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Mecamylamine Blockade of Nicotine Responses: Evidence for Two Brain Nicotinic Receptors

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COLLINS, A. C., C. B. EVANS, L. L. MINER AND M. J. MARKS. *Mecamylamine blockade of nicotine responses: Evidence for two brain nicotinic receptors.* PHARMACOL BIOCHEM BEHAV 24(6) 1767–1773, 1986.—Mice of two inbred strains, DBA and C3H, were pretreated with mecamylamine before challenge with nicotine. Mecamylamine blocked nicotine-induced seizures, enhanced startle, and alterations in respiratory rate, Y-maze activity, heart rate and body temperature. Mecamylamine blocked nicotine-induced seizures and enhanced startle with IC_{50} values of less than 0.1 mg/kg. The other nicotine effects were blocked by mecamylamine with IC_{50} values between 0.8 and 2.3 mg/kg. Strain differences in sensitivity to mecamylamine blockade were also detected. These results suggest that nicotine elicits its effects at two receptors, which may be those labeled with [^{125}I]-α-bungarotoxin and with [^{3}H]-nicotine.

Mecamylamine Nicotine Genetics Nicotine receptors

MULTIPLE forms of receptors exist for many drugs and neurotransmitters in brain. In fact, multiple forms, or subtypes, of receptors have become the rule rather than the exception. For example, the receptors for acetylcholine (ACh) have long been divided into two major classes: muscarinic and nicotinic [11]. At least two forms of the muscarinic receptor, designated M_1 and M_2, have been proposed [4,13] and nicotinic receptors may also be divided into more than one class.

The characterization of receptor subtypes has been facilitated by studies of antagonist action or binding. For example, the binding of [^{3}H]-pirenzepine has been invaluable in the study of the M_1 and M_2 receptors [13,15]. Nicotinic antagonists have also been described, and the study of the actions of compounds such as hexamethonium and decamethonium has been useful in the classification of nicotinic cholinergic receptors in the peripheral nervous system into receptors of the C-6 type, found at autonomic ganglia, and the C-10 type, found at the skeletal neuromuscular junction [6]. The snake neurotoxin, α-bungarotoxin (BTX), has been useful in the study of the C-10 nicotinic receptor subtype found in brain [23,24] but no useful ligand has been identified that is specific for the C-6 nicotinic receptor.

Recently, we [16] and others [1,25] have studied the binding of [^{3}H]-nicotine to brain membranes. Most of the data indicate that [^{3}H]-nicotine binds to a cholinergic receptor in brain and that this binding site is clearly separable from the BTX binding site. However, some investigators [2,29] have suggested that the [^{3}H]-nicotine binding site is not cholinergic, primarily because classical nicotinic receptor blockers, such as mecamylamine, inhibit nicotine binding poorly [1, 16, 25]. A recent autoradiographic analysis indicates that [^{3}H]-nicotine binds to the same sites in brain as does [^{3}H]-ACh and that these two sites are distributed differently in brain than is the BTX binding site [10]. Thus, it has been proposed [16,28] that brain has two nicotinic, cholinergic receptors, one that can be measured with [^{3}H]-nicotine or [^{3}H]-acetylcholine binding and one that can be measured with [^{125}I]-BTX binding. The nicotine binding site has a lower K_D for nicotine than does the BTX binding site. Therefore, it would be expected that responses elicited by nicotine acting at the [^{3}H]-nicotine binding site will be produced by lower doses of nicotine than those required to elicit an effect mediated by nicotine acting at the BTX binding site.

Although these binding sites have been characterized in brain, little knowledge exists with regard to their function. The BTX binding site, for example, has been studied extensively but virtually nothing is known with regard to its function. A number of electrophysiological studies have failed to detect an inhibition by BTX of ACh effects [5, 7, 14, 20]. This has raised the question as to whether the BTX binding site even has a function. It should be noted, however, that

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studies of BTX binding in homogenates indicate that 2–3 hours is required to reach equilibrium [16]. An inspection of the electrophysiological studies of BTX action indicates that BTX was challenged with ACh in the majority of studies before equilibrium had been attained and therefore before complete blockade of the receptor had occurred. Recently, we [21,22] have presented evidence which suggests that the BTX binding site found in hippocampus is associated with sensitivity to nicotine-induced seizures. These studies examined the genetic regulation of nicotine-induced seizures and brain nicotinic receptors ([^{3}H]-nicotine and [^{125}I]-BTX) in C3H and DBA mice as well as the F_1, F_2 and backcross generations derived from these two inbred mouse strains. The data obtained suggest that sensitivity to nicotine-induced seizures may be controlled by a single gene which may also control the number of hippocampal BTX binding sites.

We have also been attempting to assess the role of brain nicotine and BTX binding sites in tolerance to nicotine [18,19]. These studies have demonstrated that chronic nicotine infusion results in tolerance to nicotine as assessed by a test battery which includes measurement of the effects of nicotine on rate of respiration, startle-response, Y-maze activity and rearing, heart rate and body temperature. Tolerance to each of these measures was obtained and this tolerance was paralleled by changes (an increase) in brain [^{3}H]-nicotine binding. This finding suggests the possibility that the nicotine binding site is involved in regulating some, or all, of these responses.

Studies of receptor function have often been facilitated by studies of antagonist action. In the case of both the nicotine and BTX binding sites, it is not clear what an appropriate antagonist might be. For example, we [16] and others [1,25] have demonstrated that classical C-6 and C-10 nicotinic blockers such as hexamethonium, decamethonium, and mecamylamine are poor inhibitors of binding of [^{3}H]-nicotine or BTX to brain membranes. However, mecamylamine pretreatment blocks many of the behavioral effects of ACh, carbamylcholine, and nicotine *in situ* (see, for example [6, 9, 25–27, 30]). It has been suggested that, because mecamylamine does not block the binding of nicotinic agonists to their binding sites, it probably acts by blocking the ion channel associated with nicotinic receptors [3] and is therefore a noncompetitive antagonist.

The purpose of the study reported here was to assess the effects of mecamylamine on several behavioral and physiological responses elicited by nicotine. The responses divide into two major classes: those blocked by low doses of mecamylamine (ED_{50}<0.1 mg/kg) (seizures and enhanced startle response) and those blocked by higher doses of mecamylamine (ED_{50} around 1 mg/kg) (respiratory rate, Y-maze activity, heart rate and body temperature). The results obtained provide further evidence which supports the suggestion that nicotine elicits seizures via an effect at the BTX binding site and suggest that the effects of nicotine on the startle response may also be mediated via an effect on the BTX binding site. Most of the other nicotine responses that we have been studying may be mediated via the [^{3}H]-nicotine binding site.

METHOD

Animals

Male and female mice from two inbred strains, C3H/2Ibg and DBA/2Ibg, were used in this study. These strains have been maintained in the breeding colony at the Institute for Behavioral Genetics for at least 20 generations. All mice were weaned at 25 days of age and housed with two to five like-sex littermates. Animals were maintained on a 12-hr light/12-hr dark cycle (lights on 7 a.m. until 7 p.m.) and were permitted free access to food (Wayne Lab Blox) and water. All testing was done when the mice were between 60 and 90 days of age. Testing was conducted between 9 a.m. and 4 p.m.

Drug Administration

Nicotine and mecamylamine were administered by IP injection of drug dissolved in physiological saline. Injection volume was 0.01 ml/g for both drugs and the doses were adjusted by changing the concentration of the drugs. The nicotine was redistilled periodically.

MEASURES OF BEHAVIORAL AND PHYSIOLOGICAL RESPONSES

Test Battery

A multifactorial test battery was used to assess the response of the mice to nicotine after mecamylamine pretreatment. Mecamylamine was administered 10 min prior to nicotine injection. Five separate tests were included in the battery: respiration, startle, Y-maze, heart rate, and body temperature. Two types of results were obtained from the Y-maze: line crossings and rears. The timing of the tests was determined from the results of time course studies for the effects of nicotine on several of the components of the test battery [18]. A 1.5 mg/kg dose of nicotine was used for DBA mice and a 2.0 mg/kg dose was used for C3H mice. These doses were chosen as the "isoeffective" doses for the strains; i.e., the strains were affected to a similar extent by their respective doses of nicotine. Each test in the battery was conducted as follows:

Respiratory rate. A Respiration Rate Monitor (Columbus Instruments, Columbus, OH) was used to measure respiratory rate. Mice were placed in a glass jar, the floor of which was covered with aspen shavings. A closed system was created by placing a lid, in which a pressure transducer was mounted, on the jar. Respiratory rate was measured by recording the number of breaths/min over a 1-min period. Five separate readings, 15 sec apart, were made 1 min after the lid was put in place. These measurements were made 1 min after the injection of nicotine.

Startle response. A Columbus Instruments Responder Startle Reflex Monitor was used to quantitate the startle response elicited by a short auditory stimulus. A mouse was placed inside a Plexiglas cage (length, 5 cm; width, 14 cm; height, 16 cm), the floor of which was the sensor platform. The animal remained in the cage for 1 min before an auditory stimulus (frequency, 6250 Hz; intensity, 120 dB; duration, 50 msec) was presented. Ten stimuli, 10 sec apart, were presented during each 90-sec test period. Both response amplitude and latency were recorded after each stimulus. The sensor sensitivity was set at 5.00 (full scale, 10.00). The testing chamber was enclosed in a sound-insulated box and a low level of white noise was present at all times (volume setting was 2% of full scale). This test was conducted 2.5 min after nicotine injection.

Y-maze activity. The maze is a symmetrical Y-shaped runway. Each arm of the maze is 26 cm long, 6.1 cm wide, and 10.2 cm high. The arms are subdivided into two equal

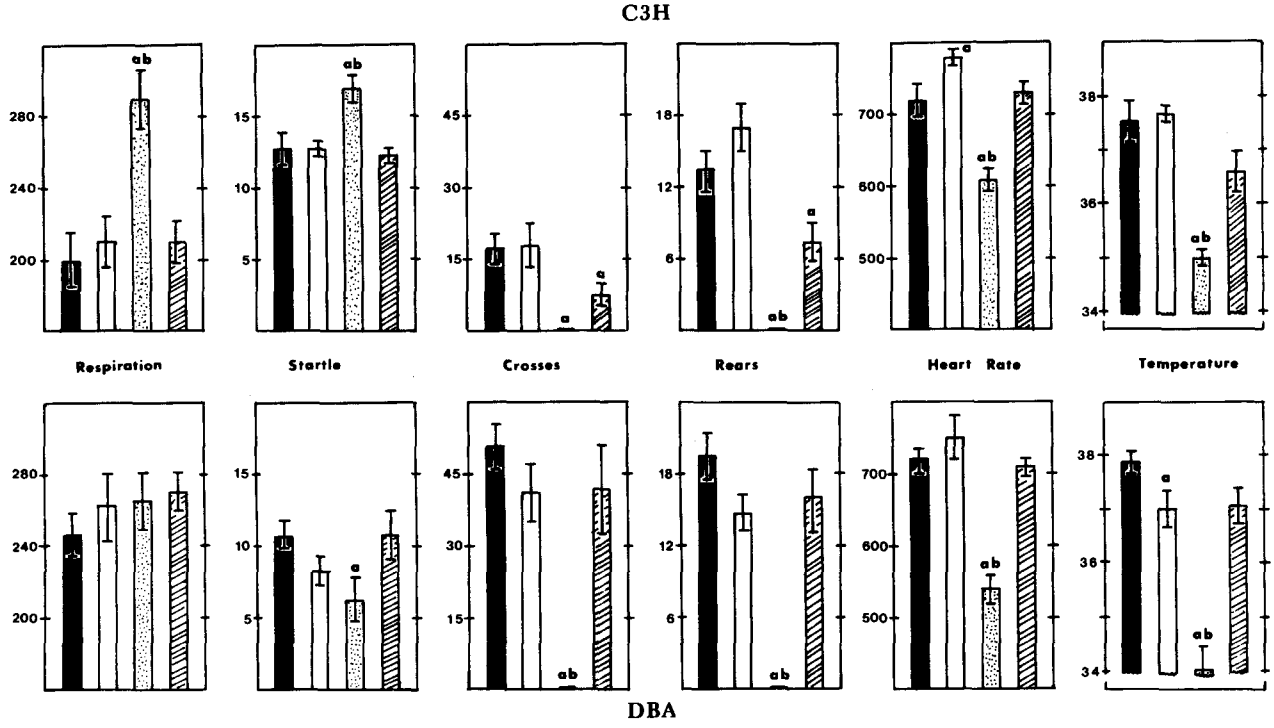

FIG. 1. Responses of C3H and DBA mice in the test battery after receiving injections of saline or mecamylamine and saline or nicotine. Mice were injected with mecamylamine or saline then 10 minutes later injected with nicotine or saline. The control group received injections of saline (closed bars). To determine the effects of mecamylamine, animals received an injection of 2.0 mg/kg mecamylamine followed 10 minutes later by saline injection (open bars). To determine the effects of nicotine, animals received an injection of saline followed 10 minutes later by 1.5 mg/kg nicotine for DBA mice or 2.0 mg/kg nicotine for C3H mice (stippled bars). Mecamylamine blockade of nicotine's effects was assessed by injecting animals with 2.0 mg/kg mecamylamine followed 10 mintues later by either 1.5 mg/kg nicotine for DBA mice or 2.0 mg/kg nicotine for C3H mice (striped bars). Drug effects were assessed on six measures, respiration (breaths per minutes), startle (total response), Y-maze crosses (total crossings), Y-maze rears (total rears), heart rate (beats per minute) and body temperature (°C). Each bar represents the mean±S.E.M. for 5–9 males per group. a: Significantly different from saline/saline group, $p<0.05$. b: Significantly different from mecamylamine/saline group, $p<0.05$.

sections. The maze is constructed of black acrylic plastic with covers of red translucent acrylic plastic. Illumination is with red light. The mouse to be tested was placed in the center of the maze and movement from one section to another was recorded for 3 min. The number of rearings occurring in the test period was also recorded. This test was conducted 4.5 min after nicotine injection.

Heart rate. After the Y-maze test was completed, the mouse was placed in a restrainer and needle electrodes were inserted through the skin. One was placed immediately behind the left foreleg and the other immediately in front of the right hindleg. The electrodes were connected through a preamplifier to an E and M physiograph (Narco Biosystems, Houston, TX). Heart rate was monitored for 6 sec and the rate was estimated by counting the number of QRS complexes. Heart rate was measured 8.5 min after the injection of nicotine.

Body temperature. Temperature was measured with a rectal probe (Bailey Instruments, Saddle Brook, NJ). The probe was lubricated with peanut oil before it was inserted 2.5 cm into the rectal cavity. Body temperature was measured 15 min after injection of nicotine.

Seizure Testing

Animals of both sexes from both strains were injected with mecamylamine or saline 10 min prior to nicotine injection. Animals of the DBA strain received 6.75 mg/kg nicotine and animals of the C3H strain received 3.75 mg/kg nicotine, the respective ED_{80} doses for nicotine-induced convulsions (i.e., the nicotine dose that elicited clonic seizures in 80% of the animals) observed in a previous study [21]. After nicotine injection, each animal was placed in a 17.5×50×20 cm metal cage and observed for 3 min. Whether a clonic seizure occurred, as well as latency to that seizure, was recorded for each animal.

Data Analysis

Test battery data were analyzed by analysis of variance (ANOVA) for each strain separately. The IC_{50} values for mecamylamine blockade of nicotine-induced seizures and nicotine's effects of the six measures of the test battery were calculated by linear regression and were compared between the strains by *t*-test. The slopes of the lines were calculated similarly and also compared between the strains by *t*-test.

RESULTS

The effects of mecamylamine (2.0 mg/kg) alone, nicotine alone and mecamylamine pretreatment followed by nicotine were compared to baseline (saline treatment) levels on the

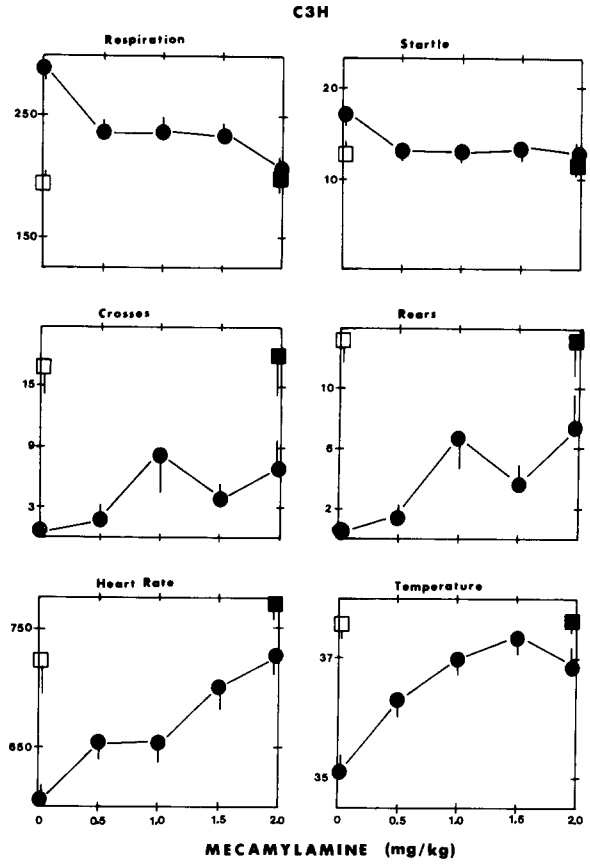

FIG. 2. Dose-response curves for mecamylamine blockade of nicotine's effects in the test battery for C3H mice. C3H mice were injected with mecamylamine in doses of 0 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg or 2.0 mg/kg. Mecamylamine was followed 10 minutes later by an injection of 2.0 mg/kg nicotine (closed circles). The mecamylamine (2.0 mg/kg)/saline groups are represented by closed squares and the saline/saline control groups are represented by the open squares. The six measures of the test battery are: respiration (breaths per minute), startle response (total responses), Y-maze crosses (total crosses), Y-maze rears (total rears), heart rate (beats per minute) and body temperature (°C). Each point represents the mean±S.E.M. for 5–9 males.

six measures of the test battery. These measures include respiration rate, startle response, Y-maze crossings and rears, heart rate and body temperature. In addition, the effects of nicotine alone on each of the six measures for each strain were compared to the effects of the mecamylamine pretreatment/nicotine treatment regimen on the six measures. The results are presented in Fig. 1. Mecamylamine alone had very little effect on any of the six measures for either strain. C3H mice had a slight elevation of heart rate over baseline levels when administered 2.0 mg/kg mecamylamine, $F(1,10)=5.40$, $p<0.05$. For DBA mice, body temperature was slightly reduced by mecamylamine from control levels, $F(1,10)=7.27$, $p<0.05$.

For C3H mice, nicotine alone had significant effects on all six measures of the test battery as compared to baseline levels. Nicotine increased both respiration levels, $F(1,9)=15.88$, $p<0.01$, and startle response, $F(1,9)=7.28$, $p<0.05$, for the C3H strain. Y-maze crossings, $F(1,9)=22.10$, $p<0.01$, and rears, $F(1,9)=61.76$, $p<0.01$, heart rate, $F(1,9)=13.00$, $p<0.01$, and body temperature, $F(1,9)=15.80$, $p<0.01$, were all significantly reduced. The pattern of results for DBA mice administered nicotine was slightly different. Y-maze crossings, $F(1,10)=88.49$, $p<0.01$, and rears, $F(1,10)=70.18$, $p<0.01$, heart rate, $F(1,10)=65.38$, $p<0.01$, and body temperature, $F(1,10)=51.46$, $p<0.01$, were all depressed when compared to control levels. Respiratory rate was slightly increased although not significantly. Whereas startle response was increased for the C3H strain, it was significantly decreased by nicotine for the DBA strain, $F(1,10)=5.46$, $p<0.05$.

To determine if mecamylamine could block the effects of nicotine on these six measures, mecamylamine (2.0 mg/kg) was administered 10 minutes prior to nicotine administration (1.5 mg/kg DBA; 2.0 mg/kg C3H). These doses of nicotine were chosen as the isoeffective doses of nicotine for the strains, i.e., the strains are affected to a similar extent by their respective doses of nicotine. Using this drug treatment regimen, mecamylamine completely blocked the effects of nicotine on four of the six tests for C3H mice. Only Y-maze crosses, $F(1,13)=4.71$, $p<0.05$, and rears, $F(1,13)=6.40$, $p<0.05$, remained significantly depressed from control levels. Y-maze rears after mecamylamine blockade, although significantly less than control levels, were significantly greater than after nicotine alone, $F(1,12)=8.02$, $p<0.05$. The effects of nicotine on respiration, startle response, heart rate and body temperature were effectively antagonized by mecamylamine pretreatment. For DBA mice, mecamylamine antagonized nicotine's effects on all five measures (nicotine had no effect on respiratory rate). It was observed that startle response, Y-maze crosses and rears, heart rate and body temperature after mecamylamine/nicotine treatment were all similar to control levels and all but startle were significantly different from the nicotine alone treatment group, [crosses, $F(1,10)=18.29$, $p<0.01$; rears, $F(1,10)=31.77$, $p<0.01$; heart rate, $F(1,10)=67.97$, $p<0.01$; temperature, $F(1,10)=27.67$, $p<0.01$].

Dose-response curves were constructed to assess mecamylamine's effectiveness at blocking nicotine's actions on the six test battery measures for each strain. The results are presented in Fig. 2 for C3H mice and Fig. 3 for DBA mice. In addition, IC_{50} values were calculated for mecamylamine blockade of nicotine's effects on all six measures for C3H mice and all but respiratory rate for DBA mice. These results are presented in Table 1. No differences were observed between C3H and DBA mice among their IC_{50} values on any of the measures. Line comparisons, testing for differences among the slopes and for coincidence of the lines, were also done among the test battery measures between the two strains. No significant differences were observed. For both C3H and DBA mice, mecamylamine was observed to effectively block 50% of nicotine's effects on heart rate and body temperature at approximately 0.9 mg/kg. Nicotine's effects on Y-maze crosses and rears were blocked by mecamylamine doses of 1.4 to 2.0 mg/kg. C3H mice had slightly but not significantly higher IC_{50} values for Y-maze crosses and rears than did DBA mice. However, because C3H activity did not return to control levels at the highest dose of mecamylamine utilized (2.0 mg/kg), these IC_{50} values may be underestimates. An IC_{50} value for mecamylamine blockade of nicotine-induced depression of the startle response, seen only in DBA mice, was calculated (1.29 mg/kg), but this value is probably not valid because the slope of the mecamylamine dose-response curve was not significantly different from zero (slope=1.76±1.12, $t(4)=1.5$, $p>0.05$).

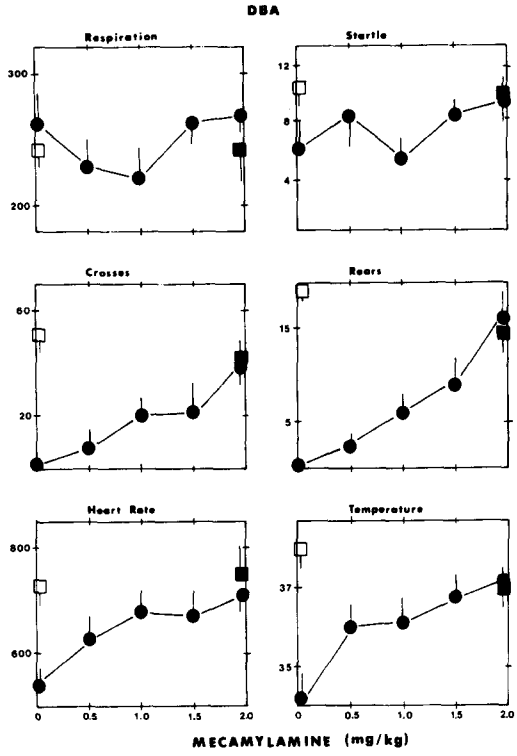

FIG. 3. Dose-response curves for mecamylamine blockade of nicotine's effects in the test battery for DBA mice. DBA mice were injected with mecamylamine in doses of 0 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg or 2.0 mg/kg. Mecamylamine was followed 10 minutes later by an injection of 1.5 mg/kg nicotine (closed circles). The mecamylamine (2.0 mg/kg)/saline groups are represented by closed squares and the saline/saline control groups are represented by the open squares. The six measures of the test battery are: respiration (breaths per minute), startle response (total responses), Y-maze crosses (total crosses), Y-maze rears (total rears), heart rate (beats per minute) and body temperature (°C). Each point represents the mean±S.E.M. for 5–9 animals.

TABLE 1

IC_{50} VALUES FOR MECAMYLAMINE BLOCKADE OF THE BEHAVIORAL EFFECTS OF NICOTINE

	IC_{50} (mg/kg)	
	C3H	DBA
Respiration	0.96 ± 0.22	—
Y-Maze Crosses	2.29 ± 0.55	1.36 ± 0.09
Y-Maze Rears	1.92 ± 0.40	1.39 ± 0.11
Heart Rate	0.89 ± 0.09	0.88 ± 0.17
Body Temperature	0.85 ± 0.48	0.96 ± 0.19
Enhanced Startle	0.085 ± 0.012	—
Clonic Seizure	0.072 ± 0.021	0.087 ± 0.012

IC_{50} values (±S.E.) are presented for mecamylamine blockade of nicotine's effects on respiration, Y-maze crosses, Y-maze rears, heart rate and body temperature. IC_{50} values were also calculated for the enhancement of startle in C3H mice and the depression of startle in DBA mice and for mecamylamine blockade of nicotine-induced seizures.

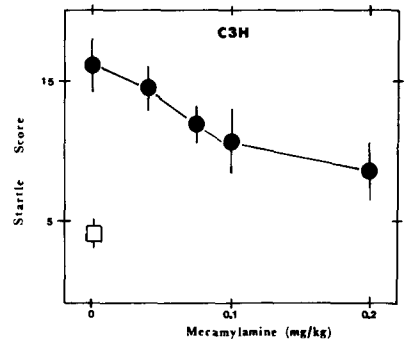

FIG. 4. Dose-response curve for mecamylamine blockade of nicotine's effects of startle response for C3H mice. C3H mice were injected with 0 mg/kg, 0.05 mg/kg, 0.75 mg/kg, 0.1 mg/kg or 0.2 mg/kg mecamylamine followed 10 minutes later by 2.0 mg/kg nicotine. 2.5 minutes later startle response was assessed as described in the Method section. Each point represents the mean±S.E.M. for 10 females.

Mecamylamine's effects on enhanced startle, seen only in C3H mice, appeared to be total at the lowest dose (0.5 mg/kg) tested in the experiments reported in Fig. 2. Therefore, startle response was assessed in C3H mice using lower doses of mecamylamine, ranging from 0.00 to 0.20 mg/kg, followed 10 minutes later by 2.0 mg/kg nicotine. These results are presented in Fig. 4. The IC_{50} value for mecamylamine blockade of nicotine's effect on startle in C3H mice was estimated to be 0.085±0.01 mg/kg.

Figure 5 presents the dose-response curves for mecamylamine blockade of nicotine-induced seizures for the C3H and DBA strains. The IC_{50} values for mecamylamine blockade were 0.072±0.021 mg/kg and 0.087±0.012 mg/kg for the C3H and DBA strains respectively. While the IC_{50} values for the two strains do not differ significantly, the slopes of the lines do differ between the strains [C3H=0.1203±0.0064 and DBA=0.2180±0.0038; $t(6)=11.28, p<0.001$]. As a consequence, there is an approximate two-fold difference in the mecamylamine dose that completely protects against nicotine-induced seizures.

DISCUSSION

The results reported here extend our earlier findings of strain differences in sensitivity to nicotine [17,21]. In addition, strain differences in sensitivity to mecamylamine were found, C3H mice were more sensitive to mecamylamine blockade of nicotine-induced seizures. Mecamylamine completely reversed the effects of nicotine on Y-maze measures in the DBA mice, but in the dose range used complete reversal was never achieved for C3H mice. In addition to strain differences in sensitivity to mecamylamine, marked differences in the IC_{50} values for the various tests were obtained. Mecamylamine blocked nicotine-induced seizures in both C3H and DBA mice with IC_{50} values of less than 0.1 mg/kg. Similarly, the nicotine-induced enhancement of the startle

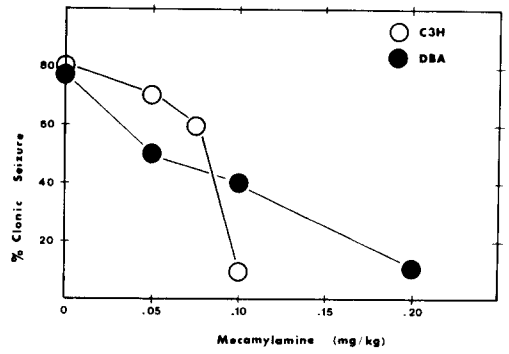

FIG. 5. Dose-response curves of mecamylamine blockade of nicotine-induced seizures in C3H and DBA mice. C3H and DBA mice were administered 0 mg/kg, 0.025 mg/kg, 0.05 mg/kg, 0.075 mg/kg, 0.1 mg/kg or 0.2 mg/kg mecamylamine followed 10 minutes later by 3.75 mg/kg nicotine for C3H (open circles) or 6.75 mg/kg nicotine for DBA (closed circles) mice. Seizure sensitivity was assessed as described in the Method section. Each point represents the proportion of mice having a clonic seizure. Both male and female mice were used and each point represents 10 animals.

response seen in C3H mice was antagonized by mecamylamine with a low IC_{50} value. IC_{50} values for mecamylamine blockade of the remainder of the responses ranged between approximately 1 and 2 mg/kg, i.e., one order of magnitude higher.

The results of the study of nicotine's actions on the startle response reported in Fig. 1 differ slightly from our previous study of this phenomenon [17]. In this earlier study, no significant effects of nicotine on the startle response were seen in DBA mice that were treated with nicotine doses ranging between 0.1 and 2 mg/kg. In view of the fact that the slope of the mecamylamine dose-response curve for antagonism of nicotine's effects on startle in DBA mice is not different from zero, we suspect that the apparent depression by nicotine of the startle response in DBA mice, reported in Fig. 1, is a spurious finding.

We have suggested in an earlier study of brain [^{3}H]-nicotine and [^{125}I]-BTX binding that nicotine may elicit its behavioral effects by interacting at these two receptor sites [16]. In this earlier study, we reported that unlabeled L-nicotine inhibits the binding of [^{3}H]-nicotine to its binding site with an IC_{50} value of 0.023 μM whereas it inhibits the binding of BTX to its binding site with an IC_{50} value of 0.81 μM. This difference approximates the difference seen between the IC_{50} values for mecamylamine blockade of nicotine-induced startle/seizures and the IC_{50} values for nicotine's effects on respiration, Y-maze crossings and rears, heart rate, and body temperature. If mecamylamine binds to a similar site on each of these nicotinic receptor classes with equal affinity, it should be more effective in blocking the effects of nicotine elicited at the lower affinity (BTX) binding site. Alternatively, the two nicotinic receptors may differ in their affinity for both nicotine and mecamylamine. In addition, if mecamylamine blocks the nicotinic receptor by blocking activated ion channels, that channel which is open for a longer period of time should be more susceptible to mecamylamine blockade. All of these possibilities remain as viable explanations for the observations reported here.

We have demonstrated in other studies [21,22] that nicotine-induced seizures, which are seen at higher doses than are the effects on Y-maze activity, heart rate and body temperature, may be regulated by a single gene. This gene may also regulate the number of BTX binding sites in the hippocampus such that a high correlation was observed between seizure sensitivity and the number of hippocampal BTX binding sites in DBA and C3H mice and in their derived F_1, F_2 and backcross generations. Sensitivity to nicotine-induced seizures did not seem to correlate with [^{3}H]-nicotine binding in any brain region. The results of the current study indicate that mecamylamine may be slightly more effective in blocking nicotine-induced seizures in C3H mice than in DBA mice. This result parallels the sensitivity of the two strains to nicotine. The strain difference in sensitivity to mecamylamine blockade of nicotine-induced seizures coupled with the low IC_{50} value for mecamylamine blockade of seizures combine to support the hypothesis that nicotine induces seizures via an effect at a lower affinity binding site, possibly the BTX site.

In studies of the development of tolerance to nicotine [18,19], we reported that chronic infusion with nicotine resulted in an increase in brain [^{3}H]-nicotine binding as well as an increase in [^{125}I]-BTX binding. Larger doses of nicotine were required to elicit changes in [^{125}I]-BTX binding. Nicotine tolerance, as measured with the Y-maze, heart rate and temperature tests, correlated better with increases in [^{3}H]-nicotine binding than with increases in [^{125}I]-BTX binding; i.e., considerable tolerance to the effects of nicotine on these tests was seen before changes in [^{125}I]-BTX binding occurred. This is consistent with nicotine eliciting its effects on these measures via an effect at a higher affinity receptor. The observation that the IC_{50} values for mecamylamine blockade of nicotine's effects on Y-maze, heart rate and body temperature were greater than the IC_{50} values for blockade of seizures and enhanced startle is also consistent with the hypothesis that nicotine elicits its effect on Y-maze and body temperature via a high affinity nicotinic receptor, assuming that mecamylamine has similar affinities for the two receptors at which nicotine may act. The heart rate effects of nicotine are likely to be mediated largely via effects at autonomic ganglia which presumably have the C-6 type nicotinic receptor.

In summary, mecamylamine blocks nicotine effects with IC_{50} values that seem to fall into two groups. These results suggest that nicotine actions in vivo are mediated by two distinct receptor systems. Whether these two receptor systems correspond to those identified by nicotine and α-BTX binding in brain must be verified.

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REFERENCES

1. Abood, L. G., D. T. Reynolds and J. M. Bidlack. Stereospecific ^{3}H-nicotine binding to intact and solubilized rat brain membranes and evidence for its noncholinergic nature. *Life Sci* **27:** 1307–1314, 1980.
2. Abood, L. G., D. T. Reynolds, H. Booth and J. M. Bidlack. Sites and mechanisms for nicotine's action in the brain. *Neurosci Biobehav Rev* **5:** 479–486, 1981.
3. Ascher, P., W. A. Large and H. P. Rang. Studies on the mechanism of action of acetylcholine antagonists on rat parasympathetic ganglion cells. *J Physiol* **295:** 139–170, 1979.
4. Birdsall, N. J. M., E. S. Hulme and J. M. Stockton. Muscarinic receptor heterogeneity. *Trends Pharmacol Sci* **5:** 4–8, 1984.
5. Carbonetto, S. T., D. M. Fambrough and K. J. Muller. Nonequivalence of α-bungarotoxin receptors and acetylcholine receptors in chick sympathetic neurons. *Proc Natl Acad Sci USA* **75:** 1016–1020, 1978.
6. Caulfield, M. P. and G. A. Higgins. Mediation of nicotine-induced convulsions by central nicotinic receptors of the 'C-6' type. *Neuropharmacology* **22:** 347–351, 1983.
7. Chou, T. C. and C. Y. Lee. Effect of whole and fractionated cobra venom on sympatheric ganglion transmission. *Eur J Pharmacol* **8:** 326–330, 1969.
8. Clarke, P. B. S. and R. Kumar. The effects of nicotine on locomotor activity in nontolerant and tolerant rats. *Br J Pharmacol* **78:** 329–337, 1983.
9. Clarke, P. B. S. and R. Kumar. Some effects of nicotine on food and water intake in undeprived rats. *Br J Pharmacol* **82:** 233–239, 1984.
10. Clarke, P. B. S., R. D. Schwartz, S. M. Paul, C. B. Pert and A. Pert. Nicotinic binding in rat brain: Autoradiographic comparison of [^{3}H]-nicotine and [^{125}I]-α-bungarotoxin. *J Neurosci* **5:** 1307–1315, 1985.
11. Dale, H. H. The action of certain esters and ethers of choline, and their relation to muscarine. *J Pharmacol Exp Ther* **6:** 147–190, 1914.
12. Duggan, A. W., J. G. Hall and C. Y. Lee. Alpha-bungarotoxin, cobra neurotoxin and excitation of Renshaw cells by acetylcholine. *Brain Res* **107:** 166–170, 1976.
13. Hammer, R., C. P. Berrie, N. J. M. Birdsall, A. S. V. Burgen and E. S. Hulme. Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature* **283:** 90–92, 1980.
14. Ko, C. P., H. Burton and R. P. Bunge. Synaptic transmission between rat spinal cord explants and dissociated cervical ganglion neurons in tissue culture. *Brain Res* **117:** 437–460, 1976.
15. Luthin, G. R. and B. B. Wolfe. Characterization of [^{3}H] pirenzepine binding to muscarinic cholinergic receptors solubilized from rat brain. *J Pharmacol Exp Ther* **234:** 37–44, 1985.
16. Marks, M. J. and A. C. Collins. Characterization of nicotine binding in mouse brain and comparison with the binding of α-bungarotoxin and quinuclidinyl benzilate. *Mol Pharmacol* **22:** 554–564, 1982.
17. Marks, M. J., J. B. Burch and A. C. Collins. Genetics of nicotine response in four inbred strains of mice. *J Pharmacol Exp Ther* **226:** 291–302, 1983.
18. Marks, M. J., J. B. Burch and A. C. Collins. Effects of chronic nicotine infusion on tolerance development and cholinergic receptors. *J Pharmacol Exp Ther* **226:** 806–816, 1983.
19. Marks, M. J. and A. C. Collins. Tolerance, cross-tolerance, and receptors after chronic nicotine or oxotremorine. *Pharmacol Biochem Behav* **22:** 283–291, 1985.
20. Miledi, R. and A. C. Szczepaniak. Effect of dendroaspis neurotoxins on synaptic transmission in the spinal cord of the frog. *Proc R Soc Lond [Biol]* **190:** 267–274, 1974.
21. Miner, L. L., M. J. Marks and A. C. Collins. Classical genetic analysis of nicotine-induced seizures and nicotinic receptors. *J Pharmacol Exp Ther* **231:** 545–554, 1984.
22. Miner, L. L., M. J. Marks and A. C. Collins. Relationship between nicotine-induced seizures and hippocampal nicotinic receptors. *Life Sci* **37:** 75–83, 1985.
23. Morely, B. J., G. E. Kemp and P. Salvaterra. α-Bungarotoxin binding sites in the CNS. *Life Sci* **24:** 859–872, 1979.
24. Oswald, R. G. and J. A. Freeman. Alpha-bungarotoxin binding and central nervous system nicotinic acetylcholine receptors. *Neuroscience* **6:** 1–14, 1981.
25. Romano, C., A. Goldstein and N. P. Jewell. Characterization of the receptor mediating the nicotine discriminative stimulus. *Psychopharmacology (Berlin)* **74:** 310–315, 1981.
26. Schechter, M. D. and J. A. Rosecrans. CNS effect of nicotine on the discriminative stimulus for the rat in a T-maze. *Life Sci* **10:** 821–832, 1971.
27. Schwab, L. S. and M. F. Kritzer. The effect of cholinergic antagonists on a central response to nicotine. *Experientia* **38:** 119–120, 1982.
28. Schwartz, R. D., R. McGee, Jr. and K. J. Kellar. Nicotinic cholinergic receptors labeled by [^{3}H]-acetylcholine in rat brain. *Mol Pharmacol* **22:** 55–62, 1982.
29. Sershen, H., M. E. A. Reith, A. Lajtha and J. Gennaro, Jr. Noncholinergic, saturable binding of (±)-[^{3}H]-nicotine to mouse brain. *J Recept Res* **2:** 1–15, 1981.
30. Tripathi, H. L., B. R. Martin and M. D. Aceto. Nicotine-induced antinociception in rats and mice: correlation with nicotine brain levels. *J Pharmacol Exp Ther* **221:** 91–96, 1982.