Pharmacology Biochemistry & Behavior, Vol. 33, pp. 469–475. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Strain Comparison of Nicotine-Induced Seizure Sensitivity and Nicotinic Receptors

LUCINDA L. MINER* AND ALLAN C. COLLINS†[1]

*Institute for Behavioral Genetics, Department of Psychology and †School of Pharmacy, University of Colorado, Boulder, CO 80309

Received 1 May 1987

MINER, L. L. AND A. C. COLLINS. *Strain comparison of nicotine-induced seizure sensitivity and nicotinic receptors.* PHARMACOL BIOCHEM BEHAV 33(2) 469–475, 1989.—Nicotine-induced seizure sensitivity was assessed in 19 inbred mouse strains. Two routes of drug administration were utilized: acute intravenous (IV) infusion and intraperitoneal (IP) injection. Dose-response curves for sensitivity to IP nicotine-induced seizures were constructed for the 19 inbred strains and a heterogeneous stock (HS/Ibg) of mice. Differences were observed among the strains both in ED_{50} values and slopes of the dose-response curves following IP injection of nicotine. ST/bJ mice were the most sensitive having an ED_{50} of 2.34 ± 0.09 mg/kg nicotine. DBA/1J mice were the most resistant strain with an ED_{50} value of 6.16 ± 0.02 m/kg nicotine. Latency to clonic seizure was measured in the 19 inbred mouse strains using the acute IV route of drug administration. Again, ST/bJ mice were the most sensitive and DBA/2Ibg were the most resistant to IV nicotine-induced seizures. Significant correlations were observed between latency to IV nicotine-induced seizures and both ED_{50} values and the slope of the dose-response curves for IP nicotine-induced seizures. However, the pattern of results differed between the two routes of drug administration. The relationship between seizure sensitivity and nicotinic receptor concentration in three brain regions, cortex, midbrain and hippocampus, was also assessed using α-bungarotoxin (BTX) as the ligand. A significant relationship was observed between BTX binding in the three brain regions and sensitivity to IV nicotine-induced seizures such that strains with greater concentrations of BTX binding sites were more sensitive to nicotine-induced seizures than were strains with lower concentrations of BTX binding sites. Because of the differences between the two routes of drug administration, it is believed that other factors possibly related to either pharmacokinetic variables or receptor dynamics (receptor desensitization, rate of binding, binding site cooperativity) are involved in nicotine-induced seizure sensitivity. By identifying extreme strains for sensitivity to nicotine-induced seizure, the importance of these variables can be assessed in future studies

α-Bungarotoxin   Nicotine   Nicotinic receptors   Seizure, nicotine-induced   Genetics of nicotine response

SEVERAL earlier studies from our laboratory have demonstrated that genetic factors influence nicotine-induced clonic seizures in the mouse. We have examined the pattern of inheritance of sensitivity to nicotine-induced seizures both after IP and IV drug administration in F1, F2 and backcross mice derived from C3H/2Ibg and DBA/2Ibg parental strains (16–18). After IP drug administration (16), the dose-response curves for the F1 and F1 × DBA backcross were indistinguishable from the DBA parental strain. The C3H strain was observed to be the most sensitive of the groups and the F1 × C3H backcross generation was only slightly more resistant than the C3H strain. A comparison of the percentage of animals seizing at a particular dose (4.0 mg/kg) and a comparison of ED_{50} values led us to conclude that seizure sensitivity is controlled by a relatively simple genetic system, possibly a single gene with two alleles, one of which was dominant towards seizure resistance.

To confirm the above observations, another classical F2 and backcross study was conducted for sensitivity to nicotine seizures after an acute IV administration (18). The advantage of this technique is that the exact dose of nicotine required to elicit a seizure in an individual animal can be determined. The results obtained from this study were slightly different from those obtained following IP nicotine administration. C3H mice were still more sensitive to nicotine seizures than were DBA mice; however, the F1 cross was more sensitive than it had been after IP drug administration. The mean dose required to elicit a seizure in the F1 generation was approximately midway between the two parental strain means. The F1 × C3H generation was as sensitive as the C3H strain, and the F1 × DBA generation was approximately midway in sensitivity between its respective parental means. The pattern obtained fits a simple additive model with no indication of dominance towards either parental strain. Thus, it appears that the factors regulating seizure sensitivity differ depending upon the mode of drug administration.

[1]Requests for reprints should be addressed to Dr. Allan C. Collins, Institute for Behavioral Genetics, Campus Box 447, University of Colorado, Boulder, CO 80309.

These studies have also suggested that sensitivity to nicotine-induced seizures may be regulated by the number of hippocampal nicotinic receptors. Recent autoradiographic and biochemical studies suggest that mammalian brain contains two classes of nicotinic receptors (3, 13, 21). One class is characterized by the binding of BTX while the other is characterized by the binding of [^{3}H]acetylcholine or [^{3}H]nicotine (3). Nicotine and acetylcholine bind to the same sites in both mouse and rat brain (3). The classical F1, F2, and backcross experiments demonstrated a significant relationship between nicotinic receptor numbers as measured by BTX binding, and sensitivity to nicotine-induced seizures, but no relationship was observed between seizure sensitivity after IP nicotine injection and the amount of nicotine binding. Animals from the segregating F2 and backcross generations which were sensitive to nicotine-induced seizures were noted to have a significantly greater concentration of BTX binding sites (greater B_{max}) in the hippocampus than animals that were less seizure sensitive (17,18). No differences were observed among these animals in either the affinity of the receptor or in the inhibition of BTX binding by nicotine. Thus, the differences observed in binding among the animals are probably reflective of differences in receptor numbers.

In order to confirm the relationship between seizure sensitivity and BTX binding and to elucidate further the genetic basis of seizure sensitivity, a strain comparison of nicotine-induced seizure sensitivity was undertaken. Sensitivity to nicotine-induced seizures either by IP injection or by IV infusion was examined utilizing mice from 19 inbred strains. In addition, the relationships between the numbers of BTX nicotinic receptors in the hippocampus, midbrain and cortex and sensitivity to IV nicotine-induced seizures were also assessed utilizing the strain comparison approach.

METHOD

Subjects

Male mice from the following inbred strains were used: A/Ibg, AKR/J, BALB/cByJ, 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. In addition, males from a heterogeneous stock (HS/Ibg) of mice were also tested. Five of these groups, A/Ibg, C3H/2Ibg, C57BL/6Ibg, DBA/2Ibg and HS/Ibg have been maintained in the breeding colony at the Institute for Behavioral Genetics (Boulder, CO) for at least 20 generations. The BALB/cByJ strain was obtained from Jackson Laboratories (Bar Harbor, ME) and has been maintained at the Institute for four generations. The remaining strains were purchased from Jackson Laboratories and were housed in our colony for a least 2 weeks prior to testing. Animals were housed with 3–4 like-sexed littermates and allowed free access to food (Wayne Lab Blox) and water. The vivarium was maintained on a 12-hr light cycle (lights on at 7 a.m. with temperature control at 21 ± 1°C). Behavioral testing was done between 0900 and 1300 hours. All animals were 60–90 days of age at time of testing.

Nicotine Administration, IP

Nicotine was obtained from Sigma Chemical Co., St. Louis, MO and was redistilled periodically. The drug was dissolved in physiological saline and administered by IP injection, 0.01 ml/g body weight. Nicotine doses for seizure testing ranged from 2.0 to 7.0 mg/kg.

Mice from each strain were given one of several doses of nicotine. After injection the individual animal was placed in a $17 \times 50 \times 20$ cm metal cage and observed for 3 minutes. This time was chosen because nicotine seizures occur very quickly after drug administration. In C3H and DBA mice, 80% of the animals that seized did so within 2 minutes (16). A dose-response curve with a minimum of three points was constructed for each inbred strain and the HS mice. For most of the data points, seven animals were tested. However, in some cases up to 14 animals were tested.

Nicotine Administration, IV

A cannula made of silastic tubing was implanted in the right jugular vein of each mouse using the method of Barr *et al.* (1). The mice were anesthetized prior to surgery with pentobarbital (45 mg/kg) and chloral hydrate (63 mg/kg). This combination of anesthetics was used because previous experience had indicated that more animals survived the surgery when lower doses of these two drugs were used together instead of higher doses of one of them. After recovery, the cannula was checked daily for clear flow with sterile saline containing sodium citrate (3 g/l).

Two days after surgery, individual mice were transferred to a clear Plexiglas cage ($25 \times 25 \times 25$ cm) and the cannula attached to thermoplastic tubing that was connected to a 1 ml syringe mounted on an infusion pump (Harvard Apparatus, South Natick, MA). Nicotine was infused at a rate of 2.0 mg/kg/min (46.6 μl/sec) until the onset of a clonic seizure; i.e., latency to seizure was determined. Six animals were used for each strain with the exception of the P/J strain where three animals were used.

After IV nicotine seizure testing, mice were sacrificed by cervical dislocation. Brains were removed, dissected and three brain regions, cortex, midbrain and hippocampus, were retained for biochemical analysis.

Tissue Preparation

After dissection, the regions were frozen in 10 volumes of Krebs-Ringer-Hepes buffer solution (KRH) of the following composition: 118 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 20 mM Hepes, pH adjusted to 7.5 with NaOH. The tissue was stored as chunks at -70°C. For preparation, the tissue was homogenized using a teflon pestle modified to fit in polypropylene 12×75 mm test tubes. The particulate fraction was prepared using the method of Romano and Goldstein (20). Before each of the 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 (13). Binding was measured in animals that had been treated once with nicotine. Although chronic nicotine treatment can elicit increased BTX binding, the doses and duration of treatment required to elicit such changes are far greater than those used here (12). Recent (unpublished) observations in our laboratory indicate that chronic injection with nicotine will not elicit a change in brain BTX binding; continuous infusion methods are required.

BTX Binding

The binding of BTX was measured as described previously (13). Binding was measured at 37°C with samples containing 100–300 μg protein in an incubation volume of 500 μl of KRH buffer. The reaction was initiated by the addition of BTX and continued for 2.5 hr. At the completion of the incubation, samples were diluted with 3 ml of ice-cold buffer containing 0.05% polyethylenimine and filtered onto Boehringer-Mannheim glass fiber filters which were soaked in buffer containing 0.5% polyethylenimine to reduce the blank. The filters were then washed four times with 3-ml aliquots of ice-cold buffer. The incubations were carried out a 37° because equilibrium binding is achieved more rapidly at this temperature than at room temperature or

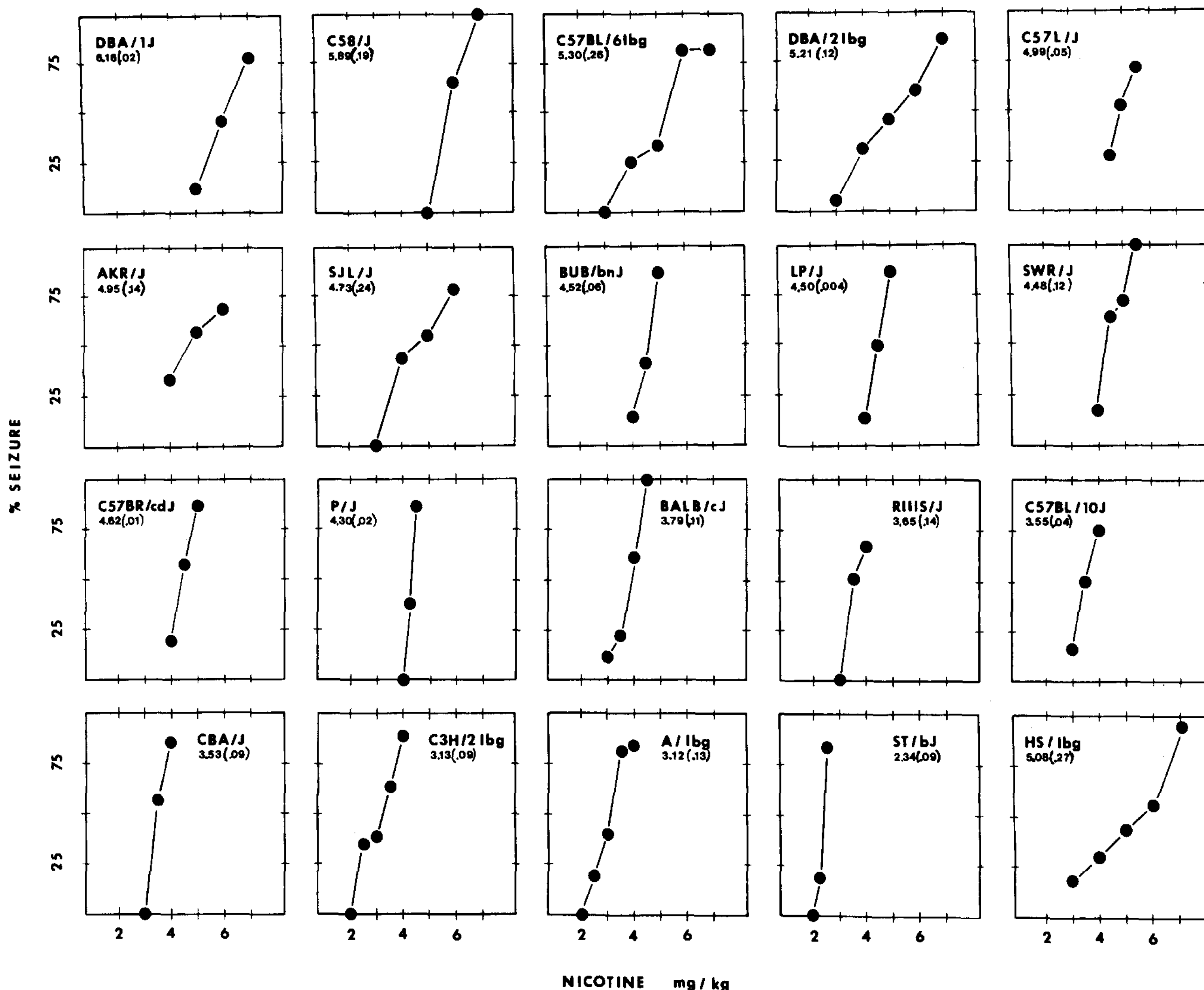

FIG. 1. Dose-response curves for nicotine-induced seizures. Nicotine was administered to 19 inbred strains and a heterogenous stock of mice (HS/Ibg). Each point represents the percent of animals tested that exhibited clonic seizures at the indicated dose of nicotine; 7–14 male mice were tested at each dose.

below, whereas the filtrations and washes used ice-cold buffer to retard dissociation of the bound ligand (13). Specific binding was calculated to be the difference in binding between samples containing no added nicotine and those containing 1 mM nicotine. A single concentration of BTX was used when saturation curves were not constructed. Saturation curves to measure K_D and B_{max} were generated by conducting the binding assays in the presence of six different concentrations of BTX and were done using tissue from the cortex. In a previous study, no differences were observed in the K_D values obtained from the three brain regions of interest (cortex, midbrain and hippocampus) in either the C3H or DBA strains (16).

After the samples were washed, the glass fiber filters were placed in polypropylene scintillation vials (7 ml) and 2.5 ml of scintillation fluid were added. Radioactivity was determined on a Beckman LS 1800 liquid scintillation spectrometer. ^{125}I was counted at 44% efficiency.

Protein was determined using the method of Lowry *et al.* (10) with bovine serum albumin as the standard. Aliquots for tissue samples were 5 μl.

Data Analysis

ED_{50} values and slopes were calculated from the dose-response curves for sensitivity to IP nicotine-induced seizures by linear regression. Dose, rather than log dose, was used because a better linear relationship was seen for untransformed data. Latency to IV seizures and BTX binding were compared among the 19 inbred strains by analysis of variance (ANOVA). Significant differences among the groups were analyzed further by Duncan's post hoc test ($p<0.05$).

Because sensitivity to IV nicotine-induced seizures and BTX binding were determined on individual animals, genetic correlations could be obtained. These were calculated as outlined by Spuhler *et al.* (22) and Kempthorne (9). The basic premise of this analysis is that the differences observed among several inbred strains provide an estimate of additive genetic variance (V_A). For this estimate to be valid, the following conditions should be met: the number of strains tested should be large, the strains should be measured concurrently, and any differences in the environment to which the strains are exposed should be minimized. Therefore, in

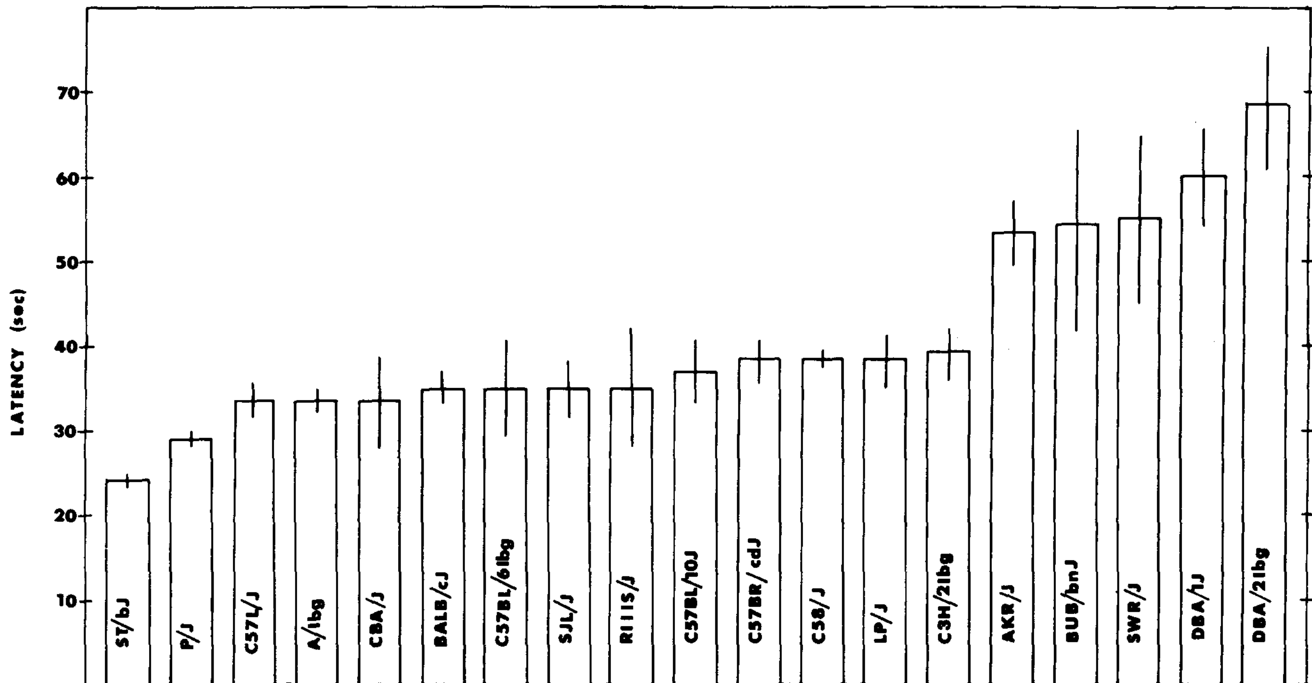

FIG. 2. Latency to IV nicotine seizures. Each bar represents the mean ± S.E.M. latency to IV nicotine seizures for six male mice from the indicated strains (P/J represents the mean of three mice).

an analysis of variance, the between inbred strain component of variance is a function only of V_A. The within strain component of variance estimates only VE (environmental variance) because any differences among members of the same strain are assumed to be due to environmental effects. In an analogous manner, the between inbred strain component of covariance in an analysis of covariance is a function of the additive genetic covariance of the two traits considered jointly. Thus, an estimate of the genetic correlation (r_A) between two traits, X and Y, can be obtained:

$$r_A = \frac{COV_{AXY}}{\sqrt{(V_{AX})(V_{AY})}}$$

where, COV_{AXY} = additive genetic covariance of X and Y; V_{AX} = genetic variance of X; and V_{AY} = genetic variance of Y.

RESULTS

Nicotine-Induced Seizure Sensitivity

Figure 1 presents the dose-response curves for nicotine-induced clonic seizures for the 19 inbred strains and HS mice used in this study following the IP administration of nicotine. The dose-response curves are arranged according to ED_{50} value from the most resistant inbred strain (DBA/1J) to the most sensitive (ST/bJ) with the HS/Ibg mice presented last. In addition, ED_{50} values and slopes calculated from these dose-response curves are listed.

Figure 2 presents the results of the strain comparison of nicotine-induced seizures after IV drug administration. Average latency to clonic seizure ranged from 24 sec for ST/bJ mice to 68 sec for DBA mice. Analysis of variance revealed significant strain differences, $F(18,110) = 4.76$, $p<0.001$. Post hoc analysis (Duncan's multiple range test) revealed that the strains could be separated into five groups. However, there is considerable overlap between three of the groups.

The relationship between seizure sensitivity after IV and IP drug administration was calculated. Significant correlations were observed between mean latency to seizure after IV nicotine infusion and the ED_{50} value calculated from the dose-response curves for each strain ($r = .52$, $p<0.05$) and the slopes of the dose-response curves ($r = -.49$, $p<0.05$).

BTX Binding

In order to compare BTX binding among the strains of mice, the dissociation constant (K_D) was determined in cortex by Scatchard analysis. No significant differences in K_D for BTX binding in the cortex were observed among any of the strains, $F(18,110) = 1.1$, $p = 0.45$. Thus, BTX bound with equal affinity to the BTX binding sites for all 19 mouse strains. Therefore, any observed differences in BTX binding should be due to differences in receptor number. Significant strain differences in BTX binding were observed in both the midbrain, $F(18,110) = 2.3$, $p<0.05$, and hippocampus, $F(18,110) = 4.9$, $p<0.01$. No differences among the strains were observed for cortical binding. Post hoc analysis for hippocampal binding revealed that the strains segregate into seven groups. The group with the lowest amount of BTX binding is characterized by the DBA/2Ibg strain, and the group with the highest number of BTX binding sites is characterized by the SJL/J strain.

Comparison of BTX Binding and Nicotine Seizure Sensitivity

Figure 3 presents the correlation between mean BTX binding in the three brain regions assayed and ED_{50} values (top panel) and slopes (bottom panel) calculated from the dose-response curves presented in Fig. 1. No significant relationships were observed between sensitivity to IP nicotine seizures and BTX binding.

Figure 4 presents the phenotypic (r_p) and genotypic (r_g) correlations between mean BTX binding in the cortex, midbrain

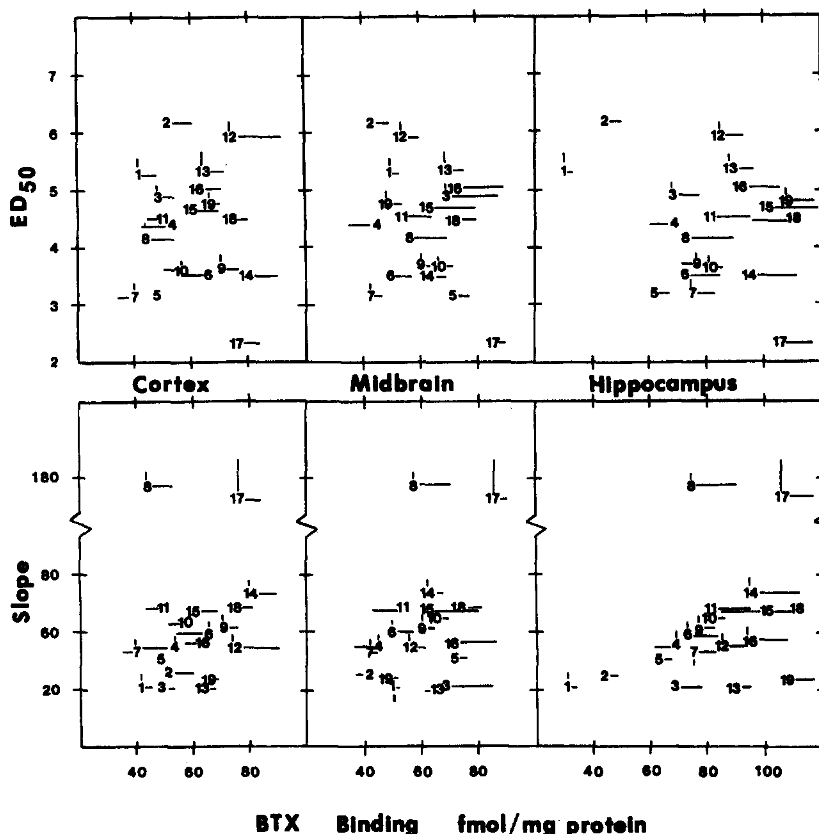

FIG. 3. A comparison of BTX binding and sensitivity to nicotine-induced seizures following IP injection. The top panels present the correlation between BTX binding in three brain regions (cortex, midbrain, hippocampus) and ED_{50} values obtained from the data presented in Fig. 1 and the bottom panels present comparisons of BTX binding and slopes of the dose-response curves. Each strain is designated by a number. The strain code is: LP (1), CBA (2), AKR (3), C57BR (4), SJL (5), C57L/6 (6), DBA/1J (7), C57BL/10 (8), ST (9), SWR (10), C58 (11), BUB (12), A/J (13), RIIIS (14), C57BL/6 (15), BALB (16), DBA/2 (17), C3H (18) and P/J (19). Each point is accompanied by a bar which represents the standard error of the mean (n=7–14 for each point).

and hippocampus and mean latency to IV nicotine seizures. Significant phenotypic correlations were obtained between mean latency to seizure and mean binding in the midbrain ($r=-.55$, $p<0.05$) and mean hippocampal binding ($r=-.64$, $p<0.05$). All of the genetic correlations were significant.

DISCUSSION

The results obtained in this 19 inbred strain comparison for IP seizures are more complex than those obtained in our earlier analysis of genetic regulation of seizures that used the DBA and C3H inbred strains as well as their derived (F1, F2 and backcross) generations (16). In addition to ED_{50} differences among the strains, there are also differences in slope, particularly for the ST/bJ and P/J strains. These differences could be influenced by pharmacokinetic variables, such as differences in drug absorption or distribution, or they could be due to differences in receptor dynamics among the strains.

Our earlier studies of nicotine-induced seizures suggested that the genetic regulation of seizures is different following IP and IV nicotine (16,18). Differences in the genetic bases of seizures induced by the different routes of administration were also observed in the present study but some similarities were also observed in that significant correlations were found following IV and IP nicotine. In general, strains with lower ED_{50} values (more sensitive to IP nicotine-induced seizures) had a lower mean latency to IV nicotine-induced seizures. In conjunction with this observation, strains which had steeper dose-response curves were more sensitive to IV nicotine-induced seizures, i.e., they had a lower mean latency. Thus, while there may be different physiological mechanisms that regulate nicotine-induced seizures following IP and IV administration, there are also common mechanisms involved. It may be that nicotine-induced seizures following IP injection are influenced by more subtle differences in receptor dynamics or pharmacokinetic variables than are IV seizures, thus leading to the seemingly more complicated pattern of results for IP seizures than for IV seizures.

The results from the present strain comparison of IV nicotine-induced seizure sensitivity and BTX binding are consistent with those of our earlier F2 and backcross analysis of nicotine-induced seizures (18). In this earlier study, the relationship between seizure sensitivity and receptors was greatest for hippocampal BTX binding. In the present study, there is evidence for a relationship with midbrain binding. We observed significant genetic correlations between seizure sensitivity and BTX binding sites in all three brain areas examined. However, the correlations involving cortical binding are probably unreliable, as evidenced by their high standard errors, and because there were no significant differences

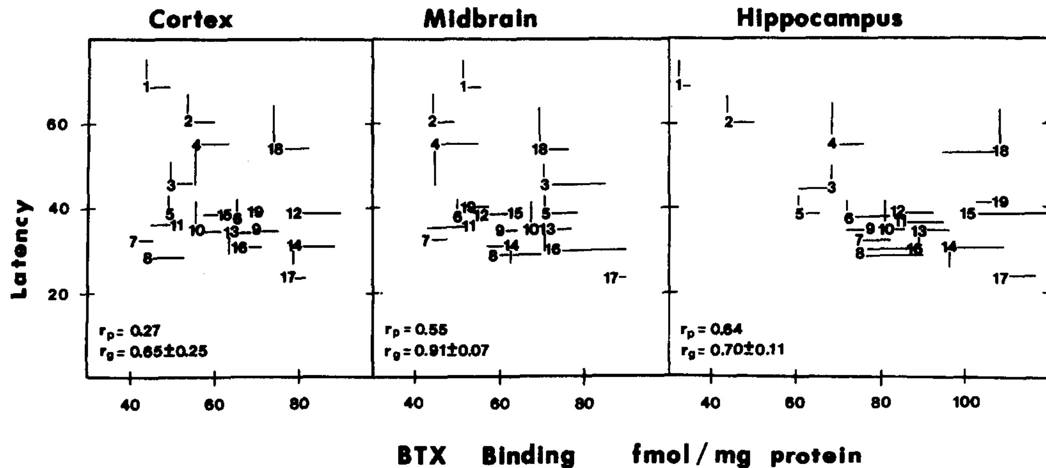

FIG. 4. A comparison of BTX binding in cortex, midbrain and hippocampus and latency to 10 nicotine-induced seizures. Each of the strains is designated by a number (see legend to Fig. 3 for the code). r_p = phenotypic correlations, r_g = genotypic correlations. n = 6 for each strain with the exception of the P strain where n = 3.

among the inbred strains tested. These results suggest that seizure sensitivity may be related to BTX binding site concentration throughout the brain, with the hippocampus possibly being a major locus of seizure control.

The hippocampus has been proposed as a major site of action in the induction of nicotine-induced seizures (2, 6, 7). Stumpf and Gogolak (23) noted that among four brain regions, hippocampus, neocortex, reticular formation and septum, nicotine elicited seizure activity in the hippocampus first. In addition, it has been demonstrated that electrical stimulation of the inferior colliculus of the rat can elicit clonic seizures (11). Thus, there is support for a relationship between seizure sensitivity and BTX binding in both the midbrain and hippocampus, but it is likely that the hippocampus is a major locus of seizure control in part because we have consistently observed a relationship between hippocampal BTX binding and nicotine-induced seizure sensitivity.

It should be pointed out that the relationship between BTX binding and seizure sensitivity is not perfect for any brain region. Because the correlations are less than 1, we must assume that factors other than receptor numbers influence seizure sensitivity. A factor that may be lowering the correlations between seizure sensitivity and BTX binding is the observation that BTX binds at both synaptic and extrasynaptic sites (5,15). It has been proposed that the extrasynaptic site is involved in signaling neuronal growth (4) and the synaptic site in cholinergic transmission (14). Clearly, the relationship between nicotine-induced seizure sensitivity and BTX binding would be better ascertained if only synaptic sites could be obtained for analysis.

The observation that the number of BTX binding sites is correlated with IV nicotine seizure sensitivity but not IP nicotine seizure sensitivity indicates that the differences in seizure sensitivity depend on route of drug administration. After IP nicotine administration the drug must first be absorbed and then transported to the brain via the circulation. Thus, a portion of the drug will be lost to metabolism as it passes through the liver and is distributed throughout the body. Mouse strains clearly could differ in these pharmacokinetic measures. Hatchell and Collins (8) observed strain differences in mice for nicotine effects on locomotor activity but these strains did not differ in nicotine metabolism. Similarly, Petersen *et al.* (19) observed no differences among several inbred mouse strains (C5BL/bIbg, C3H/2Ibg and DBA/2Ibg) in rates of nicotine metabolism. We have previously reported that there is no relationship between seizure sensitivity and either brain or blood levels of nicotine at the time of seizure in C3H and DBA mice as well as in their derived F1, F2 and backcross generations (16,18). The strains in which nicotine metabolism has actually been measured are distributed throughout the strains that we have tested and reported on here. Therefore, we suspect that metabolism differences do not play a major role in determining strain differences in seizure sensitivity. Rather, we suspect that differences in receptors is the most important parameter that regulates sensitivity to nicotine-induced seizures. The ED_{100} value for DBA/2Ibg mice is greater than 7.0 mg/kg nicotine, IP, whereas the mean dose of nicotine required to elicit a seizure given IV is approximately 2.0 mg/kg (the mean latency to seizure for DBA/2Ibg mice is 68 sec for nicotine infused at a concentration of 2.0 mg/kg/min). It may be that the more "gradual" distribution of the drug to the site of action after IP administration allows the CNS to accommodate to a potentially convulsant dose of nicotine and perhaps mouse strains differ in this ability to accommodate to nicotine. One such method of accommodation is receptor desensitizaton. Perhaps genetic factors regulate this receptor property.

The possible effects of differing pharmacokinetic variables or receptor dynamics on seizure sensitivity can be tested using the genetic tools described in this and previous studies (16–18). By observing the pattern of inheritance between two or more variables of interest such as seizure sensitivity and rate of receptor desensitization, one can determine the relationship between those two variables. The present study has identified extreme mouse strains which would be useful in future studies of this type.

ACKNOWLEDGEMENTS

This work was supported by grant DA-03194 from the National Institute on Drug Abuse. L.L.M. was supported by training grant MH-16880.

REFERENCES

1. Barr, J. E.; Holmes, D. B.; Ryan, L. M.; Sharpless, S. K. Techniques for the chronic cannulation of the jugular vein in mice. Pharmacol. Biochem. Behav. 11:115–118; 1979.
2. Brown, B. B. Relationship between evoked response changes and

behavior following small doses of nicotine. Ann. NY Acad. Sci. 142:190–200; 1967.

3. Clarke, P. B. S.; Schwartz, R. D.; Paul, S. M.; Pert, C. B.; Pert, A. Nicotinic binding in rat brain: Autoradiographic comparison of [^{3}H]-acetylcholine, [^{3}H]nicotine and [^{125}H]-α-bungarotoxin. J. Neurosci. 5:1307–1315; 1985.
4. Connolly, J. A.; St. John, P. A.; Fischbach, G. D. Extracts of electric lobe and electric organ from Torpedo Californica increase the total number as well as the number of aggregates of chick myotube acetylcholine receptors. J. Neurosci. 2:1207–1213; 1982.
5. Dun, N. J.; Karczmar, A. G. Blockade of ACh potentials by α-bungarotoxin in rat superior cervical ganglion cells. Brain Res. 196:536–540; 1980.
6. Dunlop, C. W.; Stumpf, C.; Maxwell, D. S.; Schindler, W. Modification of cortical, reticular and hippocampal unit activity by nicotine in the rabbit. Am. J. Physiol. 198:515–518; 1960.
7. Floris, V.; Morocutti, C.; Ayala, G. F. Effects of nicotine on the cortical, thalamic and hippocampal electrical activity in rabbits. J. Neuropsychiatry 5:247–251; 1964.
8. Hatchell, P. C.; Collins, A. C. The influence of genotype and sex on behavioral tolerance to nicotine in mice. Psychopharmacology (Berlin) 71:45–49; 1980.
9. Kempthorne, O. An introduction to genetic statistics. Ames, IA: Iowa State University Press; 1969.
10. Lowry, O. H.; Rosebrough, N. H.; Farr, A. C.; Randall, R. J. Protein measurement with folin reagent. J. Biol. Chem. 193:265–275; 1951.
11. McCown, T. J.; Greenwood, R. S.; Frye, G. D.; Breese, G. R. Electrically elicited seizures from the inferior colliculus: A potential site for the genesis of epilepsy. Exp. Neurol. 86:527–542; 1984.
12. Marks, M. J.; Burch, J. B.; Collins, A. C. Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. J. Pharmacol. Exp. Ther. 226:817–825; 1983.
13. Marks, M. J.; Collins, A. C. Characterization of nicotine binding in mouse brain and comparison with binding of α-bungarotoxin and quinuclidinyl benzilate. Mol. Pharmacol. 22:554–564; 1982.
14. Mashall, L. M. Synaptic localization of α-bungarotoxin binding which blocks nicotinic transmission at frog sympathetic neurons. Proc. Natl. Acad. Sci. USA 78:1948–1952; 1981.
15. Messing, A.; Gonatas, N. K. Extra-synaptic localization of α-bungarotoxin receptors in cultured chick ciliary ganglion neurons. Brain Res. 269:172–176; 1983.
16. Miner, L. L.; Marks, M. J.; Collins, A. C. Classical genetic analysis of nicotine-induced seizures and hippocampal nicotinic receptors. J. Pharmacol. Exp. Ther. 231:454–464; 1984.
17. Miner, L. L.; Marks, M. J.; Collins, A. C. Relationship between nicotine-induced seizures and hippocampal nicotinic receptors. Life Sci. 37:75–83; 1985.
18. Miner, L. L.; Marks, M. J.; Collins, A. C. Genetic analysis of nicotine-induced seizures and hippocampal nicotinic receptors in the mouse. J. Pharmacol. Exp. Ther. 239:853–860; 1986.
19. Petersen, D. R.; Norris, K. J.; Thompson, J. A. A comparative study of the disposition of nicotine and its metabolites in three inbred strains of mice. Drug Metab. Dispos. 12:725–731; 1984.
20. Romano, C.; Goldstein, A. Stereospecific nicotine receptors on rat brain membranes. Science 210:647–650; 1980.
21. Schwartz, R. D.; McGee, R.; Kellar, K. J. Nicotinic cholinergic receptors labeled by [^{3}H]acetylcholine in rat brain. Mol. Pharmacol. 22:56–62; 1982.
22. Spuhler, K.; Hoffer, B.; Weiner, N.; Palmer, M. Evidence for genetic correlation of hypnotic effects and cerebellar Purkinje neuron depression in response to ethanol in mice. Pharmacol. Biochem. Behav. 17:569–578; 1982.
23. Stumpf, C.; Gogolak, G. Actions of nicotine upon the limbic system. Ann. NY Acad. Sci. 142:143–158; 1967.