 mg/kg/inj ($p < .0001$) and 0.01 mg/kg/inj ($p = .012$). Two-way ANOVA on food-maintained responding found no significant main effects of Nicotine Dose, $F(4, 12) = 0.836$; $p = .528$, nor Anatabine Treatment, $F(3, 9) = 1.217$; $p = .3585$, and no Anatabine Treatment × Nicotine Dose Interaction, $F(12, 36) = 1.009$; $p = .461$. Each data point is the mean ± *SE* of four monkeys. * $p < .05$, ** $p < .001$, **** $p < .0001$ versus control treatment.

effects (Benowitz, 2010; Marubio et al., 2003; Picciotto et al., 1998; Watkins, Koob, & Markou, 2000). The α4β2 and α7 subunits of the nAChRs are widely distributed throughout the central nervous system (Buccafusco, 2007; Dani & Bertrand, 2007; Gotti et al., 2007) as well as on the cell bodies of mesolimbic dopamine neurons. These data converge to suggest that anatabine's effects on nicotine self-administration reflect its nicotine agonist effects, somewhat analogous to d-amphetamine's effects on cocaine self-administration in nonhuman primates (Negus & Mello, 2003a, 2003b) and human stimulant abusers (Grabowski, Rhoades et al., 2004; Grabowski, Shearer, Merrill, & Negus, 2004; Herin, Rush, & Grabowski, 2010).

However, anatabine also has a number of other effects that appear to be unrelated to nicotine's addictive properties. For example, anatabine has anti-inflammatory effects, which could reflect its agonist activity at non neuronal α7nAChR receptors (Bencherif, Lippiello, Lucas, & Marrero, 2011; Conejero-Goldberg, Davies, & Ulloa, 2008; Cui & Li, 2010; Marrero, Bencherif, Lippiello, & Lucas, 2011). Preclinical studies suggest that anatabine can reduce accumulation of brain amyloid beta as well as C-reactive protein, an inflammatory biomarker in a transgenic mouse model of Alzheimer's disease (Paris et al., 2011). Similarly, in a mouse model of multiple sclerosis, anatabine reduced the development of experimental autoimmune encephalomyelitis (Paris et al., 2013) and the severity autoimmune thyroiditis in mice (Caturegli et al., 2012). Alpha 7 nicotinic neuronal receptors may facilitate cognitive function in animal models and clinical studies (Leiser, Bowlby, Comery, & Dunlop, 2009; Levin

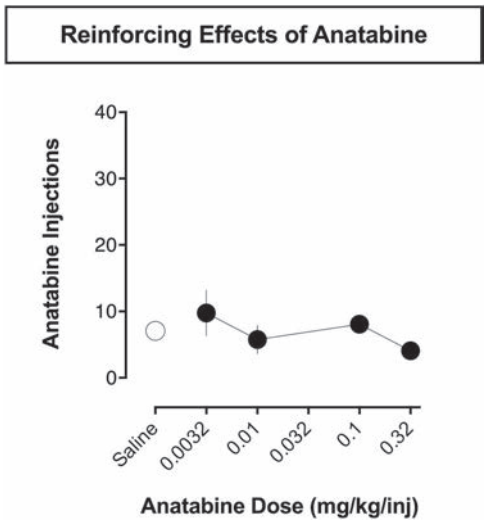

Figure 5. Anatabine and saline self-administration. Abscissa: Unit doses of anatabine available for self-administration. Points above saline show data from saline treatment sessions when saline was available for self-administration. Ordinates: number of anatabine (●) and saline (○) injections self-administered. Repeated-measures one-way ANOVA indicated that anatabine was not self-administered above saline levels, $F(4, 12) = 1.542$; $p = .252$, at doses of 0.0032–0.32 mg/kg/inj. Each data point is the mean ± *SE* of four monkeys.

& Rezvani, 2002; Levin & Simon, 1998; Rezvani & Levin, 2001). In addition, a nonselective nicotine receptor antagonist, mecamylamine, can impair cognitive function (Roegge & Levin, 2006). It was suggested that a nicotine receptor antagonist can provide a model of nicotine receptor loss seen in Parkinson disease and schizophrenia (Roegge & Levin, 2006).

The extent to which anatabine's α7 activity may also contribute to its reduction of nicotine self-administration remains to be determined. Nicotine discrimination studies in rats found that novel compounds selective for α7 and α4 nicotine receptors did not substitute for nicotine, whereas α4β2 selective compounds showed dose-dependent and complete substitution (Smith et al., 2007). In studies of α7 or β2 receptor knockout mice, oral nicotine consumption was initially greatest in α7 knockout mice and wild-type controls (Levin et al., 2009). Over the course of 5 months, the α7 knockout mice developed a persistent aversion to oral nicotine, whereas β2 receptor knockout mice increased nicotine consumption to wild type control levels (Levin et al., 2009). The contrast between these knockout mice in nicotine consumption patterns was interpreted to suggest that α7 receptor antagonists might be useful for facilitating cessation of nicotine consumption (Levin et al., 2009).

Strengths and Limitations of the Study

One limitation of this study was that only acute effects of anatabine were examined. Although acute doses are most frequently used in evaluations of candidate medications, only chronic treatment can indicate whether medication effects are sustained or diminish over time (Mello, 2005; Mello & Negus, 1996). Further studies of the effects of chronic anatabine treatment will be necessary to fully characterize its potential value as a smoking cessation aid. Despite this limitation, the study also has several strengths. Examination of anatabine's effects on both nicotine- and food-maintained responding allows us to conclude that treatment effects were selective for nicotine and did not reflect a general disruption of operant responding. Monkeys resumed baseline levels of nicotine self-administration after anatabine treatment, indicating that catheter malfunction did not account for the significant anatabine dose-related decreases in nicotine-maintained responding. Anatabine treatment was not associated with sedation or any adverse behavioral effects that could disrupt operant performance.

Translational Implications of Anatabine's Selective Reduction of Nicotine Self-Administration by Rhesus Monkeys

This nonhuman primate drug self-administration model is very useful for medication development and has good predictive validity for medication effectiveness (see for review (Haney & Spealman, 2008; Mello, 2005; Mello & Negus, 1996). Often, candidate treatment drugs are not FDA approved for evaluation in humans, so reliable animal models are one essential aspect of development. Interestingly, anatabine is available as a nutraceutical, and food supplements are not regulated by the FDA. Preliminary clinical evaluations indicate that a lozenge containing 0.1 mg of anatabine or 2 mg of nicotine each significantly reduced reports of cigarette craving by 107 heavy smokers (Lanier et al., 2013). Nicotine withdrawal scores on the Minnesota Withdrawal Behavior Rating Scale (Hughes & Hatsukami, 1986) were significantly reduced by both lozenges, but the tobacco lozenge was more effective (Lanier et al., 2013). The convergence of findings from evaluations of anatabine in monkeys in the present study, rats, and humans (Caine et al., 2014; Lanier et al., 2013) suggests that anatabine could be a useful addition to the armentarium of medications designed to treat nicotine dependence. Another important implication of this concordance between clinical and preclinical findings is that it provides an opportunity for retrospective validation of these preclinical models of drug addiction. Only a few clinically effective medications for opioid and stimulant addiction have been examined in both preclinical and clinical studies. The exceptions include buprenorphine for the treatment of opioid and opioid + cocaine abuse (Mello, Lukas, Kamien, Mendelson, & Cone, 1992; Mello & Mendelson, 1980; Mello, Mendelson, Bree, & Lukas, 1989; Mello & Negus, 1998; Mello & Negus, 2007; Montoya et al., 2004) and amphetamine for the treatment of stimulant abuse (Grabowski, Rhoades et al., 2004; Grabowski, Shearer et al., 2004; Herin et al., 2010; Negus & Mello, 2003a, 2003b; see for review Mello, 2005).

As noted earlier, nicotine addiction remains a serious public health problem. Smoking- related disorders include cancers and cardiovascular and pulmonary disease, accounting for an estimated <450,000 deaths annually in the United States alone (CDC, 2002, 2004, 2005). Relapse to cigarette smoking after treatment is common, and successful smoking cessation rates are estimated at between 5 and 18% (Messer, Trinidad, Al-Delaimy, & Pierce, 2008). It is generally agreed that more effective medications to reduce nicotine addiction are urgently needed (Henningfield et al., 2009; Lerman et al., 2007; Morgan, Backinger, Lerman, & Vocci, 2010; Pollock et al., 2009). If anatabine proves to be effective in reducing nicotine addiction during chronic treatment, and contin-

ues to have minimal abuse liability and other unwanted side effects in clinical studies, it could be useful for facilitating smoking cessation.

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