Pharmacology Biochemistry & Behavior, Vol. 30, pp. 269–278. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Genetic Influences on Acute Responses to Nicotine and Nicotine Tolerance in the Mouse

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COLLINS, A. C., L. L. MINER AND M. J. MARKS. *Genetic influences on acute responses to nicotine and nicotine tolerance in the mouse.* PHARMACOL BIOCHEM BEHAV **30**(1) 269–278, 1988.—Nineteen inbred mouse strains were tested for their relative sensitivity to nicotine's effects on respiratory rate, acoustic startle response, heart rate, Y-maze activity (crosses and rears) and body temperature. Separate animals were tested for their sensitivity to nicotine-induced seizures following IP injection or IV infusion. Dose-response curves were constructed for each measure. Large strain differences were obtained for all of these measures. Nicotine's effects on heart rate, Y-maze activity and body temperature segregated together into the various mouse strains whereas seizure sensitivity segregated independently which suggests that these responses are under separate genetic control. Evidence was obtained which suggests that nicotine-induced seizures are regulated, in part, by the number of hippocampal nicotinic receptors measured with α-bungarotoxin (BTX). Strain differences in the development of tolerance to nicotine were also observed. Four mouse strains were tested and one of these strains (C3H) did not exhibit tolerance to nicotine. The binding of (^{3}H)nicotine and (^{125}I)BTX increased in the brains of all four mouse strains. These changes may relate to tolerance in some mouse strains, but since C3H mice did not exhibit tolerance even though brain nicotinic receptors changed following chronic treatment, other explanations for the role of receptor changes in tolerance to nicotine must be sought.

Nicotine Genetics Tolerance Locomotor activity Seizures Heart rate Body temperature Nicotinic receptors

TOBACCO use is a phenomenon that appears to be restricted to humans. Russell [43] has argued that nicotine is the most important pharmacologically active agent in tobacco and that humans use tobacco in ways that will maximize the absorption of nicotine. Smokers modify their puff rate or depth of inhalation so as to regulate their nicotine intake [2, 4, 15, 48] and giving a smoker a low-nicotine cigarette results in a withdrawal syndrome [11,22]. In addition, an injected dose of nicotine serves to reduce smoking [21,24] whereas the administration of mecamylamine, a centrally acting nicotinic antagonist, results in an increase in tobacco use [47]. These observations lead to the conclusion that nicotine is the most important agent involved in the maintenance of tobacco use.

Nicotine may elicit different subjective responses in smokers and nonsmokers. A study by Johnston [21] indicated that nonsmokers generally report nicotine injection as being unpleasant whereas smokers generally report the effects as being pleasant. Therefore, it seems reasonable to suggest that an individual's response to nicotine may influence whether tobacco use will be initiated and maintained.

Another factor that may contribute to tobacco use is a genetic predisposition. Fisher [12,13] was the first to report that concordance in twins (whether the two members of a twin pair express the same trait) for smoking behavior was greater in a population of monozygotic (identical) twins than was the concordance in dizygotic (fraternal) twins. Because monozygotic twins possess an identical genotype while dizygotic twins are no more alike genetically than any other brother-sister pair, the greater concordance for smoking behavior seen in the identical twin pairs may indicate that tobacco use is influenced by genetic factors. Several more recent studies have added further support to the conclusion that genetic factors regulate smoking behavior [7, 9, 39, 46].

A major problem with all of the human genetic studies of tobacco use is that no attempts were made to quantify smoking in terms of nicotine consumption. Thus, it is not clear whether the genetic factors which seem to predispose people to smoking involve genetic regulation of response to nicotine. While we know very little about the genetic regulation of nicotine response in humans, studies with animals suggest there may be adequate reason to search for such a regulation. Several studies have detected differential effects of the same dose of nicotine in rats with high or low control locomotor activity. The Roman High Avoidance and Maudsley Nonreactive rats were selectively bred for specific traits. These animals exhibit high levels of locomotor activity, and exhibit greater stimulation of locomotor activity by

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nicotine than do low-activity lines (Roman Low Avoidance and Maudsley Reactive) [3, 16–18]. Inbred rat strains also differ with respect to the effects of nicotine on locomotor activity [36] in that strains with high control activity scores (Lister and Sprague Dawley) tend to have their activity reduced by nicotine while a low activity strain (Wistar) exhibits an increase in activity. These genetically determined differences in response to nicotine do not appear to be due to differences in the elimination of this agent although sex differences in response to nicotine seen in the rat may be explained by a difference in the rate of metabolism [41,42].

Bovet *et al.* [5] were the first to describe differences among inbred mouse strains in response to nicotine. These investigators observed strain differences in the effects of nicotine on avoidance conditioning. We [20] subsequently observed strain differences in the effects of an acute dose (1 mg/kg, IP) of nicotine on activity in a Y-maze. No significant differences among the strains in rate of nicotine elimination have been observed [38]. These results indicate that strain differences in response to nicotine in the mouse are likely due to pharmacodynamic rather than pharmacokinetic differences.

More recent studies from our laboratory have demonstrated strain differences in the effects of nicotine on open-field activity, rotarod performance, body temperature, respiration rate, and acoustic startle response [25, 29, 30] as well as nicotine-induced seizures [32]. These studies have examined nicotine effects in DBA/2Ibg, C57BL/6Ibg, C3H/2Ibg, and BALB/c mice. In general, it appears as though the C3H strain is uniquely sensitive to the stimulant effects of nicotine, with the exception of effects on respiration, and that the C57BL and DBA strains are most sensitive to depressant effects of nicotine.

Significant advances have been made in our understanding of the genetic regulation of nicotine-induced seizures. We have observed that C3H mice are very sensitive to nicotine-induced seizures, whereas DBA mice are relatively resistant and that seizure sensitivity segregates into C3H, DBA, F_1, F_2, and backcross (F_1-by-C3H and F_1-by-DBA) generations in a manner that suggests nicotine-induced seizures are regulated by a simple genetic system, perhaps a single gene [32].

In addition to our concern as to whether genetic factors influence acute responses to nicotine, we have also been concerned as to whether differences in the number or affinity of brain nicotinic receptors exist that may explain these strain differences in response to nicotine. Nicotinic receptors in the central nervous system have been measured in several ways. The binding of α-bungarotoxin (BTX) has been widely used to assay for nicotinic receptors in the brain [34,37]. As an alternative to BTX, the binding of radiolabeled nicotine itself has been studied [1, 8, 27, 40]. Acetylcholine has also been used to measure binding sites [44], and the binding properties measured with nicotine and acetylcholine, although different from those for BTX, are very similar to each other as assessed by autoradiographic techniques [6].

Our studies, to date, have not revealed any strain differences in numbers or affinity of brain nicotine binding sites, but strain differences in BTX binding have been observed, most notably in the hippocampus [25, 32, 33]. It appears that the same gene(s) that regulates nicotine-induced seizures may also regulate the number of hippocampal nicotinic receptors measured with BTX. Animals with greater numbers of hippocampal BTX binding sites are more sensitive to nicotine's seizure-inducing effects.

Another aspect of our research effort has been the analysis of tolerance to nicotine. An early study indicated that mouse strains differ in tolerance development following the chronic intraperitoneal injection of nicotine [19]. More recently, we have studied tolerance development in DBA mice that accompanies continuous chronic infusion of nicotine. The results obtained indicate that a dose-dependent tolerance develops (tolerance increases with infusion dose), and this tolerance is paralleled by an increase in the number of brain nicotine binding sites [26,28]. BTX binding also increases with chronic nicotine infusion, but these increases do not parallel tolerance development in a dose-dependent way. Another study demonstrated that the acquisition of tolerance paralleled changes in nicotine binding in a time-dependent fashion, as did the loss of tolerance: i.e., tolerance was gained and lost during and after chronic nicotine infusion, and the acquisition and loss of tolerance were paralleled by increases and the return to normal of brain nicotine binding sites [38].

The studies reported here extend our genetic analysis of acute responses to nicotine to 19 inbred strains, and data concerning the effects of genotype on the development of tolerance to nicotine are reported. Ultimately, we hope these studies will lead to animal models of smoking and nonsmoking humans.

METHOD

Animals

Male and female mice of 19 inbred strains (A/J, AKR/J, BALB/cJ, BUB/bnJ, CBA/J, C3H/2Ibg, C57BL/6Ibg, C57BL/10J, C57BR/cdJ, C57L/J, C58/J, DBA/1J, DBA/2Ibg, LP/J, P/J, RIIIS/J, SJL/J, ST/bJ, and SWR/J) were used in the acute response studies, and four inbred mouse strains (BALB/cByJ, C3H/2Ibg, C57BL/6Ibg, and DBA/2Ibg) were used in the tolerance studies. Mice of the C57BL/6Ibg, DBA/2Ibg, and C3H/2Ibg strains were bred at the Institute for Behavioral Genetics, University of Colorado, Boulder, CO. These strains have been maintained at the Institute for at least 20 generations. Mice of the BALB/cByJ strain, originally obtained from the Jackson Laboratories, Bar Harbor, ME, were also bred at the Institute, but have been maintained here for fewer than five generations. All of the other mouse strains were obtained from the Jackson Laboratories, and were allowed to acclimate to our colony for a minimum of 2 weeks before use. Mice were housed with 1–5 littermates, were provided with food (Wayne Lab Blox) and water ad lib, and were maintained on aspen bedding. Animals were 60–90 days old when tested.

Acute Response Studies

Nicotine administration. Nicotine was obtained from Sigma Chemical Co., St. Louis, MO and was redistilled periodically. Nicotine was dissolved in physiological saline and was administered by intraperitoneal injection. Injection volume was 0.01 ml/g body weight.

Testing. The responses of mice from each of the strains were tested following challenge with an acute dose of nicotine using a complete test battery consisting of the five tests (the "test battery") described below. In addition, sensitivity to nicotine-induced seizures was also determined. Dose-response curves were constructed, in each strain, for the test battery and for nicotine-induced seizures. The nicotine doses used in the test battery ranged from 0.25–4.0 mg/kg. For nicotine-induced seizures the doses ranged between 2.0 and 7.0 mg/kg. A minimum of three nicotine doses,

plus saline for the controls, were used to construct the test battery dose-response curves and the seizure dose-response curves.

For the test battery, each animal was injected with saline or nicotine and tested for its response in all of the following tests. The timing of these tests was determined from the results of a dose-response, time-course analysis of nicotine effects in four of the mouse strains used in the studies reported here [30].

Respiration. Respiratory rate was measured using a Columbus Instruments Respiration Rate Monitor. Prior to injection of nicotine, the mouse was placed in a glass jar (diameter, 10.5 cm; height, 17 cm) the bottom of which was covered with aspen shavings. After 10 min, the mouse was removed and injected with the appropriate dose of nicotine. The animal was then returned to the jar and a lid containing a pressure-sensitive transducer was placed on the jar to form a closed system. Monitoring was begun 1 min after injection of the nicotine. Respiratory rate was monitored for 1 min during which time five equally spaced recordings were made.

Startle response. The response of mice to an acoustic startle was measured using a Columbus Instruments Responder Startle Monitor. The startle reflex was measured 3 min after injection of nicotine. The mouse to be tested was placed inside a box made of acrylic plastic (length, 14 cm; width, 5 cm; height, 16 cm) and the box was covered with a lid of acrylic plastic. The bottom of the box was the sensor platform. An auditory stimulus (frequency, 6250 Hz; intensity, 120 dB; duration, 50 msec) was presented ten times, with a 10-sec interval between stimuli. Both the response time and amplitude were recorded.

Y-maze activity. Both locomotor and rearing activity were determined in a symmetrical Y-maze. The maze consists of three arms which are 26 cm long, 6.1 cm wide, and 10.2 cm high. Each arm was subdivided into two equal sections. Testing was begun 5 min after injection of nicotine by placing the mouse in the center of the maze. Testing was conducted for 3 min. Movements from one section to another were counted, as were the number of rears.

Heart rate. Heart rate was measured by placing one electrode behind the left foreleg and another in front of the right hindleg. The electrodes were connected through a preamplifier to a Narco Biosystems E & M Physiograph. Heart rate was measured for 6 sec and the rate was estimated by counting the number of QRS complexes. Heart rate was measured 9 min after injection.

Body temperature. Body temperature was measured with a Bailey Instruments rectal probe. The probe was lubricated with peanut oil and was inserted 2.5 cm into the rectal cavity. The body temperature was measured 15 min after nicotine injection.

Nicotine-induced seizures. Separate mice from each strain were also tested for their sensitivity to nicotine-induced seizures. Mice from each strain were given varying doses of nicotine ranging between 2.0 and 7.0 mg/kg. After injection, the individual animal was placed in a 17×50×20 cm metal cage and observed for 3 min. Nicotine-induced seizures occurred very quickly after drug administration, generally within 3 min.

In a second experiment, nicotine-induced seizures were measured following the IV infusion of nicotine. In these experiments, a cannula was implanted in the jugular vein, as described below, and nicotine was infused at a rate of 2 mg/kg/min until a clonic seizure occurred. Latency to seizure was, therefore, a direct measure of the seizure sensitivity of each individual mouse. This allowed a more powerful correlation analysis between seizure sensitivity and hippocampal BTX binding. After seizure testing, the animals were sacrificed, their brains removed, the hippocampus dissected out, and BTX binding measured as described below. Correlations between ED_{50} for IP seizures and BTX binding were also calculated using the BTX binding values obtained from the IV infused mice.

Tolerance Studies

Chronic nicotine infusion. Cannulas made of silastic tubing were implanted in the right jugular vein as described previously [26]. After recovery from surgery the mice were transferred to individual infusion chambers, and their cannulae attached to tubing that was connected to a 1 ml syringe mounted on a Harvard infusion pump. The animals were infused with sterile saline, at a flow rate of 35 μl/hr, for 1 day before nicotine treatment was initiated. The mice were infused with nicotine at 1 mg/kg/hr for the first day, at 2 mg/kg/hr for the second day, and at 3 mg/kg/hr for the next 10 days. Control animals were infused for the same time period with saline.

Tolerance testing. Two hours after termination of saline or nicotine infusion, the animals were tested for their responses to an IP injection of saline or nicotine (1 or 2 mg/kg) using the test battery described above. After completion of the tolerance test the animal was sacrificed and its brain was removed for measurement of brain nicotinic receptors.

Receptor Measurements

Tissue preparation. After completion of the behavioral tests, the mouse was sacrificed by cervical dislocation and its brain was removed. Subsequently, the brain was dissected into seven regions: cortex, cerebellum, hindbrain (pons-medulla), hypothalamus, hippocampus, striatum and midbrain (midbrain areas remaining after removal of the hypothalamus, hippocampus and striatum). The cerebellum was discarded because of its low level of cholinergic activity. The tissue pieces were placed in 10 volumes of HEPES-buffered Ringer's solution (NaCl, 118 mM; KCl, 4.8 mM; $CaCl_2$, 2.5 nM; $MgSO_4$, 1.2 mM; HEPES, 20 mM; pH adjusted to 7.5 with NaOH; and were then frozen at −70°. On the day of assay, the samples were thawed and homogenized with a glass-Teflon homogenizer. The particulate fraction was prepared using the method of Romano and Goldstein [40]. Before each of the three centrifugation steps, the homogenates were incubated for 5 min at 37°C to promote the dissociation of any nicotine that may have been in the tissue [26].

L-(^{3}H)nicotine binding. The binding of L-(^{3}H)nicotine was measured using a modification of the method of Romano and Goldstein [40], as described previously [27]. A single concentration of radiolabeled nicotine (9.6±0.2 nM) was used for these assays in all brain regions. Specific binding was determined as the difference in binding between samples containing no nonradioactive nicotine and those containing 10 μM unlabeled L-nicotine.

α-(^{125}I)BTX binding. The binding of (^{125}I)BTX was measured as described previously [27]. A single concentration of (^{125}I)BTX was used (0.98±0.04 nM). Specific binding was determined from the difference in binding obtained in the presence or absence of 1 mM L-nicotine.

Both of the receptor binding assays were carried out, as noted above, using a single ligand concentration. Our earlier

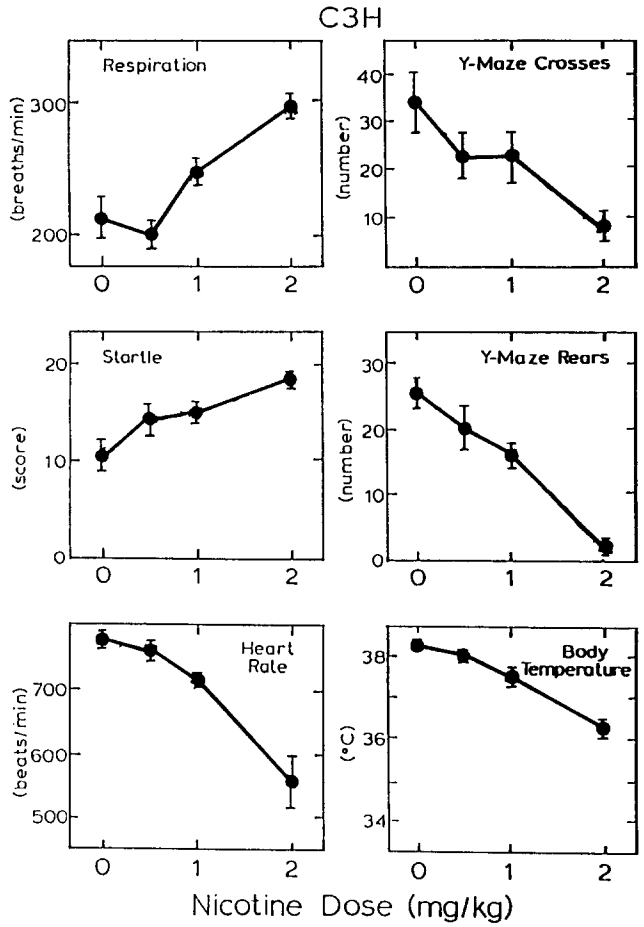

FIG. 1. Nicotine effects on the test battery in C3H mice. Male C3H mice were injected with saline (0 mg/kg nicotine) or 0.5, 1 or 2 mg/kg nicotine. The effects of these treatments were measured as described in the Method section. Each point represents the mean±SEM of 10 mice.

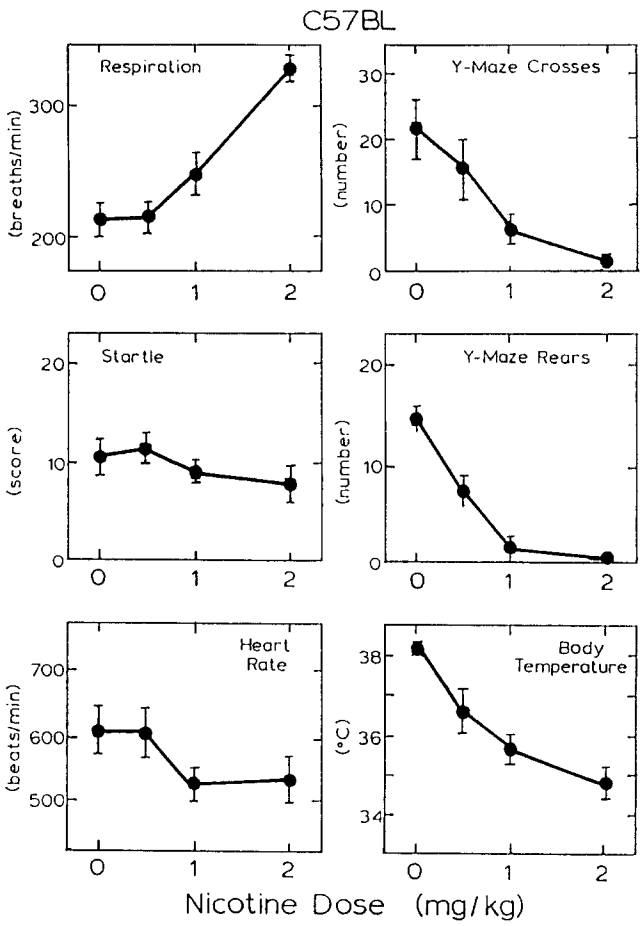

FIG. 2. Nicotine effects on the test battery in C57BL mice. Male C57BL mice were injected with saline (0 mg/kg nicotine) or 0.5, 1 or 2 mg/kg nicotine. The effects of these treatments were measured as described in the Method section. Each point represents the mean±SEM of 10 mice.

studies of the effects of chronic nicotine treatment on brain nicotinic receptors have demonstrated that such treatment elicits a change in the maximal number of binding sites (B_{max}) whereas the affinity (K_d) is unaffected [26, 28, 38]. Therefore, the use of a single ligand concentration should provide a reliable estimate of the effects of chronic nicotine infusion on the number of brain nicotinic receptors.

Protein assay. Protein was measured using the method of Lowry *et al.* [23], with bovine serum albumin as the standard.

Scintillation counting. After the samples were washed, the glass fiber filters were placed in polypropylene scintillation vials (7 ml) and 2.5 ml of scintillation fluid (toluene, 1.35 liters; Triton X-100, 0.9 liters; 2,5-diphenyloxazole, 10.5 g) were added. The samples were mechanically shaken for 30 min and radioactivity was determined on a Beckman LS 1800 liquid scintillation spectrometer. Tritium was counted at 40% efficiency and ^{125}I was counted at 44% efficiency.

RESULTS

Acute Response Studies

Figures 1 and 2 present representative data obtained in the study of nicotine effects on the test battery in the 19 inbred strains. In these studies, mice were injected with a single dose of nicotine and the entire test battery run as described in the Method section. Figure 1 presents the results obtained with C3H mice and Fig. 2 presents the results obtained with C57BL mice. These strains are representative of the diversity in response that was seen. C3H mice show both stimulant and depressant responses following the injection of nicotine (Fig. 1). Increases in respiration rate and in the acoustic startle response were elicited by nicotine whereas Y-maze crosses and rears, heart rate, and body temperature were all depressed by nicotine. These effects increased with an increase in nicotine dose. C57BL mice (Fig. 2) were affected in a similar fashion except this mouse strain did not exhibit an increase in acoustic startle following nicotine.

Figure 3 presents representative dose-response curves for nicotine-induced seizures in four of the inbred mouse strains. The dose-response curves varied in two respects: the dose required to elicit seizures in 50% of the animals (ED_{50}) and in the slope. Most of the dose-response curves were relatively shallow, but the curves obtained for some of the strains, such as the P/J strain, were extraordinarily steep suggesting a threshold phenomenon regulates seizure sensitivity.

Certain similarities were observed among the various

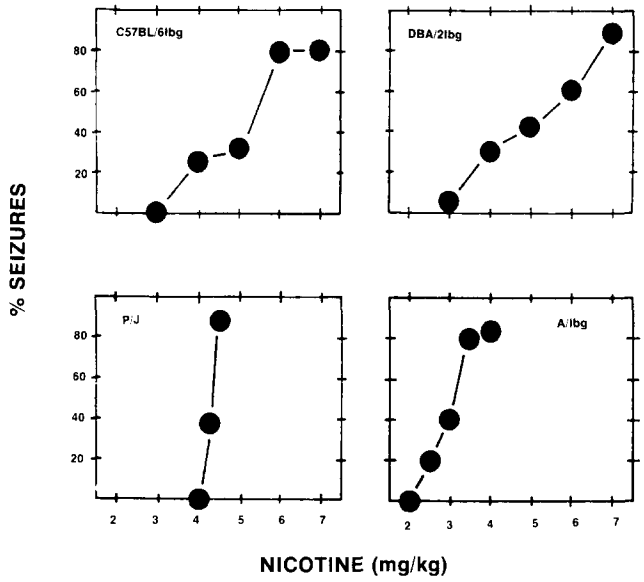

FIG. 3. Dose-response curves for nicotine-induced seizures in four mouse strains. Male mice of four mouse strains (C57BL/6Ibg, DBA/2Ibg, P/J and A/Ibg) were injected with one of several doses of nicotine. Whether a mouse seized or not during the ensuing 3 min was recorded. The percent of animals that seized at each nicotine dose is reported. Each point represents the results obtained from testing 8–12 animals.

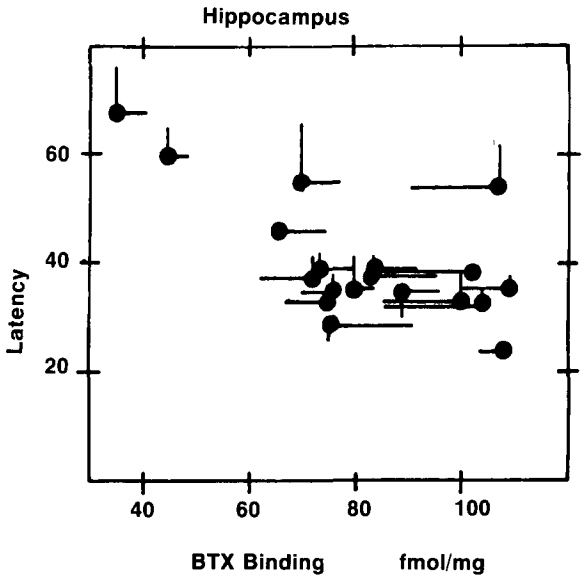

FIG. 5. Correlation between latency to nicotine-induced seizures and hippocampal BTX binding. Nicotine was infused into the jugular veins of male mice from 19 inbred mouse strains at the rate of 2 mg/kg/min. Latency to seizure, a measure of the dose required to elicit a seizure, was measured and BTX binding was subsequently measured in the hippocampi obtained from these animals. The correlation coefficient between latency and BTX binding is −0.64.

components of the test battery. Most notably, the relative sensitivities of the 19 mouse strains to the effects of nicotine on the two Y-maze measures (crosses and rears), heart rate, and body temperature seemed to segregate together. Therefore, an overall ED_{50} value was calculated for each of these measures. These overall ED_{50} values are presented for each

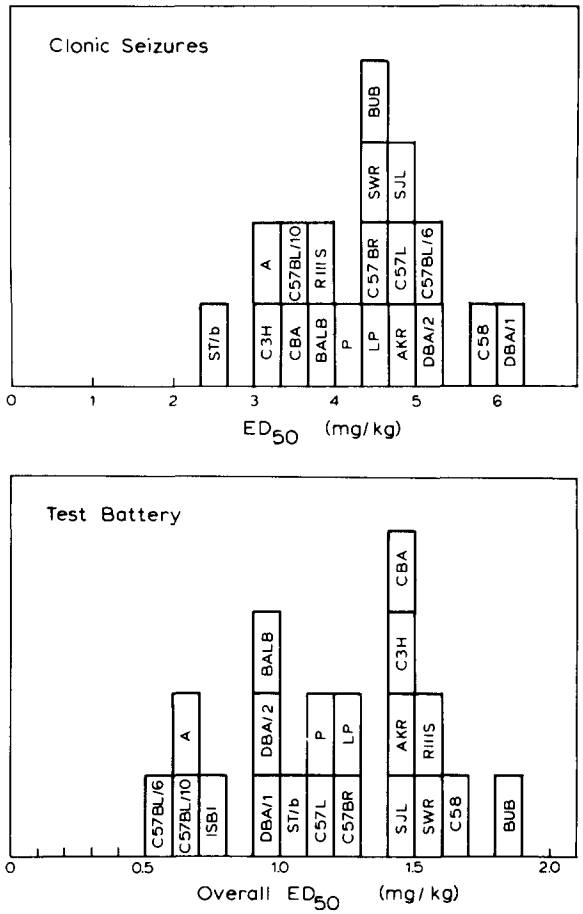

FIG. 4. Segregation analysis of the responses of 19 inbred mouse strains to the effects of nicotine on the test battery and nicotine-induced seizures. ED_{50} values were calculated from dose-response curves constructed from the studies of nicotine-induced seizures in 19 inbred mouse strains. These are reported in the upper panel of Fig. 4. The lower panel of Fig. 4 presents the relative strain sensitivities to the effects of nicotine on Y-maze crosses and rears, heart rate, and body temperature as measured by the calculation of an "overall" ED_{50} value. This overall ED_{50} consists of the arithmetic average of the nicotine dose required to decrease the Y-maze activities (crossings and rears) by 50%, the dose required to decrease heart rate by 100 beats per minute, and the dose required to decrease body temperature by 2 degrees Centigrade.

mouse strain in the lower panel of Fig. 4. The upper panel of Fig. 4 presents the calculated ED_{50} values for nicotine-induced seizures following IP injection of nicotine. The correlation coefficient for ED_{50} for clonic seizures and the overall ED_{50} is 0.10 which indicates that different genes likely regulate sensitivity to nicotine-induced seizures and sensitivity to the effects of nicotine on the Y-maze, heart rate, and body temperature tests. This finding also suggests that the strain differences do not result from pharmacokinetic (metabolic rate) differences.

Sensitivity to nicotine-induced seizures was also measured following the IV infusion of nicotine, and BTX binding was measured in the hippocampi of these animals. Figure 5 presents the results of these experiments. A high correlation ($r=-0.64$; $p<0.05$) was seen between latency to nicotine-induced seizures (a measure of the dose required to elicit a seizure) following IV administration and the number of hip-

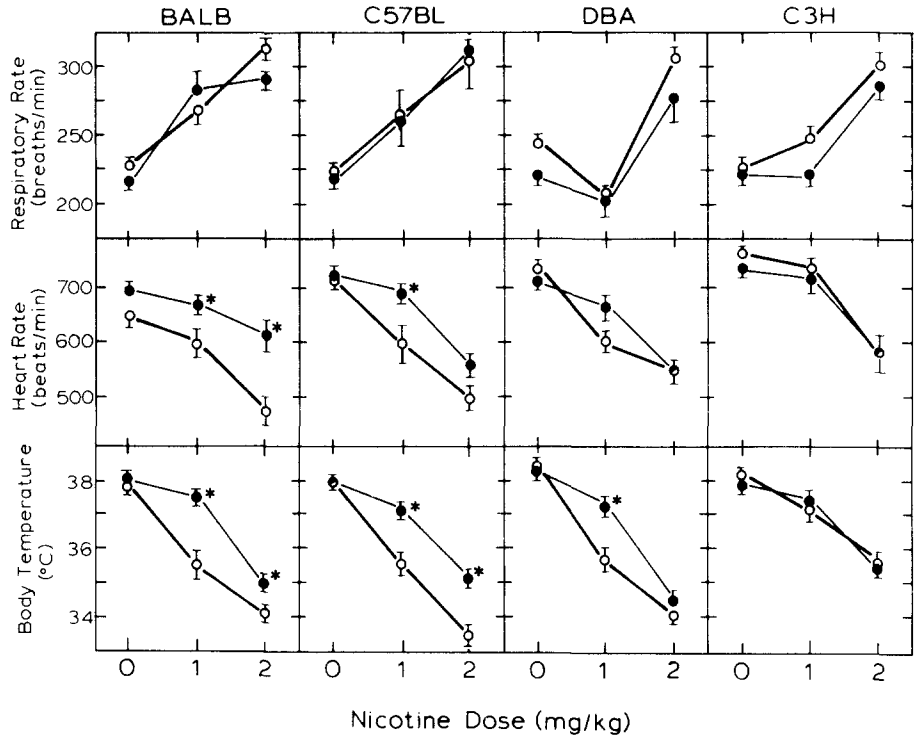

FIG. 6. Nicotine tolerance in four mouse strains. Mice of the four strains were infused with saline (○) or 3 mg/kg/hr nicotine (●) for 10 days and tested for their responses to nicotine (0, 1.0 or 2.0 mg/kg) on the respiratory rate, heart rate and body temperature tests 2 hours after infusion was stopped. Each point represents the mean±SEM of results obtained in 9 animals. Significant differences between saline- and nicotine-infused mice ($p<0.05$) are marked with an *.

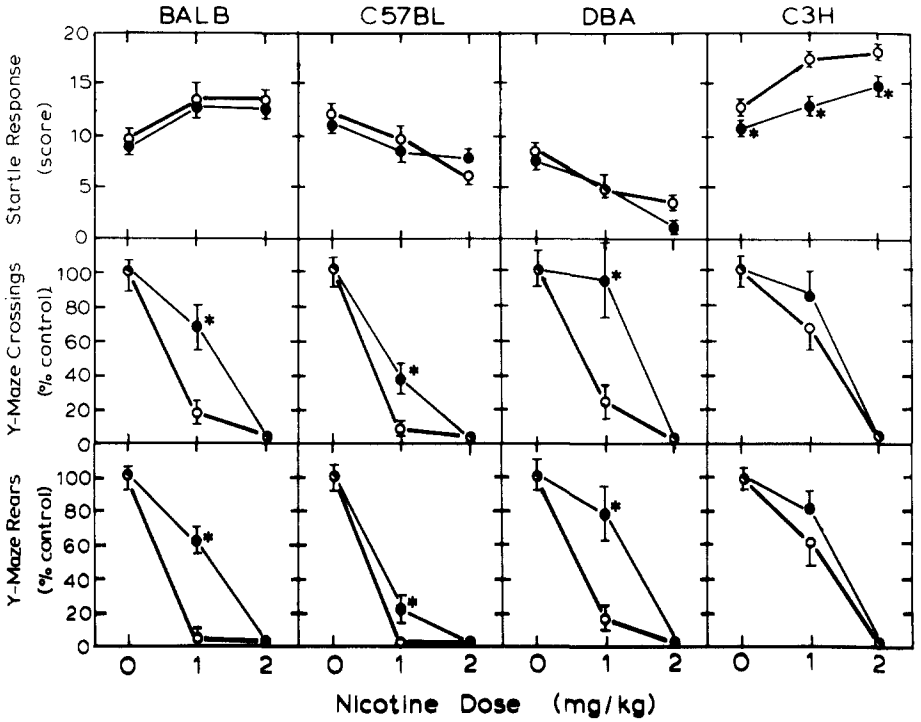

FIG. 7. Nicotine tolerance in four mouse strains. Mice of the four strains were infused with saline (○) or 3 mg/kg/hr nicotine (●) for 10 days and tested for their responses to nicotine (0, 1.0 or 2.0 mg/kg) on the acoustic startle response. Y-maze crossings, and Y-maze rears 2 hours after infusion was stopped. Each point represents the mean±SEM of results obtained in 9 animals. Values differing between saline and nicotine-infused mice ($p<0.05$) and marked with an *.

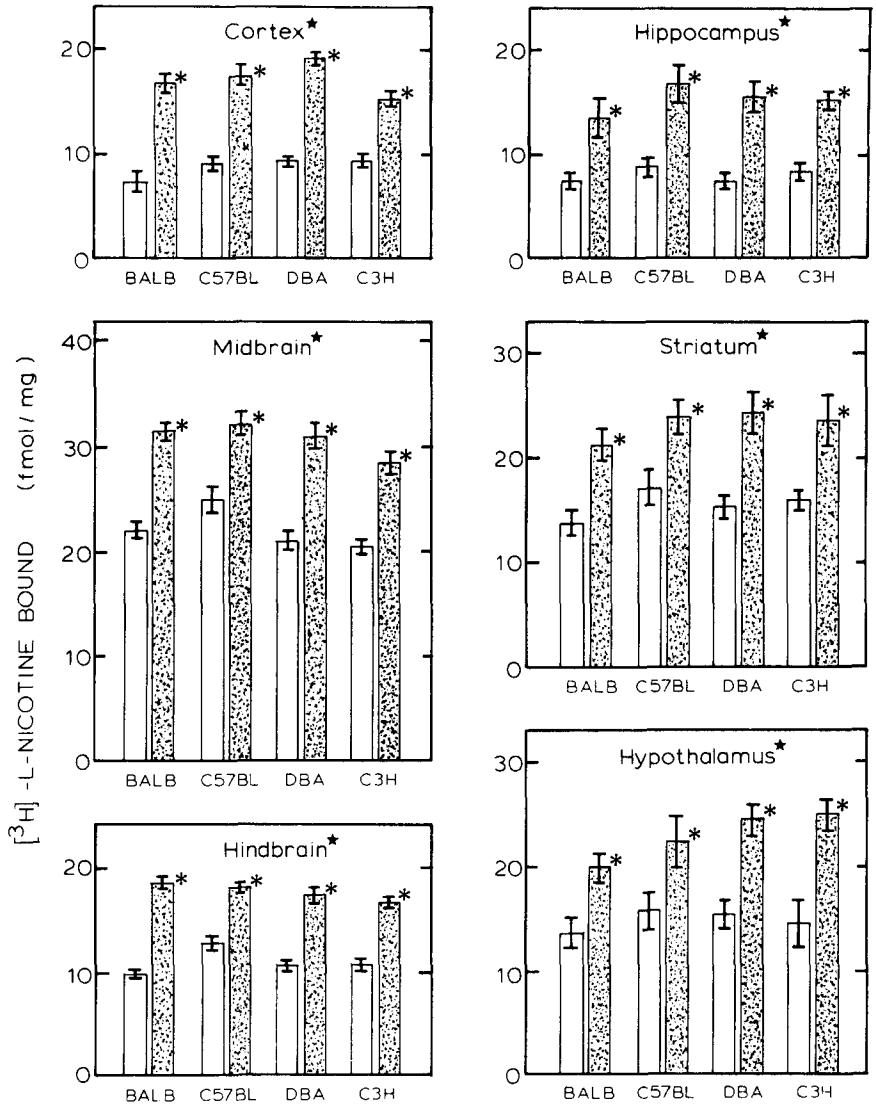

FIG. 8. Binding of L-(³H)nicotine in six brain regions. The binding of L-(³H)nicotine was measured in six brain regions obtained from saline- (open panels) and nicotine- (stippled panels) infused mice of the BALB, C57BL/6Ibg, DBA/2Ibg and C3H/2Ibg strains. Each value represents the mean±SEM of 9–17 determinations. Those values for nicotine-treated animals differing significantly ($p<0.05$) from saline-treated controls are marked with an *.

pocampal BTX binding sites. When the binding results reported in Fig. 5 were correlated with sensitivity to seizures, as measured by the ED_{50} value determined following IP nicotine administration, a lower ($r=-0.30$; $p>0.05$) and nonsignificant correlation was obtained. This suggests that some factor, other than the number of binding sites, becomes important when nicotine is given IP.

Tolerance Studies

Figures 6 and 7 present the results of studies where the responses to challenge doses of nicotine were measured in BALB, C57BL, DBA and C3H mice that had been infused chronically with a nicotine dose of 3 mg/kg/hr for 10 days. Mice were tolerance tested 2 hr after cessation of chronic nicotine infusion. Figure 6 presents the results obtained with the respiration rate, heart rate and body temperature tests. BALB, C57BL and DBA mice developed significant tolerance to the effects of a challenge dose (1 or 2 mg/kg) of nicotine on heart rate and body temperature. No evidence for tolerance was seen with C3H mice. Similarly, none of the strains developed tolerance to the respiratory stimulant effects. Figure 7 presents the results for the two Y-maze tests and for the startle response test. BALB, C57BL and DBA mice were tolerant to the Y-maze depressant effects of nicotine whereas mice of the C3H strain did not develop tolerance following chronic nicotine infusion. C3H mice, however, did develop tolerance to the enhanced startle response elicited by nicotine in this strain. The other mouse strains did not exhibit an alteration in startle response following an acute dose of nicotine, and no change in this measure was seen following chronic nicotine infusion. In summary, the BALB, C57BL and DBA mice developed tolerance to four of the six measures (heart rate, body temperature, Y-maze crosses, Y-maze rears) whereas C3H mice devel-

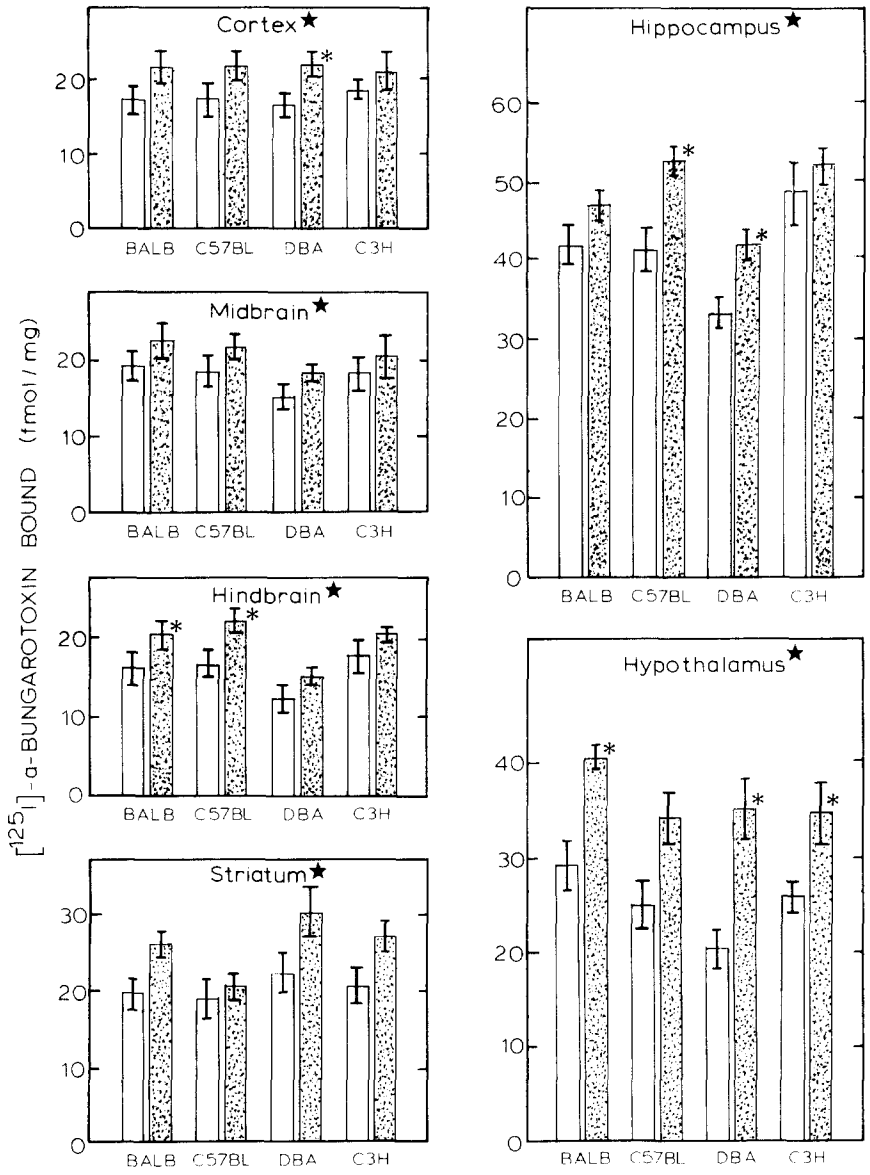

FIG. 9. Binding of (^{125}I)BTX in six brain regions. The binding of BTX was measured in six brain regions obtained from saline- (open panels) and nicotine- (stippled panels) infused mice of the BALB, C57BL/6Ibg, DBA/2Ibg and C3H/2Ibg strains. Each value represents the mean±SEM of 8 determinations. Those values for nicotine-treated animals differing significantly ($p<0.05$) from saline-treated controls are marked with an *.

oped tolerance only to the enhanced startle response. Therefore, we conclude, as is the case for acute sensitivity, that genetic factors regulate the development of tolerance to nicotine.

Figure 8 presents the results of those studies where (^{3}H)nicotine binding was measured in six brain regions obtained from BALB, C57BL, DBA and C3H mice that had been chronically infused with saline or 3 mg/kg/hr nicotine. A comparison of the saline-infused data indicates that these four strains do not differ in (^{3}H)nicotine binding in these six regions. Chronic nicotine infusion resulted in significant increases in (^{3}H)nicotine binding in every brain region in each mouse strain. Therefore, the failure of C3H mice to manifest tolerance to nicotine cannot be explained by an inability to up-regulate (^{3}H)nicotine binding sites.

Figure 9 presents the results of comparable experiments where BTX binding was measured in the same six brain regions in saline- and nicotine-infused BALB, C57BL, DBA and C3H mice. Chronic nicotine infusion may have resulted in an increase in brain BTX binding sites, but this effect was not as robust as was the effect on (^{3}H)nicotine binding and was not as widespread among the brain regions. Most importantly, the failure of C3H mice to develop tolerance to nicotine does not seem to be readily explained by effects, or lack of effects, on BTX binding.

DISCUSSION

The pharmacology of nicotine is extraordinarily complex in that many systems are affected by this compound and both

stimulant and depressant effects are seen. In general, we observed stimulation of respiration and, when the dose was high enough, seizures occurred following acute nicotine administration. In some strains, such as the C3H/2Ibg, nicotine treatment resulted in an enhanced startle response whereas in the vast majority of the strains tested nicotine did not alter this measure. Nicotine depressed Y-maze crosses and rears, heart rate, and body temperature in all of the mouse strains, but the strains varied significantly in the dose required to elicit measurable effects. However, similar ED_{50} values were obtained within a strain for these four tests which suggests similar genetic regulation.

By and large, seizure sensitivity seemed to segregate independently of sensitivity to nicotine's effects on the Y-maze tests, heart rate and body temperature. A careful examination of the respective ED_{50} values for nicotine-induced seizures following IP administration of nicotine and the overall test battery will reveal that strains sensitive to one effect are not necessarily sensitive to another effect. This observation rules out strain differences in metabolism as being entirely responsible for the strain differences in response. If pharmacokinetic differences were responsible for the strain differences in sensitivity that we observed, it would be expected that a given strain would be uniformly sensitive, resistant or intermediate in response. As noted previously, nicotine metabolism and distribution do not differ in several of the inbred mouse strains that we have tested [38]. These strains vary markedly in their relative sensitivity to nicotine, but they do not differ in rate of nicotine metabolism. Therefore, it seems likely that pharmacodynamic, rather than pharmacokinetic, differences are the primary cause of strain differences in sensitivity to nicotine.

Our analysis of L-(^{3}H)nicotine binding has not revealed any strain differences, thus far. We have reported a comparison of nicotine binding in four of the strains: BALB/cByJ, DBA/2Ibg, C57BL/6Ibg and C3H/2Ibg previously [25], and are currently making such measurements in the remaining 15 strains that we have examined behaviorally. The data currently available do not suggest that strain differences in response to nicotine will be explainable on the basis of the number of nicotine binding sites as measured in six brain regions. It may be that a finer analysis, such as that afforded by quantitative autoradiography, will be required to identify strain differences in nicotine binding. Alternatively, it may be that genetic factors regulate the functional status of brain nicotinic receptors.

We have had some success in identifying the cause of strain differences in sensitivity to nicotine-induced seizures in that those mouse strains with greater numbers of hippocampal BTX binding sites are more sensitive to nicotine-induced seizures. However, the correlation between seizure sensitivity and hippocampal BTX binding is considerably less than 1.0 which suggests other factors regulate sensitivity. Among the possibilities that we have been exploring is a genetic influence on desensitization of nicotinic receptors. We have observed (unpublished) that pretreatment with subseizure doses of nicotine results in a decreased sensitivity of DBA mice to a subsequent injection with a dose of nicotine that normally elicits seizures: the dose-response curves for nicotine-induced seizures shift to the right in DBA mice that have been pretreated with nicotine. No such shift was seen with C3H mice. The causes of this behavioral desensitization are, at this time, unknown, but it is clear that genetic factors regulate this response. If receptor densensitization underlies behavioral desensitization, it may be that some mouse strains have receptors that desensitize and/or resensitize more rapidly than do receptors in other mouse strains. Such a phenomenon could contribute substantially to individual differences in response to nicotine.

The studies of nicotine tolerance provided additional evidence that genetic factors regulate nicotine response. All of our earlier studies [26, 28, 31] used DBA/2Ibg mice, and the results obtained indicated that tolerance to nicotine increased with an increase in nicotine dose, that this tolerance was paralleled by increases in brain (^{3}H)nicotine binding, and that tolerance was gained and lost at the same rate as (^{3}H)nicotine binding increased and decreased. These results provided compelling evidence that suggested increases in nicotine binding underlie tolerance to nicotine. The results obtained in the present study for the C3H mice shed doubt on this hypothesis. Since C3H mice did not develop tolerance even though brain (^{3}H)nicotine binding increased following chronic nicotine treatment, it seems necessary to re-examine the role of changes in receptor numbers in nicotine tolerance.

The finding that brain nicotine and BTX binding sites increase following chronic nicotine infusion is a surprising one. We [26] have discussed potential reasons for this in the past. The hypothesis that we favor most is that nicotine administration results in receptor desensitization; this is equivalent to inactivation of the receptor. As a consequence of this inactivation the synthesis of new receptors, or the degredation of old receptors, may be changed in such a way that increases in receptor numbers result. However, the functional status of these receptors is unknown. It may be that chronically treated animals have more total receptors but fewer functional receptors. Along these lines, Simasko *et al.* [45] have recently reported that the chronic treatment of PC12 cells with nicotinic agonists results in a slowly reversible or irreversible inactivation of the nicotinic receptors, as measured by agonist-induced increases in ion flux. It may be that all mouse strains have nicotinic receptors that desensitize and inactivate as a consequence of nicotine administration. Those mouse strains such as the C3H that do not appear to develop tolerance to nicotine may have nicotinic receptors that regain functionality more quickly.

Clearly there is no shortage of potential explanations for the strain differences in sensitivity to an acute dose of nicotine and in tolerance development that we have seen. Perhaps identifying inbred mouse strains that differ in response to nicotine and establishing why these differences exist will prove to be valuable in identifying reasons that underlie differences among humans in response to nicotine and may explain why people do and do not smoke.

ACKNOWLEDGEMENTS

The authors wish to thank Jerry Stitzel and David Gaffney for technical assistance with some of the experiments. This work was supported by DA-03194 and AFOSR 82-0300.

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