Sensitivity to the Seizure-Inducing Effects of Nicotine is Associated with Strain-Specific Variants of the \( \alpha_5 \) and \( \alpha_7 \) Nicotinic Receptor Subunit Genes

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Accepted for publication November 7, 1997

This paper is available online at http://www.jpet.org

Restriction fragment length polymorphisms (rflps) have been identified for the nicotinic ACh receptor subunit genes \( \alpha_5 \) and \( \alpha_7 \) between two mouse strains (C3H/2ibg and DBA/2ibg) that differ in sensitivity to the convulsant effects of nicotine. In the study reported here, F2 animals derived from these two parental strains were tested for their sensitivity to the convulsant effects of nicotine as measured by seizure frequency and overall sensitivity score. Subsequently, the animals were genotyped for the \( \alpha_5 \) and \( \alpha_7 \) rflps. In addition, levels of \( \alpha \)-bungarotoxin (\( \alpha \)-BTX) binding were measured in four brain regions (colliculi, hippocampus, hypothalamus and striatum) to determine whether there is a correlation among \( \alpha \)-BTX binding levels, sensitivity to nicotine and nicotinic ACh receptor subunit genotype. A significant relationship was observed between \( \alpha_5 \) and \( \alpha_7 \) genotype and sensitivity to nicotine. In addition, the \( \alpha_7 \) rflp significantly correlated with levels of \( \alpha \)-BTX binding in hippocampus, colliculi and striatum. The \( \alpha_5 \) rflp did not correlate with \( \alpha \)-BTX binding in any brain region. Levels of \( \alpha \)-BTX binding did not correlate with nicotine-induced seizure sensitivity or overall nicotine sensitivity score in any of the four brain regions examined.

Genetic components appear to influence smoking behavior (Carmelli et al., 1992; Heath and Madden, 1994; Heath et al., 1995). Similarly, animal studies have demonstrated that sensitivity to the effects of nicotine on a variety of behavioral and physiological measures is influenced by the genetic constitution of the animal (Marks et al., 1989; Marks et al., 1991; Miner and Collins, 1989; Robinson et al., 1996). Although these studies have demonstrated a clear influence of genetics on nicotine sensitivity, the genes responsible for these differences in sensitivity have not been identified. Because the effects of nicotine are presumably mediated through nAChRs, it is possible that differences in sensitivity to nicotine in both humans and mice are due, at least in part, to differences in the genes that code for the various nicotinic receptor subunits.

Eleven (10 in mammals) neuronal nAChR subunits have been identified: \( \alpha_2 \) to \( \alpha_9 \) (\( \alpha_8 \) is found only in chickens) and \( \beta_2 \) to \( \beta_4 \) (Lindstrom, 1995; Elgoyhen et al., 1994). In mice, rflps have been identified for several of the genes that encode nAChR subunits, including the genes for \( \alpha_5 \) and \( \alpha_7 \) (Bessis et al., 1990; Eng et al., 1991; Nagavarapu and Boyd, 1995; Stitzel et al., 1995). The \( \alpha_7 \) subunit is thought to be the major \( \alpha \)-BTX-binding subunit in the mammalian brain (Séguela et al., 1993), although some reports suggest that the \( \alpha_5 \) subunit can bind \( \alpha \)-BTX (McLane et al., 1990; McLane et al., 1991).

Previous studies have shown that nicotine-induced seizures correlates with levels of the nAChR subtypes that binds \( \alpha \)-BTX with high affinity in the hippocampus (Miner et al., 1986; Miner et al., 1985; Miner et al., 1984; Miner and Collins, 1989), the brain region where nicotine-induced seizures are believed to be initiated (Dunlop et al., 1960; Floris et al., 1964; Brown, 1967; Stumpf and Gogolak, 1967). In general, animals with higher levels of hippocampal \( \alpha \)-BTX binding were found to be more sensitive to the convulsant effects of nicotine. It has also been demonstrated that the \( \alpha_7 \) rflp is linked to levels of \( \alpha \)-BTX binding in hippocampus (Stitzel et al., 1996). In addition, the rflps for the \( \alpha_5 \) and \( \alpha_7 \) nAChR subunit genes have been found between strains that differ in sensitivity to the convulsant effects of nicotine (Nagavarapu and Boyd, 1995; Stitzel et al., 1995). RNAs for both \( \alpha_5 \) and \( \alpha_7 \) nAChR subunit genes are found in the hippocampus (Wada et al., 1990; Marks et al., 1992; Marks et al., 1996; Séguela et al., 1993). Overlap in the distribution of these two nAChR subunit RNAs in the hippocampus occurs only in the pyramidal cell layer of the CA1 region, a region.

**ABBREVIATIONS:** \( \alpha \)-BTX, \( \alpha \)-bungarotoxin; ANOVA, analysis of variance; KRH, Krebs-Ringer’s HEPES; MLA, methyllycaconitine; nAChR, nicotinic ACh receptor; rflp, restriction fragment length polymorphism.
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particularly prone to excitotoxicity due to epileptiform activity (Meldrum, 1991).

Despite the correlation between the amount of α-BTX binding in hippocampus and seizure sensitivity, some evidence suggests that α-BTX-sensitive nAChRs may not mediate nicotine-induced seizures. Gasior et al. (1996) demonstrated that methyllycaconitine (MLA), an antagonist selective for a-α-BTX-sensitive nAChRs, did not block nicotine-induced convulsions. Some of the results of Miner et al. (1985) also indicated that the correlation that has been observed between seizure sensitivity and α-BTX binding levels among populations and inbred mouse strains does not persist in certain populations. When segregating populations from a classic genetic cross (backcross to parentals and F2 animals) were compared for seizure sensitivity and α-BTX binding levels, a significant correlation between the two measures was observed when all three populations were grouped together. However, no significant relationship between the two measures was observed when the most genetically diverse population, the F2 animals, was examined alone. This finding suggests that the relationship between seizure sensitivity and α-BTX binding may be casual rather than causal. The lack of relationship between nicotine-induced seizures and levels of hippocampal α-BTX binding observed in the F2 population also may be due to an insufficient number of animals having been tested—34 F2 animals were tested in the study by Miner et al. (1985)—for this genetically heterogeneous population.

The studies reported here attempt to determine whether there is a relationship between nAChR subunit genotype and seizure phenotype in F2 animals derived from a C3H/2ibg by DBA/2ibg cross. This investigation also examines whether a significant relationship exists between nicotine-induced seizure sensitivity and levels of α-BTX binding in four brain regions of F2 animals when a significantly larger number of animals is surveyed. Mice were injected with a high dose of nicotine (4 mg/kg) and observed for seizure activity as well as evaluated for overall response to the nicotine challenge. Subsequently, responses for each animal were compared to their respective nAChR α5 and α7 genotypes. Sensitivity to nicotine and genotype were also compared with levels of α-BTX binding in colliculi, hippocampus, hypothalamus and striatum. The comparison of nicotine sensitivity phenotype vs. nAChR subunit genotype indicated that both of the nAChR subunit genes examined are linked to sensitivity to the high-dose effects of the drug. In addition, a confirmation of the linkage between α7 genotype and levels of hippocampal α-BTX binding was observed, whereas no relationship was seen between levels of α-BTX binding in any brain region examined and nicotine-induced seizure sensitivity.

Materials and Methods

Animals. C3H/2ibg and DBA/2ibg mice were maintained at the specific pathogen-free mouse colony at the Institute for Behavioral Genetics. F2 progeny were generated from a classic genetic cross between these two inbred strains. Offspring of the matings were weaned at 25 days of age and housed with 1 to 5 same-sex litter mates. Both male and female mice were used for all experiments. Mice were maintained on a 12-h light/12-h dark cycle (lights on between 7 a.m. and 7 p.m.) and had free access to food (Teklad 22/5 rodent diet, Harlan, Madison, WI) and water.

Behavioral testing. F2 (n = 122) animals (males and females) derived from a classic genetic cross between C3H/2ibg and DBA/2ibg were injected i.p. with a dose of nicotine (4 mg/kg) that results in nearly 100% seizure frequency in C3H/2ibg animals (ED50 = 3.1 mg/kg for nicotine-induced seizures) but rarely induces seizures in DBA/2ibg mice (ED50 = 5.5 mg/kg). After injection, animals were placed in a clear Plexiglas container measuring 30 × 30 × 30 cm and observed for 3 min. The symptoms elicited by nicotine included straub tail, head and body tremors, loss of righting response, wild running, clonic seizures and tonic seizures. Animals were scored for whether they had seizures and also received an overall sensitivity score. For the overall sensitivity score, animals were rated on a scale of 1 to 5 as follows: 1, no effect or mild head tremors; 2, more severe tremors, including body tremors, and/or near loss of righting response; 3, any combination of severe tremors, wild running and complete loss of righting response; 4, clonic seizures; 5, tonic seizures. All testing was videotaped, and all borderline cases were reevaluated.

DNA isolation. Spleens were quick-frozen in isopentane kept at −70°C and subsequently crushed into a fine powder with a mortar and pestle. The crushed spleens were placed into 3 ml of a solution containing 20 mM Tris (pH 7.5), 100 mM NaCl, 1 mM EDTA, 0.5% SDS and 10 μg/ml proteinase K and were incubated for 3 to 5 h at 56°C. After incubation, the samples were sequentially extracted with equal volumes of phenol/phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1). Genomic DNA was precipitated from the final aqueous phase by the addition of two volumes of 95% ethanol. The DNA precipitate was spooled out with a glass rod, washed with 70% ethanol, dried and resuspended in TE (10 mM Tris, pH 8, and 1 mM EDTA). The DNA was allowed to resuspend slowly for several days at 4°C. DNA concentrations were estimated by measuring the absorbance at 260 nm of a 1:100 dilution of 2 to 4 replicates of each sample.

Southern transfer and hybridization. Genomic DNA (10 μg) was digested with 10 to 20 units of the appropriate restriction endonuclease and electrophoresed on an 0.8% agarose gel. The gel was subsequently transferred to a nylon membrane (Hybond-N, Amersham Corp., Arlington Heights, IL) by capillary action as described elsewhere (Sambrook et al., 1989). Once the transfer was complete, the membrane was placed in a UV transilluminator (Stratagene, La Jolla, CA) to link the DNA covalently to the membrane. Membranes were prehybridized for 30 min at 65°C in Rapid Hyb (Amersham Corp., Arlington Heights, IL) hybridization solution (0.2 ml/m²/cm² Radiolabeled (32P-dCTP; New England Nuclear, Boston, MA), full-length α5 and α7 cDNA probes were generated by the random priming method (Feinberg and Vogelstein, 1983) using a commercially available kit (Decaprime II, Ambion, Austin, TX) and subsequently added to the solution. Hybridization was continued for 3 h. After hybridization, membranes were washed at increasing stringencies until background was not detectable with a Geiger counter. For most experiments, the final wash was at 65°C and consisted of 0.5 × SSC (1 × SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0) and 0.1% sodium dodecyl sulphate. Membranes were then exposed to X-ray film (Kodak XAR-5) at −70°C with intensifying screen for 1 to 14 days.

Tissue preparation for ligand binding. Each mouse was sacrificed by cervical dislocation, and its brain and spleen were removed. The brains were placed on an ice-cold platform and dissected into four regions: hippocampus, striatum, hypothalamus and colliculi (superior and inferior). The brain regions were placed in 10 volumes of 0.1 × KRH (11.8 mM NaCl, 0.48 mM KCl, 0.25 mM CaCl2, 0.12 mM MgSO4 and 2.0 mM HEPES, pH 7.5) and homogenized using a Teflon pestle. The homogenates were then incubated for 5 min at 37°C and subsequently centrifuged for 20 min at 18,000 × g. After centrifugation, pellets were resuspended in 10 volumes of fresh 0.1 × KRH, and the foregoing procedure was repeated a total of four
times. Samples were stored at −20°C as a pellet until use. On the
day of receptor-ligand binding, pellets were resuspended in 10 vol-
umes of ice-cold distilled water.

**Ligand binding.** The binding of α-bungarotoxin to brain mem-
branes was measured as described previously (Marks and Collins,
1982) with modifications for filtration by means of a 96-well cell
harvester. [125I]-α-Bungarotoxin (230–285 Ci/mmol; Amersham, Ar-
lington Heights, IL) binding was carried out at 37°C for 4 h in 96-well
microtiter plates in a final volume of 100 μl. The average concen-
tration of [125I]-α-bungarotoxin used was 2.7 ± 0.28 nM. Nonspecific
binding was determined by the addition of 1 mM L-nicotine during
the incubation. Samples were filtered through two filters, a Gelman
type A/E filter and a MFS GB100R filter, with a cell harvester
(Inotech, Lansing MI). The Gelman A/E filter was presoaked in 1×
KRH/0.5% polyethylenimine, and the MFS GB100R filter was pre-
soaked in 5% nonfat dry milk/1× KRH before filtration. Individual
filters were placed into 5-ml culture tubes and counted on a Packard
Auto-Gamma 5000 gamma counter.

Protein levels were measured by the method of Lowry (Lowry et
al., 1951).

**Statistics.** One-way ANOVA was used for all statistical analyses.
Duncan’s Multiple Range test was utilized for post-hoc analysis.

## Results

**Behavioral testing.** The sensitivity of individual F2 ani-
mals to the convulsant effects of nicotine was determined after an i.p.
injection of nicotine at a dose (4 mg/kg) that elicits seizures in 100% of animals of the C3H parental strain but rarely induces seizures in the DBA parental strain. Of 122 F2 animals tested, 24 (19.7%) exhibited clonic or tonic seizure activity. These results are consistent with previous studies that have shown that resistance to the convulsant effects of nicotine is inherited in a dominant fashion (where 25% of the animals would be expected to have seizures) (Miner et al., 1984).

These mice were also scored for their overall sensitivity to the high-dose effects of nicotine on a scale of 1 (least sensitive) to 5 (tonic seizure) as described in “Materials and Methods” (fig. 1). Of the mice that did not show nicotine-induced seizure activity, 35.4% (34/96) were scored as least sensitive (a score of 1), 26.0% (25/96) were scored as moderately sen-
sitive (a score of 2) and 38.5% (37/96) were scored as sensitive
but without the appearance of convulsions (a score of 3). Of the 24 mice that seized, 21 had clonic seizures (a score of 4), and the remaining 3 had clonic/tonic seizures (a score of 5). Two of the animals were not assigned sensitivity scores because they died after testing without showing signs of sei-
zure.

**Comparison of genotype and nicotine sensitivity.** It
is possible that the differences in sensitivity to the high-dose
effects of nicotine are due to a strain-specific variant of a gene or
genes that code(s) for subunits of the nAChR family. Pre-
viously, a rflp has been described for the nAChR subunit gene as determined by rflp analysis. The α5 and α7 polymorphisms were
measured with the restriction endonucleases EcoRV and PvuII, respec-
tively, as described in “Materials and Methods.” Size standards (HindIII digested Lambda DNA) are indicated by lines adjacent to the autoradiograms. For the α5 polymorphism, the standards shown are, from top to bottom, 23,130 bp, 9416 bp, 6557 bp and 4361 bp. The size standards shown for the α7 polymorphism are, from top to bottom, 4361 bp, 2322 bp, 2027 bp and 564 bp.

Fig. 1. Distribution of nicotine sensitivity scores across the F2 popula-
tion. Animals were injected i.p. with 4 mg/kg nicotine and scored for their
sensitivity to nicotine as described in “Materials and Methods.” The number of animals rated for each sensitivity score is shown.

Fig. 2. Restriction fragment length polymorphisms for the nicotinic ACh receptor subunit genes α5 and α7. Representative autoradiograms of F2 animals homozygous for the DBA variant (DD), heterozygous (HD) or homozygous for the C3H variant (HH) for either the α5 or the α7 subunit gene as determined by rflp analysis. The α5 and α7 polymorphisms were
measured with the restriction endonucleases EcoRV and PvuII, respec-
tively, as described in “Materials and Methods.” Size standards (HindIII digested Lambda DNA) are indicated by lines adjacent to the autoradiograms. For the α5 polymorphism, the standards shown are, from top to bottom, 23,130 bp, 9416 bp, 6557 bp and 4361 bp. The size standards shown for the α7 polymorphism are, from top to bottom, 4361 bp, 2322 bp, 2027 bp and 564 bp.

higher frequency (40%) than animals homozygous for the
DBA variant of α5 (7.7%) (table 1). Heterozygotes for the α5
rflp had a seizure frequency (10.2%) essentially the same as
the α5 DBA variant homozygotes. Animals homozygous for the
C3H variant of α7 also had an increased seizure frequency
(32.4%) compared with the α7 heterozygotes (18.2%) and the
α7 DBA variant homozygotes (4%). However, unlike
the a5 heterozygotes, the a7 heterozygotes had a seizure frequency midway between the two homozygous populations.

A comparison of overall seizure sensitivity score with genotype gave similar results (fig. 3). C3H-like homozygotes had significantly higher seizure frequency than their heterozygous counterparts (a5: 3.16 ± 0.17; a7: 2.88 ± 0.17) or their DBA-like homozygotes (a5: 2.22 ± 0.14; a7: 2.38 ± 0.16) or their DBA-like homozygous counterparts (a5: 2.02 ± 0.2; a7: 2.04 ± 0.2) for both a5 and a7. Once again, heterozygotes for the a5 rflp had an average sensitivity score very similar to that of the a5 DBA-like homozygotes, and a7 heterozygotes had an average score midway between those of the two a7 homozygous populations.

When the a5 and a7 genotypes of the animals were combined and compared with seizure frequency and overall nicotine sensitivity, the two nAChR loci appeared to act in combination relative to these measures. For example, animals homozygous for the C3H variant of both a5 and a7 rflps exhibit a higher seizure frequency (54.5%) and average sensitivity score (3.54 ± 0.16) (fig. 4) than did those animals homozygous for the C3H variant of either a5 (40%; 3.16 ± 0.17) or a7 (32.4%; 2.88 ± 0.17) alone (compare table 1 with table 2 and fig. 3 with fig. 4). In addition, animals homozygous for the DBA variant of both nAChR loci had a lower seizure frequency (no animals seized) than animals homozygous for the DBA variant of a5 (7.7%) or a7 (4%) alone. Although the overall sensitivity score for the combined DBA-like homozygotes (1.83 ± 0.4) was lower than that of animals homozygous for the DBA variant of a5 (2.02 ± 0.2) or a7 (2.04 ± 0.2) alone, the difference was not statistically significant.

**Comparison of α-BTX binding levels in brain with nAChR subunit genotype and nicotine sensitivity.** Previous findings have indicated that sensitivity to the convulsant effects of nicotine may not correlate with levels of α-BTX binding in the hippocampus in F2 animals even though the two measures do significantly correlate across populations and between inbred mouse strains (Miner et al., 1985). In addition, a7 genotype has been shown to exhibit a significant effect on levels of α-BTX binding in several brain regions including hippocampus (Stitzel et al., 1996). Therefore, levels of α-BTX binding were measured in four brain regions (colliculi, hippocampus, hypothalamus and striatum) and compared with a5 genotype, a7 genotype, seizure frequency and sensitivity score for those animals tested for their high-dose nicotine sensitivity to nicotine. As previously demonstrated, a7 genotype was significantly associated with levels of α-BTX binding in hippocampus (F(2,118) = 10.96, P < .0001), colliculi (F(2,119) = 6.36, P < .005) and striatum (F(2,118) = 6.29, P < .005) (fig. 5A). No significant relationship between a5 genotype and levels of α-BTX binding was observed (fig. 5B).

In accordance with an earlier study by Miner et al. (1985), levels of α-BTX binding were not significantly different in the hippocampus or any other brain region between the F2 animals that had seizures and those that did not after a challenge dose of nicotine (fig. 6). Moreover, there was no significant relationship between α-BTX binding levels in any brain region and nicotine sensitivity scores (fig. 7).

**Discussion**

This study demonstrated an association between sensitivity to the high-dose effects of nicotine, as measured by seizure frequency and overall sensitivity score, and polymorphisms for the nAChR subunit genes, a5 and a7, in F2 animals.
derived from a cross between C3H/2ibg and DBA/2ibg mice. The α5 genotype appears to have a dominant effect relative to the sensitivity measures; α5 heterozygotes exhibit seizure frequencies and overall sensitivity scores that are indistinguishable from the DBA variant homozygotes. On the other hand, α7 heterozygotes had seizure frequencies and average sensitivity scores intermediate between those of animals of the homozygous α7 genotypes. This result indicates that the inheritance of high-dose sensitivity with respect to α7 genotype is additive. Together, the α5 and α7 genotypes act in combination with respect to nicotine sensitivity. The observation that dominance and additive effects are seen with the α5 and α7 genotypes, respectively, is consistent with previous classic genetic cross studies that established that the inheritance of sensitivity to nicotine-induced seizures has both additive and dominance components (Miner et al., 1984). To the best of our knowledge, this is the first study to find linkage between specific genetic loci and sensitivity to nicotine.

This study also confirmed the linkage of nAChR α7 subunit genotype with levels of α-BTX binding in several brain regions, which suggests that the strain-specific variants of the α7 gene have a significant influence on determining levels of α-BTX binding in a brain region-specific fashion.

No correlation between levels of hippocampal α-BTX binding and nicotine-induced seizure sensitivity was observed in this study. This result is in agreement with a previous study by Miner et al. (1985) that indicated that such a relationship does not exist in F2 animals even though a relationship is seen across populations and between inbred mouse strains.

An explanation for the apparent correlation between nicotine-induced seizure sensitivity and levels of hippocampal α-BTX binding across populations but not among individuals is that the two measures are controlled by different genes that are linked. However, it is possible for seizure susceptibility and levels of α-BTX binding to be linked to the same gene if one of the measures is influenced by a second gene that does not affect the other measure. This is, in fact, what we observe for high-dose sensitivity and binding levels of α-BTX. Both measures are linked to the α7 locus, whereas only the high-dose sensitivity measures are linked to the α5 locus. Moreover, there is a significant effect of α5 genotype on seizure frequency and sensitivity score among animals of the same α7 genotype; the seizure frequency and average sensi-
are linked to seizure susceptibility raises two questions: Does nicotine sensitivity score for animals of a given nicotine-induced seizure sensitivity vary significantly depending on the genotype of the animal (table 2; fig. 4). No such effect of \( \alpha_5 \) genotype is seen for levels of \( \alpha \)-\( \beta \)-TX binding (data not shown).

The observation that both \( \alpha_5 \) and \( \alpha_7 \) nAChR subunit genes are linked to seizure susceptibility raises two questions: Does nicotine-induced seizures in F2 animals, but only in one subregion of the hippocampus (out of 13 subregions examined), the ventral portion of the stratum lacunosum-moleculare of the CA1. Therefore, the association between nicotine-induced seizure sensitivity and levels of \( \alpha \)-\( \beta \)-TX binding may exist in F2 animals, but only in the hippocampal subregion where \( \alpha_5 \) and \( \alpha_7 \) are co-localized. These observations suggest that nicotine-induced seizures may be mediated by a novel \( \alpha_5/\alpha_7 \) heteromeric nAChR or by two distinct nAChRs: a heteromeric nAChR containing \( \alpha_5 \) and a homomeric/heteromeric nAChR containing \( \alpha_7 \). If \( \alpha_5/\alpha_7 \) hetero-oligomeric nAChR does exist and does mediate nicotine-induced convulsions, it may have a pharmacology distinct from that of classic neuronal \( \alpha \)-\( \beta \)-TX-sensitive nAChRs because nicotine-induced seizures do not appear to be blocked by methyllycaconitine (Gasior et al., 1996). The possibility that co-expression of an \( \alpha_5 \) subunit with the \( \alpha_7 \) subunit may lead to the formation of a hetero-oligomeric nAChR with unique pharmacology and function is not implausible, given the recent finding that the \( \alpha_5 \) nAChR subunit can alter the pharmacology and electrophysiological properties of an \( \alpha_4/\beta_2 \) nAChR subtype when co-expressed with these nAChR subunits in vitro (Ramirez-Latorre et al., 1996).

The lack of inhibition of nicotine-induced seizures by MLA may also indicate that the convulsions induced by nicotine are the result of inactivation/desensitization, rather than activation, of the affected nAChR by nicotine. In fact, MLA alone will induce seizures very similar in appearance to nicotine-induced seizures (P. Dobelis, unpublished data). In an effect consistent with the idea that nicotine-induced seizures may be due to inactivation/desensitization of the \( \alpha \)-\( \beta \)-TX-sensitive nAChR, \( \alpha \)-\( \beta \), the classic antagonist of this subtype of nAChR, induces seizures when administered intracerebroventricularly (Cohen et al., 1981).

Even though the nAChR subunit genes for both \( \alpha_5 \) and \( \alpha_7 \) are linked to sensitivity to the high-dose effects of nicotine, we cannot rule out the possibility that genes linked to these nAChR genes, and not the \( \alpha_5 \) and \( \alpha_7 \) nAChR genes themselves, are responsible for the associations between nAChR genotype and nicotine sensitivity. Judging on the basis of physical maps from chicken, rat and human, \( \alpha_5 \) is part of a gene cluster that includes the nAChR \( \alpha_3 \) and \( \beta_4 \) genes (Courtier et al., 1990; Boulter et al., 1990; Raimondi et al., 1992). Consequently, if such a cluster were to exist in mice also, it would be impossible to rule out the involvement of either or a native neuronal nAChR exist that is made up of these two nAChR subunits? And if so, does this subtype of nAChR mediate nicotine-induced seizures? RNAs for both subunits are found in the hippocampus (Wada et al., 1990; Marks et al., 1992; Marks et al., 1996), the brain region where nicotine-induced seizures are believed to be initiated (Dunlop et al., 1960; Floris et al., 1964; Brown, 1967; Stumpf and Gogolak, 1967). \( \alpha_7 \) RNA is localized in the pyramidal cell layers CA1, CA2 and CA3 and in the polymorphic areas of the dentate gyrus, whereas \( \alpha_5 \) RNA is restricted to the pyramidal cell layer of CA1. Thus overlap in the localization of \( \alpha_5 \) and \( \alpha_7 \) RNAs occurs in the CA1 region of the hippocampus, a region that is especially prone to excitotoxicity due to epileptiform activity (Meldrum, 1991). In addition, a preliminary quantitative autoradiographic study (L. Caton, unpublished data) indicates that nicotine-induced seizure sensitivity may, in fact, be correlated with levels of \( \alpha \)-\( \beta \)-TX binding and overall nicotine sensitivity score, as described in "Materials and Methods." Animals were separated according to overall sensitivity score, and sensitivity score vs. levels of \( \alpha \)-\( \beta \)-TX binding were subsequently determined in four brain regions of these animals as described in "Materials and Methods."
both of these nAChR genes in regulating sensitivity to the high-dose effects of nicotine. This is especially true in light of the fact that a small subpopulation of hippocampal neurons appear to express a receptor that has a pharmacology quite similar to that of α3β4-containing nAChRs (Alkondon and Albuquerque, 1993).

Recently, it has been shown that a mutation in the nAChR α4 subunit gene is linked to nocturnal frontal lobe epilepsy in humans (Steinleit et al., 1995). This finding suggests that nicotinic cholinergic systems may play a role in at least some forms of epilepsy. Whereas the data described in the present study demonstrate that the nAChR α5 and α7 subunit genes are associated with nicotine-induced convulsions, other studies that sought to identify loci that are linked to epilepsy in mouse models detected loci proximal to both the nAChR α5 and α7 genes. Frankel et al. (1995) found a single locus, termed sfz1, linked to handling-induced convulsions. This locus is mapped to within 2 centiMorgans of the nAChR α7 subunit gene on chromosome 7. An investigation by Rise et al., (1991) described a locus, E1I, that is linked to seizure susceptibility. This locus maps to within 4 centiMorgans of the nAChR α3 locus on chromosome 9, which implicates both α5 and β4 as a result of linkage. Thus, in addition to being linked to nicotine-induced seizure sensitivity, the nAChR α5 and α7 subunit genes may be considered as candidates for genes that regulate general epileptic activity. The α7 nAChR subunit is particularly intriguing in light of the observation that mice engineered to lack a functional α7 subunit have epileptiform-like wave patterns in hippocampus (Orr-Urtreger et al., 1996).

Although polymorphisms have been identified for the nAChR α5 and α7 subunit genes and these polymorphisms were found to be linked to sensitivity to the high-dose effects of nicotine, the exact nature of the polymorphism is not yet known for either gene. A point mutation in α7 between C3H/2Ibg and DBA/2Ibg mice has been identified in the protein-coding portion of the gene, but this mutation does not alter the amino acid sequence (Stitzel et al., 1996). However, mutations need not alter the open reading frame of a gene to have a profound effect on behavior. For example, the mouse mutant spastic, which is a prototype of inherited myoclonus, is due to the insertion of a LINE-1 element into intron 6 of the β subunit of the glycine receptor (Kingsmore et al., 1994). Efforts are under way to identify the region or regions of the α7 gene that are polymorphic between C3H/2Ibg and DBA/2Ibg mice. A murine nAChR α5 cDNA has recently been cloned (J. Stitzel, unpublished data), and RNAs from C3H/2Ibg and DBA/2Ibg mice are being investigated for differences in α5 sequence. Identification and characterization of the polymorphic region of each gene is essential to determine whether variations in these genes are, in fact, involved in determining strain-specific differences in sensitivity to nicotine.

References


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