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Effect of Nicotine Pretreatment on Nicotine-Induced Seizures

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MINER, L. L. AND A. C. COLLINS. *Effect of nicotine pretreatment on nicotine-induced seizures.* PHARMACOL BIOCHEM BEHAV **29**(2) 375–380, 1988.—Inactivation of the nicotinic receptor via the process of desensitization has been well characterized for the nicotinic receptor in vitro, but potential behavioral manifestations of desensitization have received little study. To test whether behavioral desensitization occurs, C3H and DBA mice were pretreated with subseizure-producing doses of nicotine and nicotine-induced seizure sensitivity was determined at various time intervals after pretreatment. Fifteen minutes after nicotine pretreatment, DBA mice were significantly less sensitive to nicotine-induced seizures than were saline pretreated mice after both IP and IV administration. Seizure sensitivity returned to baseline levels at 60 minutes after pretreatment for the IP route of administration and at 30 minutes for the IV route of administration. Sensitivity to nicotine-induced seizures was altered for C3H mice under only one experimental condition; 7.5 minutes after IP injection with 2.0 mg/kg nicotine. Thus, DBA mice display a marked behavioral desensitization as a result of nicotine pretreatment whereas C3H mice do not. These results, in conjunction with our previous studies, indicate that nicotine-induced seizure sensitivity may be influenced by a minimum of two factors: the concentration of nicotinic receptors in the brain and possibly the ability of these receptors to inactivate in the presence of nicotine.

Desensitization Nicotine Seizures, nicotine-induced

SEVERAL earlier studies from our laboratory have demonstrated that C3H/2Ibg and DBA/2Ibg mice differ in their sensitivity to nicotine-induced seizures [11–13]. This difference in seizure sensitivity does not appear to be due to differences in the rate of metabolism of nicotine [14] or in levels of nicotine found in brain [8,13]. Rather, it appears to be related to the number of nicotinic receptors in the central nervous system, most notably in the hippocampus, such that animals with a greater number of α-bungarotoxin (BTX) binding sites are more sensitive to nicotine-induced seizures than are animals that have fewer BTX binding sites.

Other factors may also contribute to seizure sensitivity. In a recent study of sensitivity to nicotine-induced seizures after acute intravenous (IV) administration, we observed a qualitative difference in seizure sensitivity [13]. In this study, nicotine was administered IV until the onset of a seizure, at which time the drug infusion was immediately discontinued. DBA mice regained normal body posture and appeared normal shortly after the infusion was stopped (at the onset of myoclonus) whereas C3H mice did not. The C3H mice often died even though the drug infusion had been stopped. This suggests that in some mouse strains, such as the C3H, once a threshold of excitation is reached, a complete seizure will develop whereas in other strains, such as the DBA, other processes can interact which serve to decrease CNS excitability.

It is possible that differences in seizure sensitivity are due to differences in receptor desensitization such that less sensitive mouse strains have receptors that desensitize following nicotine binding and that the receptors of more sensitive strains do not desensitize, or do so slowly. Desensitization of the nicotinic receptor was first described by Katz and Thesleff [6] who proposed a model for this phenomenon. Their model suggests that after an agonist binds to the receptor, the receptor is activated which results in the opening of an ion channel. This active complex subsequently is converted into a desensitized (nonfunctional) state with the agonist still bound. After the agonist dissociates from the receptor, the receptor returns to the resting or activatable state, i.e., it resensitizes. Desensitization has been demonstrated for nicotinic receptors in various preparations, including at the motor end plate [6], adrenal chromaffin cells [2,3], the PC-12 clonal line [7, 17, 18], and in Renshaw cells [4], but it has not been directly demonstrated in mammalian brain most likely because of the relatively small number of nicotinic receptors present. Evidence exists which suggests that desensitization of brain nicotinic receptors occurs. Barass *et al.* [2] have demonstrated that pretreating mice with nicotine (0.8 mg/kg) resulted in a dramatic increase in the LD_{50} for nicotine, and Stolerman *et al.* [19] have observed that pretreating rats with 0.75 mg/kg nicotine resulted in a 2.4-fold increase in the ED_{50} for nicotine-induced decreases in locomotor activity. Dunlop

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et al. [5] have obtained evidence which suggests that desensitization for nicotine-induced seizures also occurs. These investigators tested the effects of repeated doses of nicotine on electroencephalographic (EEG) recordings from electrodes implanted in the rabbit hippocampus. If the first dose of nicotine elicited a seizure, subsequent administrations up to 45 minutes later had no effect on the EEG even when the dose of nicotine was doubled.

The purpose of the present study was to determine whether behavioral desensitization can be demonstrated for nicotine-induced seizures and whether C3H and DBA mice differ in behavioral desensitization, if it exists. Mice from both strains were treated with nicotine at various time points prior to assessing nicotine-induced seizure sensitivity. According to the receptor desensitization model, the initial exposure to the agonist should induce receptor inactivation via the process of desensitization which should result in the subsequent administration of nicotine being less effective. The interaction between sensitivity to flurothyl-induced seizures and nicotine-induced seizures was also examined to address the question of whether any effects of nicotine generalized to seizures induced by other agents. Flurothyl was chosen as the convulsant for these studies because its actions appear to be due to a nonspecific effect on membrane excitability rather than to an interaction with a specific neurotransmitter system [5].

METHOD

Subjects

Mice from the C3H/2Ibg and DBA/2Ibg strains were utilized. Mice from the DBA and C3H strains have been maintained in the breeding colony at the Institute for Behavioral Genetics (Boulder, CO) for greater than 20 generations. All animals used ranged in age from 60–90 days of age. Equal numbers of male and female mice were used in all experiments.

Nicotine Administration, IP

Nicotine was obtained from Sigma Chemical Co. (St. Louis, MO) and was redistilled periodically. Nicotine was dissolved in physiological saline, neutralized with HCl and given in a concentration of 0.01 ml/g. Nicotine pretreatment doses were 0.0, 1.0 and 2.0 mg/kg.

To test the effects of nicotine pretreatment on sensitivity to nicotine-induced seizures, mice from the C3H and DBA strains were pretreated with subseizure doses of nicotine, 7.5 or 15 minutes prior to seizure testing for C3H mice and 15, 30 or 60 minutes prior to seizure testing for DBA mice. These times were chosen on the basis of preliminary studies which suggested that effects may be seen at these time points. Dose-response curves for sensitivity to nicotine-induced seizures after nicotine pretreatment were constructed for each strain at each pretreatment dose at each time period following pretreatment.

Nicotine Administration, IV

Cannulas made of silastic tubing were implanted in the right jugular vein of either C3H or DBA mice using the method of Barr *et al.* [1]. The surgery was performed on each mouse under anesthesia (pentobarbital, 45 mg/kg, and chloral hydrate, 63 mg/kg). This mixture of anesthetics was used because our experience has been that fewer animals die than is obtained when higher doses of pentobarbital alone are used. The cannula contained sterile saline and 3 g/l sodium citrate as an anticoagulant.

Two days after surgery, individual mice were transferred to a clear Plexiglas cage (25×25×25 cm) and the cannula was attached to thermoplastic tubing which was connected to a 1 ml syringe mounted on an infusion pump (Harvard Apparatus, South Natick, MA). To test the effects of nicotine pretreatment on sensitivity to IV nicotine-induced seizures, mice from the C3H and DBA strains were infused for either 5 or 10 seconds with a 2.0 mg/kg/min concentration of nicotine (0.167 or 0.333 mg/kg respectively) or with saline (rate of infusion was 46.6 μl/min). The drug infusion was then stopped for 15 or 30 minutes for DBA mice and 7.5 or 15 min for C3H mice. After the prescribed time had elapsed, the infusion was reinitiated and continued until the occurrence of a seizure. Latency from the time of recontinuing the drug infusion until a clonic seizure occurred was measured. Control animals were pretreated with IV saline for 10 seconds at a rate of 46.6 μl/min, 15 minutes before nicotine infusion for DBA mice and 7.5 minutes before nicotine for C3H mice. Nicotine was infused for all seizure testing at a concentration of 2.0 mg/kg/min which was accomplished by infusing at a rate of 46.6 μl/min.

Flurothyl-Induced Seizures

Susceptibility to seizures induced by inhalation of flurothyl (bis[2,2,2-trifluroethyl] ether) (Armageddon Chemical Co., Durham, NC) was determined according to the method described by Smolen *et al.* [16]. Each mouse was placed individually into a modified 435 ml ointment jar with a screw cap lid which had a rubber septum in the middle. A Plexiglas support located 15 mm below the septum contained a 1 cm square piece of Whatman No. 1 filter paper. Flurothyl was injected through the septum in a dose of 5 μl. During the test sessions, the chamber was kept air tight. At the end of the session a water aspirator was used to flush the chamber with air through two glass tubes mounted in the lid. Latencies to the first instance of myoclonus and clonus were recorded to the nearest second. For any animal that did not seize, a time of 300 seconds was assigned. The flurothyl pretreatment control group consisted of placing mice in the jar for 5 minutes, but no flurothyl was administered. Nicotine sensitivity was measured after this treatment.

Data Analysis

ED_{50} values of the dose-response curves for the effect of nicotine pretreatment on sensitivity to nicotine-induced seizures were calculated by linear regression. Drug pretreated animals were compared to saline pretreated by *t*-test. The effect of nicotine pretreatment on IV seizures was analyzed by analysis of variance (ANOVA). Similarly, the effects of nicotine pretreatment on latency to flurothyl seizures were assessed by ANOVA. The effects of flurothyl-induced seizures on sensitivity to IP nicotine-induced seizures was assessed by chi square. No significant sex differences were observed; therefore all data presented are for male and female mice combined.

RESULTS

Figure 1 presents the results for DBA mice pretreated with either saline or nicotine (1.0 or 2.0 mg/kg), 15, 30 or 60 minutes prior to seizure testing. The ED_{50} values were calculated for each dose-response curve and are presented in

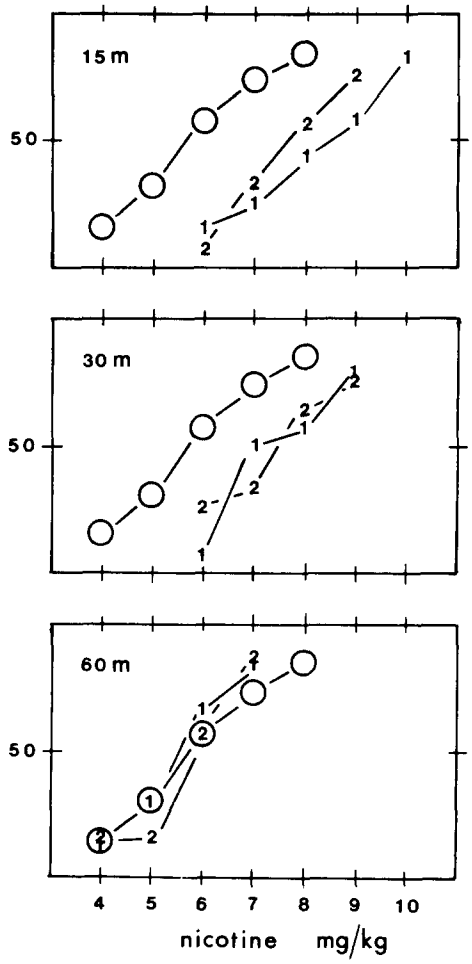

FIG. 1. Dose-response curves for the effect of nicotine pretreatment on nicotine-induced seizures. Male and female DBA mice were administered either 0.0 (open circles), 1.0 or 2.0 mg/kg nicotine 15 (top panel), 30 (middle panel) or 60 (bottom panel) minutes prior to administering a convulsant dose of nicotine.

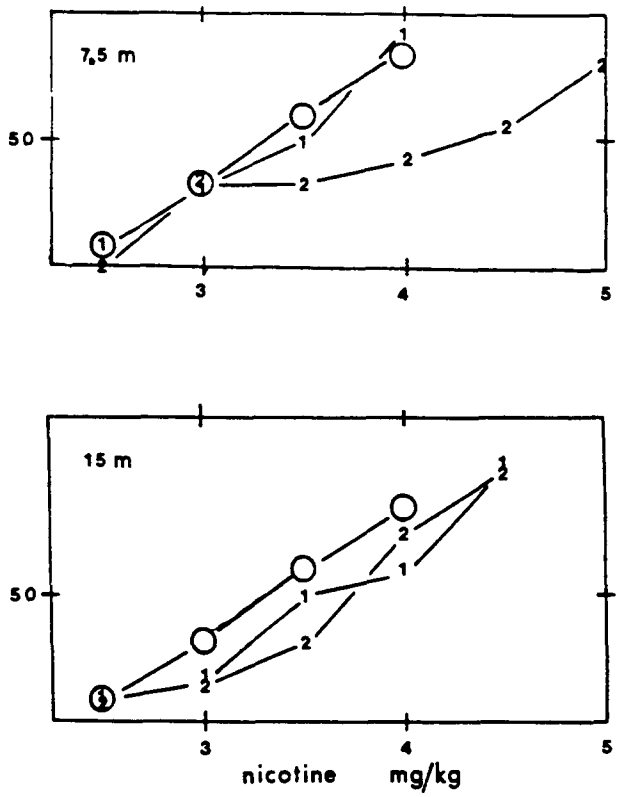

FIG. 2. Dose-response curve for the effects of nicotine pretreatment on nicotine-induced seizures in C3H mice. Male and female C3H mice were administered either 0.0 (open circles), 1.0 or 2.0 mg/kg nicotine either 7.5 (top panel) or 15 (bottom panel) minutes prior to administering a convulsant dose of nicotine.

TABLE 1

ED_{50} VALUES OF DOSE-RESPONSE CURVES FOR DBA AND C3H MICE AFTER NICOTINE PRETREATMENT

Strain	Time—Dose of Pretreatment	ED_{50}
DBA	control	5.82 ± 0.14
C3H	control	3.34 ± 0.05
C3H	7.5 min—1.0 mg/kg	3.33 ± 0.09
C3H	7.5 min—2.0 mg/kg	4.08 ± 0.15
DBA	15 min—1.0 mg/kg	8.30 ± 0.16
C3H	15 min—1.0 mg/kg	3.57 ± 0.11
DBA	15 min—2.0 mg/kg	7.79 ± 0.07
C3H	15 min—2.0 mg/kg	3.57 ± 0.11
DBA	30 min—1.0 mg/kg	7.61 ± 0.30
DBA	30 min—2.0 mg/kg	7.50 ± 0.50
DBA	60 min—1.0 mg/kg	5.50 ± 0.13
DBA	60 min—2.0 mg/kg	5.76 ± 0.61

Table 1. There were no significant differences in ED_{50} values among the saline pretreated animals at 15, 30 or 60 minutes so these data were combined for the control dose-response curve.

The ED_{50} values for the animals pretreated with nicotine (1.0 and 2.0 mg/kg) 15 and 30 minutes before seizure testing were compared to the control ED_{50} value and were observed to be significantly greater [15 minutes: $t(8)=11.81$, and $t(7)=10.72$ for 1.0 and 2.0 mg/kg pretreatment respectively; 30 minutes $t(7)=5.77$, and $t(7)=6.22$ for 1.0 and 2.0 mg/kg respectively; $p<0.01$ in all cases). The ED_{50} values for animals pretreated 60 min before seizure testing did not differ from control ED_{50} values.

Figure 2 presents the results for C3H mice pretreated with 0.0, 1.0 and 2.0 mg/kg nicotine 7.5 and 15 minutes prior to seizure testing. As was the case with the DBA strain, no significant differences in ED_{50} values were observed for animals pretreated with saline 7.5 or 15 minutes prior to seizure testing. Therefore, these data were combined for the control dose-response curve. ED_{50} values are presented in Table 1.

The dose-response curve for the group pretreated with 2.0 mg/kg nicotine 7.5 minutes before seizure testing differed from the control group in both ED_{50} value and slope (ED_{50}: $t(7)=2.5$, $p<0.05$; slope: $t(7)=11.5$, $p<0.01$). This group had a significantly shallower slope and a significantly greater ED_{50} value than did all the other treatment groups (Fig. 2).

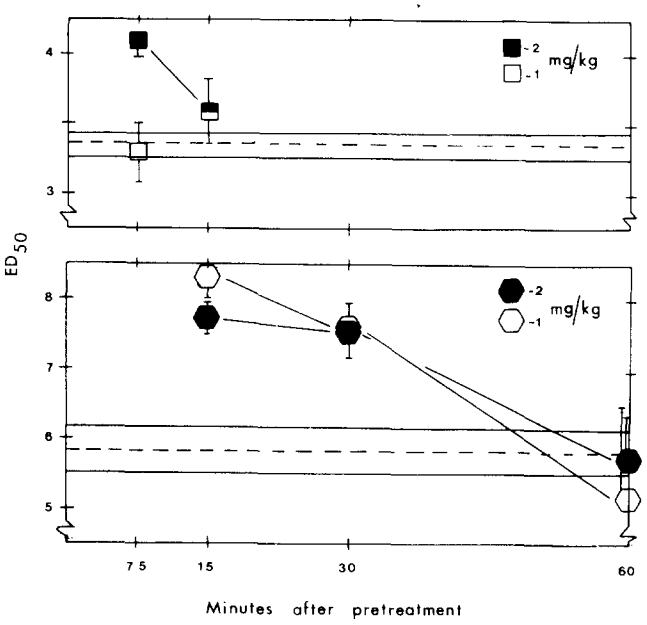

FIG. 3. Time course for return of seizure sensitivity. ED_{50}±S.E. value is plotted versus time after nicotine pretreatment (either 1.0 mg/kg, open symbols or 2.0 mg/kg, closed symbols) for C3H mice (top panel) and DBA mice (bottom panel). The saline pretreated control ED_{50} is plotted as the dashed line with the standard error represented as the solid lines.

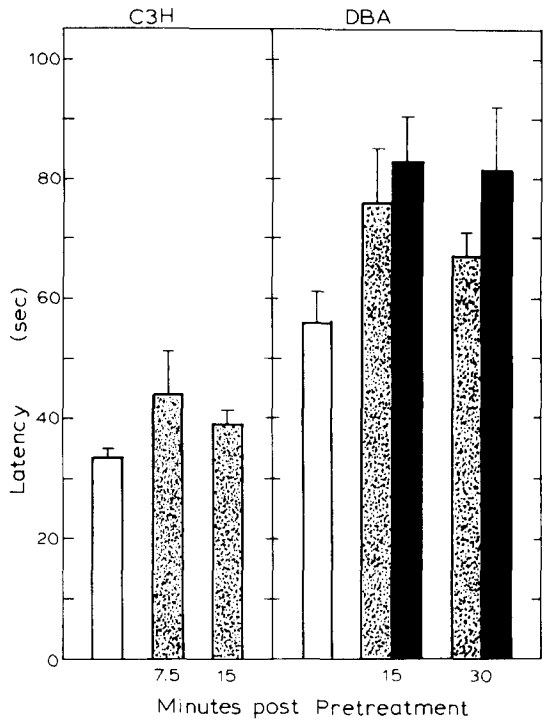

FIG. 4. Effect of nicotine pretreatment on sensitivity to IV nicotine seizures. C3H were pretreated with 0.167 mg/kg and DBA mice were pretreated with either 0.167 (stippled bars) or 0.333 mg/kg (solid bars) at 7.5 or 15 minutes prior to seizure testing for C3H mice and 15 or 30 minutes for DBA mice.

No differences were observed for any of the other treatment groups.

The time course for the return of seizure sensitivity to baseline sensitivity was examined by plotting ED_{50} value versus pretreatment time. These results for both the DBA and C3H strains are presented in Fig. 3. The half-time for the return of normal behavioral sensitivity was calculated from these data. For the DBA strain, the half-time was 39 minutes for the 1.0 mg/kg pretreatment group and 40 minutes for the 2.0 mg/kg pretreatment group. Because only one treatment group differed for C3H mice (2.0 mg/kg 7.5 min pretreatment), the half-time for return to baseline sensitivity was not calculated. However, it is clear that it would be less than 15 minutes which would be substantially different from the half-time calculated for DBA mice.

The results for nicotine pretreatment on nicotine seizures induced by IV drug administration for DBA and C3H mice are presented in Fig. 4. As can be seen, a shift in sensitivity for DBA mice was noted such that animals pretreated with nicotine are more resistant to the convulsant effects of nicotine than are control animals. For the 15 minute pretreatment group, those animals pretreated with nicotine had a significantly longer latency to seizure than saline pretreated animals, $F(2,21)=3.77$, $p<0.05$. By 30 minutes, however, sensitivity had essentially returned to baseline levels as there was no significant difference between nicotine pretreated and control groups.

The results for C3H mice are different than those for DBA mice. There was no significant difference between animals pretreated with saline and those pretreated with nicotine for 5 seconds for either the 7.5 or 15 minute latency groups. Interestingly, no animal survived the 10 second nicotine pretreatment regimen. All animals pretreated with 0.33 mg/kg

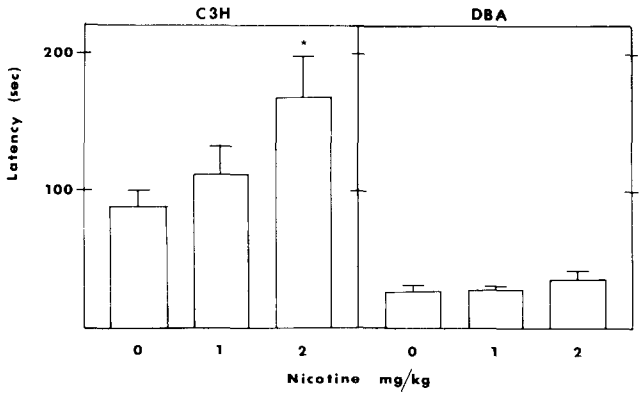

FIG. 5. Effect of nicotine pretreatment on flurothyl-induced seizures. Mice from the DBA and C3H strains were pretreated with either 0.0, 1.0, or 2.0 mg/kg nicotine 7.5 minutes for C3H and 15 minutes for DBA mice prior to administration of 5 μl flurothyl. *$p<0.01$.

nicotine seized within 1 minute after termination of pretreatment.

Figure 5 presents the results for the effects of nicotine pretreatment on sensitivity to flurothyl-induced seizures in both DBA and C3H mice. In this study, C3H and DBA mice were pretreated with either 0, 1 or 2 mg/kg nicotine IP 7.5 minutes for C3H and 15 minutes for DBA mice prior to

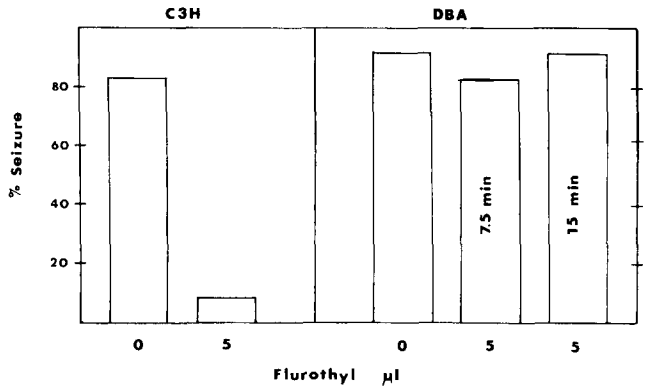

FIG. 6. Effect of flurothyl seizures on nicotine seizures. Mice from the C3H and DBA strains were administered a convulsant dose of flurothyl 7.5 minutes for C3H and 7.5 or 15 minutes for DBA prior to administration of an ED_{90} dose of nicotine (4.0 mg/kg, C3H and 7.0 mg/kg, DBA). Control animals were placed in the testing chamber but not administered flurothyl.

flurothyl treatment. These time points were chosen because they represent the time at which we observed maximal change in seizure sensitivity for the two strains following IP nicotine injection. A two-way ANOVA revealed a significant strain difference, $F(1,47)=38.3$, $p<0.001$, with the DBA strain seizing at a shorter latency than the C3H strain. A similar strain difference was seen following pretreatment with 1.0 nicotine with the DBA mice showing a shorter latency than the C3H mice, $F(1,47)=35.1$, $p<0.01$. It was also observed that 2.0 mg/kg nicotine increased the latency to seizures, $F(1,47)=6.7$, $p<0.05$. The significant dose × strain interaction indicates that the increased latency to seizures was greater for C3H mice than for DBA mice, $F(1,47)=4.6$, $p<0.05$.

Figure 6 presents the results for the effect of flurothyl seizures on nicotine seizures. At 7.5 minutes after flurothyl, C3H mice show a significant decrease in the percentage of nicotine seizures, $\chi^2=8.89$, $p<0.01$. No such decrease was observed for DBA mice at either 7.5 or 15 minutes after a flurothyl seizure.

DISCUSSION

The results from these experiments clearly indicate a strain difference in the effects of nicotine pretreatment on sensitivity to nicotine-induced seizures. Mice from the DBA strain injected IP with either a 1.0 or 2.0 mg/kg dose of nicotine were less sensitive to the convulsant effects of nicotine than were DBA mice that had been pretreated with saline at both 15 minute and 30 minutes. At 60 minutes after pretreatment, there was no difference in seizure sensitivity between saline pretreated and nicotine pretreated DBA mice. C3H mice didn't show a dramatic change in seizure sensitivity following nicotine pretreatment. Only the 2.0 mg/kg-7.5 min pretreatment group was observed to be significantly less sensitive to nicotine-induced seizures.

The results obtained following the IV administration of nicotine were similar to those obtained after IP injection. DBA mice were less sensitive to nicotine-induced seizures after nicotine pretreatment whereas C3H mice showed no such shift in seizure sensitivity. This shift was most pronounced after the highest pretreatment dose. After IP nicotine administration the half-time for return to control sensitivity in DBA mice was estimated at approximately 40 minutes. For IV administration, however, latency to seizures after nicotine pretreatment was back to baseline levels at 30 minutes following pretreatment with the 1 mg/kg dose suggesting a half-time between 15 and 30 minutes. This difference in half-times may be due to pharmacokinetic differences between the two routes of drug administration, particularly in the rates of drug absorption and distribution. Alternatively, a higher pretreatment dose was used in the IP experiments. It may be that the larger dose of nicotine resulted in greater receptor desensitization and thereby a more dramatic change in seizure susceptibility ensued.

An unexpected observation of the IV study was that C3H mice may be more seizure sensitive than originally believed. After a pretreatment dose of 0.33 mg/kg nicotine, all animals were observed to seize within a minute even though there was no sign of seizure during pretreatment. In our previous studies [10,11] the mean convulsant dose for C3H mice was 1.3± 0.11 mg/kg following IV infusion. In those studies, infusion was continued until the onset of a clonic seizure. Thus, it appears that we overestimated the dose required to elicit a seizure in C3H mice. It appears as though C3H mice have a low threshold for nicotine-induced seizures and that once that threshold is crossed there is no preventing a seizure even though it may take some time for the visible signs to develop. This may hold true for DBA mice but there were no indications of severe tremors (a precursor to seizures) after either of the pretreatment doses.

It is clear from these experiments that the reduced sensitivity to nicotine seizures observed in DBA mice 15 minutes after nicotine pretreatment is not generalizable to convulsions elicited by flurothyl. Nicotine pretreatment did not alter sensitivity to flurothyl-induced seizure in DBA mice. However, C3H mice did exhibit an effect of nicotine pretreatment on sensitivity to flurothyl-induced seizures in the group that was tested 7.5 minutes after 2.0 mg/kg nicotine pretreatment. A significant decrease in sensitivity to flurothyl seizures was observed after the 2.0 mg/kg pretreatment dose. Similarly, in the flurothyl seizure/nicotine seizure experiment a significant alteration in sensitivity to nicotine-induced seizures was noted in C3H mice only. Therefore, it seems reasonable to assert that the observed behavioral desensitization seen in DBA mice for nicotine-induced seizures is due to a specific effect on nicotinic systems and that the interactions between nicotine and flurothyl are different in C3H and DBA mice.

As noted previously, a possible explanation for the reduced sensitivity to nicotine-induced seizures after nicotine pretreatment seen in DBA mice is receptor desensitization. It may be that the initial, subseizure dose inactivated those receptors that regulate seizures. If this is the case, it seems possible that the DBA and C3H mice differ in some parameter related to either the desensitization or resensitization of brain nicotinic receptors.

In vitro studies of desensitization of the nicotinic receptor report that the onset of desensitization occurs within the tens of seconds time range [15,17] and, the half-time for recovery from desensitization is in the 1 to 2 minute time range [15]. These times are inconsistent with the present results. Simasko and coworkers [15] have indicated that there may be two processes involved in the loss of nicotinic receptor function in PC-12 cells. The first process may be the classically described receptor desensitization and the second process was termed inactivation by these investigators. Re-

ceptor inactivation in the PC-12 cell line has a slower onset (approximately 15 min) than desensitization and does not appear to recover within a 2 hour time frame. This time course for receptor inactivation more closely coincides with the time course for the behavioral desensitization we observed in DBA mice, although we do observe recovery of sensitivity at 1 hour. Based on the comparison of the time scales observed in vitro and in vivo, rather than differing in the abilities of the receptors to desensitize, C3H and DBA mice may actually differ in the longer term process of receptor inactivation.

In summary, the results presented here demonstrate that C3H and DBA mice differ not only in sensitivity to nicotine-induced seizures but also in a behavioral desensitization that results from pretreatment with nicotine. The latter differences may be due to differences in receptor desensitization or inactivation. Clearly, more direct methods will be required to test these possibilities, but gaining an understanding of the mechanisms that underlie the behavioral desensitization phenomenon may prove to be of critical importance in understanding the relationship between nicotinic receptors and behavior.

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