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Differential Antagonism and Tolerance/Cross-Tolerance Among Nicotinic Acetylcholine Receptor Agonists: Scheduled-Controlled Responding and Hypothermia in C57BL/6J Mice

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Abstract

The tobacco dependence pharmacotherapies varenicline and cytisine act as partial $\alpha 4\beta 2$ nAChR agonists. However, the extent to which $\alpha 4\beta 2$ nAChRs mediate their in vivo effects remains unclear. Nicotine, varenicline, cytisine, and epibatidine were studied in male C57BL/6J mice for their effects on rates of fixed ratio responding and rectal temperature, alone and in combination with the nonselective nAChR antagonist mecamylamine and the $\alpha 4\beta 2$ nAChR antagonist DH β E. The effects of nicotine, varenicline, cytisine, epibatidine, and cocaine were assessed before and during chronic nicotine treatment. The rate-decreasing and hypothermic effects of nicotine, varenicline, cytisine, and epibatidine were antagonized by mecamylamine (1 mg/kg), but only the effects of nicotine and epibatidine were antagonized by DH β E (3.2 mg/kg). Chronic nicotine produced 4.7 and 5.1 fold rightward shifts in the nicotine dose-effect functions to decrease response rate and rectal temperature, respectively. Nicotine treatment decreased the potency of epibatidine to decrease response rate and rectal temperature 2.2 and 2.9 fold, respectively, and shifted the varenicline dose-effect functions 2.0 and 1.7 fold rightward, respectively. Cross-tolerance did not develop from nicotine to cytisine. These results suggest that the in vivo pharmacology of tobacco cessation aids cannot be attributed to a single nAChR subtype; instead, multiple receptor subtypes differentially mediate their effects.

Keywords

nicotine; varenicline; cytisine; epibatidine; tolerance; cross-tolerance; simple-schedule responding; hypothermia; dihydro- β -erythroidine; mecamylamine

Introduction

Approximately five million deaths annually are attributed to tobacco use (World Health Organization, 2011). Nicotine is considered to play a prominent role in tobacco use, and produces reinforcing effects that are thought to be mediated by the $\alpha 4\beta 2$ subtype of nicotinic acetylcholine receptor (nAChR; Picciotto, et al., 1998; Tapper et al., 2004; Hoffman and Evans, 2013)..

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The most effective tobacco cessation pharmacotherapy on the market currently is varenicline (Chantix); however, approximately 75% of individuals taking varenicline relapse to using tobacco products within a year (Hays et al., 2008). In vitro, varenicline is a low efficacy $\alpha 4\beta 2$ nAChR agonist (Rollema et al., 2007), and in vivo, varenicline has been demonstrated to antagonize the discriminative stimulus effects of nicotine in rats (LeSage et al., 2009; Jutkiewicz, et al., 2011; Desai and Bergman, 2010). However, varenicline has also been demonstrated to have agonist activity at $\alpha 7$ nAChRs, $\alpha 3\beta 4$ nAChRs, and 5HT₃ receptors (Grady et al., 2010; Lummis et al., 2011). Actions at multiple receptors including nAChR and non-nAChR are consistent with studies suggesting that low efficacy at $\alpha 4\beta 2$ nAChRs cannot account for all of the behavioral effects of varenicline (Cunningham and McMahon, 2011; Cunningham and McMahon, 2013; Rodriguez et al., 2014).

Most in vivo studies examine the effects of nAChR ligands under acute or subchronic conditions of drug exposure. Chronic exposure to nicotine has been demonstrated to upregulate $\alpha 4\beta 2$ nAChRs to a greater extent than other nAChR subtypes (Olale et al., 1997; Fenster et al., 1999; Buisson and Bertrand, 2001; Nashmi et al, 2007). Chronic exposure to varenicline also upregulates nAChRs, with greater upregulation of $\alpha 3\beta 4$ and $\alpha 7$ nAChRs than following chronic nicotine (Marks et al., 2015). The extent to which chronic treatment and corresponding subtype-specific alterations in nAChR expression differentially impact the behavioral effects of nAChR drugs has not been established. Despite receptor upregulation, there is marked desensitization in nAChR function as a consequence of chronic nicotine treatment, which typically leads to the development of tolerance in humans (Perkins et al., 1994) and rats (Rosecrans et al., 1989). Furthermore, repeated exposure to varenicline produces cross-tolerance to the effects of nicotine on y-maze crosses, rearing, and body temperature in mice (Marks et al., 2015). The extent to which cross-tolerance develops from nicotine to varenicline has yet to be characterized.

Receptor theory suggests that a low efficacy agonist occupies more receptors than a higher efficacy agonist at equal levels of effect (Kenakin 1997; Kenakin 2002). If a particular nAChR subtype solely mediated the effects of agonists with different efficacies, then loss of function at this subtype following chronic agonist exposure would likely have a greater impact on the effects of a low efficacy agonist relative to an agonist with higher efficacy. Therefore, if the primary mechanism of action of varenicline is low efficacy at $\alpha 4\beta 2$ nAChRs, then cross-tolerance from nicotine to varenicline might be greater than tolerance to nicotine. However, as the literature presents more data suggesting that nicotine and varenicline differ in receptor selectivity, the degree of cross-tolerance from varenicline to nicotine might depend on the extent to which nicotine and varenicline share binding sites.

Operant responding (Cunningham and McMahon, 2011) and hypothermia in C57BL/6J mice (Rodriguez et al., 2014) have been used to examine the receptor pharmacology of nicotine and other nAChR agonists, as well as tolerance to nicotine (Rosecrans et al., 1989). The present study was conducted to measure both in vivo effects and had two main objectives: 1) identify the extent to which $\alpha 4\beta 2$ nAChRs mediate the rate-decreasing and hypothermic effects of nicotine, varenicline, cytisine, and epibatidine; and 2) examine the extent to which differential antagonism by the selective $\alpha 4\beta 2$ nAChR antagonist dihydro- β -erythroidine (DH β E) predicts the degree of cross-tolerance that develops from nicotine to varenicline,

cytisine, and epibatidine. Epibatidine was previously demonstrated to fully substitute for a nicotine discriminative stimulus in C57BL/6J mice (Rodriguez et al., 2014), consistent with similar mechanisms at nAChRs. Therefore, the expectation was that tolerance to nicotine would be accompanied by cross-tolerance from nicotine to epibatidine. In contrast, the degree of cross-tolerance to varenicline and cytisine was less certain. If nicotine has higher efficacy than varenicline and cytisine, then greater cross-tolerance to varenicline and cytisine would be expected. However, lesser cross-tolerance from nicotine would be expected in the event that nAChR subtypes differentially mediate the effects of the three drugs.

Methods

Animals

Eight-week-old male C57BL/6J mice (n=8; Jackson Laboratories, Bar Harbor, ME) were housed individually with a 10/14-hour light/dark schedule. Mice were food restricted to 85% of their free-feeding body weight, with access to food (Dustless Precision Pellets Grain-Based Rodent Diet, Bio-Serv, Frenchtown, NJ) immediately following operant experimental sessions. Water was available ad libitum. Ambient room temperature was 23°C. Mice were maintained and experiments were conducted in accordance with The University of Texas Health Science Center at San Antonio and the National Institute of Health's Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 2011).

Apparatus

Operant conditioning chambers (ENV-307A-CT, MedAssociates, St. Albans, VT) were kept in ventilated, sound-attenuating boxes. Each chamber contained a house light, a recessed 2.2 cm-diameter hole for reinforcer presentation on one wall, and three identical holes horizontally arranged and spaced 5.5 cm apart on the opposite wall. The center of each hole was 1.6 cm from the floor. Only the center hole was illuminated in the current study. When the center hole was illuminated, disruption of a photobeam in the hole resulted in access to 0.01 cc of 50% v/v condensed milk/water through the hole on the opposite wall. The operant conditioning chambers were connected to a computer through an interface (MED-SYST-8, MedAssociates). Med-PC software (MedAssociates) controlled experimental events and provided a record of responses. Rectal temperature was measured with a digital thermometer (BAT7001H, Physitemp, Clifton, NJ) attached to a rectal probe designed for mice. The probe was 2 cm long and had a diameter 0.7112 mm along its entire length except for the tip, which was 1.651 mm in diameter (RET-2-ISO, Physitemp, Clifton, NJ). The probe was inserted 2 cm into the rectum.

Experimental Procedure

After habituation to the housing room for 7 days, experimental sessions were conducted during the light period at the same time each day, 7 days per week. Mice were placed in the operant chamber for 60 min where the center hole was illuminated. A single nose poke into the center hole resulted in 10-s access to milk. During the presentation of milk, the house light was illuminated while the light in the center hole was extinguished. Responses in the center hole during milk presentation had no programmed consequence. After 6 of 8 mice

obtained 100 reinforcers per session for three consecutive sessions, the response requirement was systematically increased to a fixed ratio 20 (FR20). Sessions were shortened to 25 min, and saline was administered at the beginning of a 10-min pretreatment period; during the pretreatment, both the house light and the center hole were not illuminated and responses in the center hole had no programmed consequence. Following the pretreatment, the light in the center hole was illuminated, signaling the start of 15 min during which milk was available under the FR20 schedule. Rectal temperature was measured immediately before saline administration and after the operant sessions.

Drug tests were conducted when the rate of responding of 6 of 8 mice were within $\pm 20\%$ of the baseline, defined as the running average of the previous 5 sessions, for each of 5 consecutive, or 6 of 7, sessions. One dose or dose combination was administered per drug test, and all mice were tested on the same day. After a drug test, daily sessions with saline were conducted; before the next drug test, response rate for 6 of 8 mice was required to vary by no more than $\pm 20\%$ of the 5-day running baseline for at least 2 out of 3 sessions. Rectal temperature was measured prior to drug or saline administration, the mice were placed in the operant chamber for the 25-min behavioral session, and immediately afterward rectal temperature was measured again. Dose-effect functions for rate-decreasing effects and hypothermia were generated in the following order: nicotine alone and in combination with the non-selective nAChR antagonist mecamylamine or the $\alpha 4\beta 2$ nAChR antagonist DH β E, varenicline alone and in combination with mecamylamine or DH β E, cytisine alone and in combination with mecamylamine or DH β E, epibatidine alone and in combination with mecamylamine or DH β E, and cocaine alone. Dose-effect functions for a drug were initiated only after every mouse received all doses of the previous test drug. On days of drug administration, doses were administered in a counterbalanced order among mice. After all dose-effect functions were determined and prior to chronic nicotine treatment, 1 mg/kg nicotine was tested again in each mouse.

Chronic nicotine treatment consisted of three doses of 1.78 mg/kg nicotine administered 90 min apart, for a total of 5.34 mg/kg nicotine per day. Rectal temperature was measured immediately before and 30 min after each dose. For the first 10 days of chronic nicotine treatment, the third nicotine dose was administered at the beginning of operant sessions. However, on day 11, operant sessions (beginning with the 10-min pretreatment) were conducted 1 h after the second nicotine dose, 30 min prior to the third nicotine dose. This change was made because 2 of the 8 mice responded at very low rates 10 min after the third nicotine dose. From day 11 onward, sessions were conducted until the response rate of 6 of 8 mice did not vary by $\pm 20\%$ of the 5-day running baseline for each of 5 consecutive, or 6 of 7, sessions. Thereafter, dose-effect functions were generated for nicotine, epibatidine, varenicline, cytisine, and cocaine. To account for any order effects during chronic nicotine treatment, drug order was counterbalanced among mice, except for cocaine, which was tested last in all mice. Only two mice received the same drug during any given session. After chronic nicotine treatment was discontinued, saline was administered instead of nicotine three times daily, 90 min apart, for 15 consecutive days to provide a control for the chronic nicotine treatment.

Drugs

The drugs included nicotine hydrogen tartrate salt (Sigma-Aldrich, St. Louis, MO), varenicline dihydrochloride (National Institute on Drug Abuse, Rockville, MD), $\pm$ epibatidine dihydrochloride hydrate (Sigma-Aldrich), cytosine (Atomole Scientific, Hubei, China), mecamylamine hydrochloride (Waterstone Technology, LLC, Carmel, IN), dihydro- β -erythroidine hydrobromide (DH β E; Tocris Biosciences, Bristol, UK), and cocaine hydrochloride (Sigma-Aldrich). Nicotine, varenicline, epibatidine, cytosine, and DH β E were administered subcutaneously. Mecamylamine and cocaine were administered intraperitoneally. All drugs were administered in a volume of saline equivalent to 10 ml/kg. Drug doses were expressed as the weight of the salt forms except for nicotine dose, which was expressed as the base weight.

Data Analyses

Response rate and rectal temperature are expressed as the mean $\pm$ standard error of the mean (S.E.M). Prior to chronic treatment, response rate is expressed as a percentage of control for each mouse, defined as the mean response rate from the previous 5 saline training sessions. During chronic nicotine treatment, response rate is expressed as a percentage of the mean response rate from the 5 saline training sessions immediately preceding the first day of chronic nicotine treatment. Rectal temperature is expressed as a change from the mean of the 5 daily saline measurements preceding a drug test or first day of chronic treatment.

Straight lines were fitted to dose-effect data using linear regression (GraphPad Prism version 5.0 for Windows, San Diego, CA). The linear portion of a dose-effect function for operant responding, as determined per individual mouse, included not more than one ineffective dose (i.e. response rate greater than 80% of control) up to and including the smallest dose that decreased response rate to less than 20% of control. The linear portion of a dose-effect function for hypothermia included not more than one ineffective dose (i.e. temperature decreased by less than 1°C) up to and including the smallest dose that decreased temperature by more than 5°C. For cocaine, all doses were included in the analyses.

The effects of drugs alone and in combination with an antagonist were analyzed by fitting lines to individual data simultaneously. A significant effect of drug was evidenced by a slope that was significantly different from 0, as determined by an *F*-ratio test using GraphPad. The slopes and intercepts of different functions were compared with an *F*-ratio test. If the *F*-ratio value was significant, then the dose-effect functions were considered significantly different from each other. If the slopes of the two lines were not significantly different, then the common best-fitting slope was used to calculate ED₅₀ values, potency ratios, and 95% confidence limits (Tallarida, 2000). The ED₅₀ values for hypothermic effects were calculated by expressing data at each dose as a percentage of the maximum effect determined separately for each test drug (i.e. effect of largest dose studied). If the 95% confidence limits of a potency ratio did not include 1, then ED₅₀ values were considered significantly different. In order to compare changes in potency as a function of chronic nicotine treatment, potency ratios of ED₅₀ values determined before and during chronic nicotine treatment (i.e., magnitudes of tolerance and cross-tolerance) were calculated for each drug by fitting straight lines to dose-effect data separately for each mouse using linear

regression. The potency ratios of each drug were compared with repeated measures one-way ANOVA followed by a Tukey's multiple comparisons test. The effects of 1 mg/kg nicotine determined at the beginning of the study and immediately before initiation of daily nicotine treatment were compared with a paired two-tailed Student's t-test.

Daily nicotine or saline treatment data were divided into bins of 5 sessions for further analysis. A one-way repeated measures ANOVA was used to analyze the effects of daily nicotine treatment on operant responding recorded once per day. A two-way repeated measures ANOVA was used to examine changes in the hypothermic effects of each dose of 1.78 mg/kg nicotine administered three times daily 90-min apart and as a function of changes in the effects of each dose across days ($p < 0.05$).

Results

Rate-Decreasing and Hypothermic Effects of Nicotine, Epibatidine, Varenicline, and Cytisine Prior to Chronic Nicotine Treatment

Nicotine dose-dependently decreased rate of responding, with an ED_{50} value of 0.438 (95% confidence limits: 0.366-0.523) mg/kg (Figures 1a and 2a, top panels, circles) (Table 1). Nicotine decreased rectal temperature by 5.85°C at a dose of 1.78 mg/kg nicotine (Figures 1a and 2a, bottom panels, circles). The ED_{50} value for nicotine to produce hypothermia was 0.707 (0.594-0.841) mg/kg. Sensitivity to the rate-decreasing ($t_7 = 0.71$, $p = 0.50$) and hypothermic effects ($t_7 = 1.47$, $p = 0.19$) of 1 mg/kg nicotine was not significantly different when determined at the beginning of the study and immediately before initiating chronic nicotine treatment. Epibatidine decreased response rate with an ED_{50} value of 0.00260 (0.00221-0.00306) mg/kg (Figures 1b and 2b, top panels, circles). Epibatidine decreased rectal temperature by 3.42°C at a dose of 0.0056 mg/kg (Figures 1b and 2b, bottom panels, circles). The ED_{50} for epibatidine to produce hypothermia was 0.00297 (0.00178-0.00497). Varenicline decreased response rate with an ED_{50} value of 2.79 (2.39-3.25) mg/kg (Figure 1c and 2c, top panels, circles). Varenicline decreased rectal temperature by 3.53°C at a dose of 10 mg/kg (Figures 1c and 2c, bottom panels, circles). The ED_{50} of varenicline to produce hypothermia was 4.72 (4.09-5.45) mg/kg. Cytisine decreased rate of responding with an ED_{50} value of 3.85 (3.27-4.53) mg/kg (Figures 1d and 2d, top panels, circles). Cytisine, up to the largest dose that could be tested to avoid lethality (5.6 mg/kg), did not significantly decrease rectal temperature ($F_{1,7} = 1.31$, $p = 0.29$) (Figures 1d and 2d, bottom panels, circles).

Antagonism of the Rate-Decreasing and Hypothermic Effects of Nicotine, Epibatidine, Varenicline, and Cytisine

Mecamylamine (1 mg/kg) alone did not significantly alter response rate or rectal temperature (Figure 1a, leftmost square). Mecamylamine (1 mg/kg) did significantly antagonize the rate-decreasing effects of nicotine as evidenced by a 2.8 (2.1-3.7) fold rightward shift in the nicotine dose-effect function (Figure 1a, top panel) (Table 1). Mecamylamine (1 mg/kg) also antagonized the hypothermic effects of nicotine as evidenced by a 3.7 (2.9-4.7) fold shift in the nicotine dose-effect function (Figure 1a, bottom panel). Mecamylamine (1 mg/kg) antagonized the rate-decreasing effects of epibatidine ($F_{2,38} = 41.8$, $p < 0.0001$) (Figure 1b, top panel) and the hypothermic effects of epibatidine ($F_{1,41} = 12.8$,

$p=0.0009$) (Figure 1b, bottom panel). Mecamylamine (1 mg/kg) also significantly antagonized the rate-decreasing effects of varenicline ($F_{2,38}=21.3$, $p<0.0001$) and the hypothermic effects of varenicline ($F_{2,52}=63.3$, $p<0.0001$) (Figure 1c, top and bottom panels, respectively). Because epibatidine (up to 0.1 mg/kg) and varenicline (up to 10 mg/kg) did not produce greater than 50% effect in the presence of mecamylamine, potency ratios were not calculated. Mecamylamine (1 mg/kg) produced a 1.8 (1.5-2.2) fold rightward shift in the cytisine dose-response function for producing rate-decreasing effects (Figure 1d, top panel). Mecamylamine (1 mg/kg) in combination with 10 mg/kg cytisine decreased rectal temperature by 4.05°C (Figure 1d, bottom panel). The same dose of mecamylamine and a smaller dose of cytisine (5.6 mg/kg) did not significantly alter rectal temperature when administered separately or together.

DH β E (3.2 mg/kg) alone did not significantly modify response rate or rectal temperature (Figure 2a, leftmost squares). DH β E (3.2 mg/kg) produced a significant 3.0 (2.4-3.8) fold rightward shift in the nicotine dose-response function for rate-decreasing effects (Figure 2a, top panel), and a significant 3.6 (2.9-4.5) fold rightward shift in the nicotine dose-effect function for hypothermia (Figure 2a, bottom panel). DH β E (3.2 mg/kg) produced a significant 1.9 (1.6-2.3) fold rightward shift in the dose-response function for epibatidine to produce rate-decreasing effects (Figure 2b, top panel). DH β E (3.2 mg/kg) significantly antagonized the hypothermic effects of epibatidine ($F_{2,44}=17.6$, $p<0.0001$); a potency ratio was not calculated because epibatidine (up to 0.01 mg/kg) in the presence of DH β E did not produce greater than 50% of the maximum hypothermic effect of epibatidine alone (Figure 2b, bottom panel). DH β E did not significantly modify the dose-response function of varenicline to produce rate-decreasing effects ($F_{2,41}=3.16$, $p=0.053$), and did not significantly antagonize the hypothermic effects of varenicline, as evidenced by a potency ratio (95% confidence limits) of 1.0 (0.8-1.2) (Figure 2c). DH β E (3.2 mg/kg) did not antagonize the rate-decreasing effects of 5.6 mg/kg cytisine. DH β E (3.2 mg/kg) and cytisine (5.6 mg/kg) did not significantly alter rectal temperature when administered separately or together (Figure 2d).

Chronic Nicotine Treatment: Tolerance and Cross-Tolerance

Mean response rate across days 1-5 of daily nicotine treatment was 25% of control after the third daily dose of nicotine (Figure 3, top panel, leftmost circle). While tolerance developed to the rate-decreasing effects of the third daily dose ($F_{5,75}=14.5$, $p<0.0001$), response rate remained at 39% of control for days 6-10. When the timing of the operant session was changed to begin before the third nicotine dose (i.e. 1 h after the second nicotine dose) on day 11, response rate was 77% of control on days 11-15, 89% of control on days 16-20, and 104% of control on days 21-25 (Figure 3, top panel, squares). Response rate during daily nicotine treatment remained stable for the remainder of daily dosing (i.e. through day 80). The hypothermic effects of nicotine varied significantly as a function of daily dose ($F_{2,10}=11.09$; $p<0.01$) and consecutive days of treatment ($F_{15,75}=23.90$; $p<0.0001$); moreover, there was an interaction between daily dose and consecutive days of treatment ($F_{30,150}=5.19$; $p<0.0001$). On days 1-5 of treatment, the first daily dose of 1.78 mg/kg nicotine decreased rectal temperature by 2.46°C; hypothermia after the first daily dose was significantly greater than hypothermia after the second daily dose (i.e. 1.59°C) and the third

daily dose (i.e. 1.36°C) (Figure 3, bottom panel, leftmost points). The magnitude of hypothermia produced by each daily nicotine dose significantly decreased over consecutive days of treatment; on treatment days 11-80, each dose had minimal effects on rectal temperature.

On days 15-21 of chronic nicotine treatment, the response rate of 6 of 8 mice did not vary by more than $\pm 20\%$ of the 5-day running average for 6 out of 7 days; tests with various doses of drugs were initiated on day 22 (Figure 3, top panel). Chronic nicotine treatment produced a 4.7 (3.8-5.8) and a 5.1 (4.1-6.3) fold rightward shift in the nicotine dose-effect functions for rate-decreasing effects and hypothermia, respectively (Figure 4a) (Table 1). Significant cross-tolerance developed from nicotine to epibatidine for both rate-decreasing and hypothermic effects as evidenced by 2.2 (1.8-2.8) and 2.9 (2.2-3.9) fold rightward shifts, respectively (Figure 4b). Cross-tolerance from nicotine developed to the rate-decreasing and hypothermic effects of varenicline as evidenced by 2.0 (1.6-2.5) and 1.7 (1.4-2.1) fold rightward shifts, respectively, in the varenicline dose-effect functions (Figure 4c). Cross-tolerance from nicotine failed to develop to cytisine for rate-decreasing effects ($F_{2,32}=1.79$, $p=0.18$), and doses of cytisine that were ineffective before chronic nicotine treatment continued to be ineffective during nicotine treatment (Figure 4d). Cross-tolerance failed to develop to the rate-decreasing of cocaine ($F_{2,29}=2.08$, $p=0.14$), and the effects of cocaine on rectal temperature did not significantly vary as a function of chronic nicotine treatment ($p>0.05$) (Figure 4e). The magnitudes of tolerance and cross-tolerance varied significantly among drugs for both rate-decreasing effects ($F_{2,12}=17.46$, $p<0.01$) and hypothermia ($F_{2,12}=32.73$, $p<0.0001$). For both effects, the magnitude of tolerance to nicotine was greater than the cross-tolerance that developed to epibatidine and varenicline ($p<0.05$); however, cross-tolerance from nicotine to epibatidine was not significantly different from cross-tolerance from nicotine to varenicline.

Discussion

Nicotine, epibatidine, and varenicline produced significant decreases in rectal temperature at doses that were approximately 2 fold greater than the doses needed to decrease responding under an FR20 schedule of food presentation in mice. The rate-decreasing and hypothermic effects of nicotine, epibatidine, and varenicline were significantly antagonized by mecamylamine, and the rate-decreasing effects of cytisine were also antagonized by mecamylamine. This suggested that the observed effects of nicotine, epibatidine, varenicline, and cytisine were due primarily to binding at nAChRs. DH β E (3.2 mg/kg) significantly antagonized the effects of nicotine and epibatidine, but not those of varenicline and cytisine. Differential antagonism by DH β E suggests that $\alpha 4\beta 2$ nAChRs mediate the rate-decreasing and hypothermic effects of nicotine and epibatidine, and play a less prominent role in mediating the same effects of varenicline and cytisine. Doses of DH β E larger than 3.2 mg/kg were not combined with varenicline and cytisine for two main reasons. First, 5.6 mg/kg DH β E alone decreases operant response rate in mice (unpublished observation). Second, lethality results from 10 mg/kg DH β E in combination with doses of varenicline and cytisine that are required to decrease response rate and rectal temperature in mice. The current results do not exclude the possibility that varenicline and cytisine have effects at $\alpha 4\beta 2$ nAChRs; however, $\alpha 4\beta 2$ nAChRs do not appear to mediate the effects of

varenicline and cytisine under the present experimental conditions. Prior to chronic nicotine treatment, cytisine was not tested alone at a dose of 10 mg/kg for fear of toxicity. However, when 10 mg/kg cytisine was tested in combination with mecamylamine (1 mg/kg), rectal temperature was decreased 4.1°C, suggesting that 10 mg/kg cytisine alone might also decrease rectal temperature.

Tolerance that developed to the rate-decreasing and hypothermic effects of nicotine was accompanied by cross-tolerance from nicotine to epibatidine. That DH β E antagonized the rate-decreasing and hypothermic effects of nicotine and epibatidine suggests that chronic nicotine treatment results in loss of function at $\alpha 4\beta 2$ nAChRs. However, cross-tolerance to epibatidine was significantly less than tolerance to nicotine. In vitro, epibatidine has higher efficacy than nicotine at $\alpha 4\beta 2$ nAChRs (Grady et al., 2010) which, according to receptor theory (Kenakin, 1997) would predict lesser cross-tolerance to the higher efficacy nAChR agonist (i.e. epibatidine) than tolerance to a lower efficacy agonist (i.e. nicotine). It is also possible that nicotine and epibatidine differ in selectivity for nAChR subtypes. In vitro, both nicotine and epibatidine have similar binding profiles, though epibatidine has overall greater affinity for $\alpha 4\beta 2$ and $\alpha 6\beta 2$ nAChRs than nicotine and is less selective than nicotine for $\alpha 4\beta 2$ versus $\alpha 6\beta 2$ nAChRs (Grady et al., 2010). It is presently unclear to what extent such differences in $\beta 2^*$ nAChR selectivity might underlie the different magnitudes of tolerance to nicotine and cross-tolerance to epibatidine.

Failure of DH β E to antagonize varenicline led to the prediction that little to no cross-tolerance from nicotine would develop to varenicline. This prediction was correct inasmuch as cross-tolerance to varenicline was significantly lower than tolerance to nicotine. Less cross-tolerance to varenicline than tolerance to nicotine is consistent with differential binding to $\alpha 4\beta 2$ nAChRs. Because mecamylamine antagonized both the rate-decreasing and hypothermic effects of varenicline, cross-tolerance from nicotine to varenicline appears to reflect overlapping binding at nAChRs aside from $\alpha 4\beta 2$. However, varenicline is also a 5-HT $_3$ receptor agonist (Lummis et al., 2011). If the effects of varenicline are mediated by non-nAChRs, then this could account for the differential tolerance and cross-tolerance in nicotine-treated mice. The current results do not discount evidence that the behavioral effects of varenicline in rats are consistent with low efficacy at $\alpha 4\beta 2$ nAChRs (LeSage et al., 2009; Jutkiewicz, et al., 2011; Desai and Bergman, 2010). However, the current results do suggest that low efficacy at $\alpha 4\beta 2$ nAChRs cannot explain fully the behavioral effects of varenicline in all situations (Cunningham and McMahon, 2013). Because DH β E consistently fails to antagonize the effects of varenicline in mice (Cunningham and McMahon, 2013), it will be important to determine whether or not the apparent non- $\alpha 4\beta 2$ nAChR pharmacology of varenicline generalizes to other species.

Similar to varenicline, cytisine was not antagonized by DH β E. However, unlike varenicline, cross-tolerance failed to develop from nicotine to cytisine. While differences in the receptor pharmacology of nicotine and cytisine have been previously observed in vivo (Chandler and Stolerman, 1997), antagonism of cytisine by mecamylamine suggests that nAChRs mediate the rate-decreasing effects of cytisine. The lack of cross-tolerance from nicotine to cytisine could indicate that the current parameters of chronic nicotine dosing do not produce loss of function at the nAChR subtypes that mediate the effects of cytisine.

Cross-tolerance failed to develop from nicotine to the effects of cytosine and cocaine on operant response rate. These results suggest that the tolerance and cross-tolerance developing from chronic nicotine treatment is to some extent pharmacologically selective and related to adaptations at nAChRs. The absence of cross-tolerance to cocaine also suggests that the decreased potency of nicotine, varenicline, and epibatidine is not due entirely to behavioral tolerance. If behavioral tolerance accounted for the decreased sensitivity to the rate-decreasing and hypothermic effects of drugs, then similar decreases might have been expected for all of the drugs tested regardless of their underlying pharmacology.

Tobacco use remains a significant problem in the world (WHO, 2011). Thus, the search for pharmacotherapies that will effectively reduce tobacco use remains an important public health goal. Understanding the mechanisms of current pharmacotherapies for tobacco cessation is imperative so that more effective drugs might be developed. This study demonstrates that $\alpha 4\beta 2$ nAChRs mediate the effects of nicotine and epibatidine, but not all effects of varenicline and cytosine. Furthermore, the degree of cross-tolerance to epibatidine, varenicline, and cytosine was significantly less than tolerance to nicotine, indicative of a difference in efficacy at $\alpha 4\beta 2$ nAChRs or selectivity at multiple nAChR subtypes. This study suggests that cross-tolerance will develop to varenicline in heavy tobacco users. However, notwithstanding overlap in the receptor pharmacology of the common smoking cessation aids nicotine, varenicline, and cytosine, nAChRs aside from $\alpha 4\beta 2$ appear to differentially mediate their in vivo effects.

Supplementary Material

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Figure 1.

The rate-decreasing effects (top panels) and hypothermic effects (bottom panels) of nicotine (a), epibatidine (b), varenicline (c), and cytisine (d) alone (circles) and in combination with 1 mg/kg mecamylamine (squares). Ordinate: response rate expressed as a percentage of the saline control (top panels), and rectal temperature expressed as a change from the saline control in °C (bottom panels). Abscissa: drug dose in mg/kg body weight.

Figure 2.

The rate-decreasing effects (top panels) and hypothermic effects (bottom panels) of nicotine (a), epibatidine (b), varenicline (c), and cytosine (d) alone (circles) and in combination with 3.2 mg/kg DHβE (squares). Ordinate: response rate expressed as a percentage of the saline control (top panels), and rectal temperature expressed as a change from the saline control in °C (bottom panels). Abscissa: drug dose in mg/kg body weight.

Figure 3.

Response rate (top panel) and change in rectal temperature (bottom panel) during daily treatment with 3 doses of 1.78 mg/kg nicotine administered 90 min apart. For days 1-10, the third dose was administered at the beginning of operant conditioning sessions. From session 11 onward (vertical dashed line), operant conditioning sessions began 1 h after the second nicotine dose. Temperature was measured 30 min after each dose throughout the experiment. Only the effects of 1.78 mg/kg nicotine are shown, i.e. data for other doses and drugs are omitted. Ordinate: response rate expressed as a percentage of the saline control calculated prior to chronic treatment (top panels), and rectal temperature expressed as a change from the control in °C determined during daily treatment with saline (bottom panels). Abscissa: 5-day averages starting with the first day of chronic nicotine treatment.

Figure 4.

The rate-decreasing effects (top panels) and hypothermic effects (bottom panels) of nicotine (a), epibatidine (b), varenicline (c), cytisine (d), and cocaine (e) before (open circles) and during chronic nicotine treatment (filled circles). Ordinate: response rate expressed as a percentage of the saline control (top panels), and rectal temperature expressed as a change from the saline control in °C (bottom panels). Abscissa: drug dose in mg/kg body weight.

Table 1

ED₅₀ values, potency ratios, and corresponding 95% confidence limits for nicotine, epibatidine, varenicline, and cytosine alone, in combination with 1 mg/kg mecamylamine, in combination with 3.2 mg/kg DHβE, or during chronic nicotine treatment. Potency ratios are not shown for treatments that are not significantly different from control or when ED₅₀ values are not calculated due to the maximum effect being less than 50%.

Drug	Response Rate		Temperature	
	ED ₅₀ Value (mg/kg)	Potency Ratio	ED ₅₀ Value (mg/kg)	Potency Ratio
Nicotine Control	0.438 (0.366-0.523)		0.707 (0.594-0.841)	
+ 1 Mecamylamine	1.22 (0.985-1.52) *	2.8 (2.1-3.7)	2.63 (2.24-3.09) *	3.7 (2.9-4.7)
+ 3.2 DHβE	1.31 (1.12-1.54) *	3.0 (2.4-3.8)	2.51 (2.08-3.03) *	3.6 (2.9-4.5)
Chronic Nicotine Treatment	2.05 (1.83-2.30) *	4.7 (3.8-5.8)	3.56 (3.03-4.18) *	5.1 (4.1-6.3)
Epibatidine Control	0.00260 (0.00221-0.00306)		0.00297 (0.00178-0.00497)	
+ 1 Mecamylamine	N/C *	N/C	N/C *	N/C
+ 3.2 DHβE	0.00501 (0.00450-0.00558) *	1.9 (1.6-2.3)	N/C *	N/C
Chronic Nicotine Treatment	0.00580 (0.00493-0.00682) *	2.2 (1.8-2.8)	0.00921 (0.00752-0.0113) *	2.9 (2.2-3.9)
Varenicline Control	2.79 (2.39-3.25)		4.72 (4.09-5.45)	
+ 1 Mecamylamine	N/C *	N/C	N/C *	N/C
+ 3.2 DHβE	N/S	N/S	N/S	N/S
Chronic Nicotine Treatment	5.56 (4.48-6.90) *	2.0 (1.6-2.5)	8.17 (6.54-10.2) *	1.7 (1.4-2.1)
Cytosine Control	3.85 (3.27-4.54)		N/A	
+ 1 Mecamylamine	6.94 (6.24-7.71) *	1.8 (1.5-2.2)	N/A	N/A
+ 3.2 DHβE	N/S	N/S	N/A	N/A
Chronic Nicotine Treatment	N/S	N/S	N/A	N/A

N/C Not calculated; did not produce greater than 50% of the maximum effect

N/S Not significant vs. control

N/A Not applicable; control not associated with a significant decrease

* Significant vs. respective control (p<0.05)