Potential Role of the α4 and α6 Nicotinic Receptor Subunits in Regulating Nicotine-Induced Seizures¹

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ABSTRACT

Several studies have shown that genetic factors influence sensitivity to nicotine-induced seizures in the mouse. We used recombinant inbred (RI) strains derived from the Long-Sleep (LS) and Short-Sleep (SS) mouse lines to assess the possibility that polymorphisms associated with one or more of the nicotinic receptors cosegregate with differential sensitivity to nicotine-induced seizures. Restriction fragment length polymorphisms (RFLPs) associated with the α2, α3, α4, α5, and α6 nicotinic receptors were identified in the LS and SS mouse lines, but the RI strains were polymorphic for only the α4 and α6 RFLPs. The RI strains were tested for sensitivity to nicotine-induced seizures. Strain and gender effects on seizure sensitivity were obtained as assessed by ED₅₀ values and latency to seizure. Those RI strains with the LS-like α4 RFLP were, on average, more sensitive to nicotine-induced seizures than were those RI strains with SS-like α4 RFLP. The α6 nicotine receptor may also play a role in modulating nicotine-induced seizures, but this effect is markedly influenced by gender. Females of the RI strains with the LS-like α6 RFLP were more sensitive to nicotine than were females of the strains with the SS-like α6 RFLP. Similar trends were seen in the males, but these trends were not significant. Thus, these strain differences may be due to polymorphisms associated with both the α4 and α6 nicotinic receptors, but gender also plays an important role in regulating sensitivity to nicotine-induced seizure.

A very high percentage (80–90%) of alcoholics smoke, whereas 25 to 30% of all adult Americans are smokers (Batel et al., 1995). Unfortunately, very few studies have attempted to provide an explanation for this often-made observation. One possibility relates to the finding that both alcoholism and tobacco (nicotine) abuse appear to be regulated, in part, by genetic factors (see Heath and Madden, 1995, for a recent review), and recent reports suggest that one or more of the genes that influence alcohol use also influence tobacco use (Madden et al., 1995, 1997). Thus, it may be that the co-use and abuse of alcohol and nicotine has a genetic basis. Sensitivity to one or more of the behavioral effects of alcohol may be an important genetically determined factor that influences the development of alcoholism (Moss et al., 1989; Schuckit and Smith, 1996). Apparently, individuals who are less sensitive to the intoxicating effects of alcohol are more likely than alcohol-sensitive individuals to become alcoholics by their early 30s. Similarly, several studies have suggested that sensitivity to the behavioral and physiological actions of nicotine influences whether experimentation with tobacco progresses to tobacco abuse (reviewed in Pomerleau, 1995). These results suggest that identifying the factors that influence sensitivity to alcohol and nicotine may help identify the genes that regulate addiction to alcohol and tobacco.

A series of studies carried out in our laboratory have attempted to determine, using rodent (mouse and rat) models, whether common genes regulate sensitivity to alcohol and nicotine. Most of these studies assessed the sensitivity to nicotine of two mouse lines that were selectively bred for differential sensitivity to the depressant effects of alcohol (de Fiebre et al., 1987, de Fiebre and Collins, 1992). These two mouse lines, designated Long-Sleep (LS) and Short-Sleep (SS), were selectively bred for differences in duration of ethanol-induced loss of the righting response or sleep-time (McClearn and Kakihana, 1973). Outbreeding was maintained as the LS and SS mice were being selectively bred. One outcome of this procedure is that the ethanol-sensitive LS mice should differ from the ethanol-resistant SS mice at the genes that affect the duration of ethanol-induced sleep time. Thus, asking whether the LS and SS mice also differ in sensitivity to nicotine addressed the question of whether one or more of the genes that influence sensitivity to the anesthetic effects of alcohol also influence sensitivity to nicotine. The LS mice are slightly more sensitive than the SS mice to several behavioral and physiological effects produced by

ABBREVIATIONS: LS, Long-Sleep; SS, Short-Sleep; RI, recombinant inbred; RFLP, restriction fragment length polymorphism; GABA, γ-aminobutyric acid.
nicotine injection (de Fiebre et al., 1987; de Fiebre and Collins, 1992). This difference in sensitivity is not likely due to differences in nicotine metabolism because no overall differences in nicotine metabolism were found between the LS and SS mice (de Fiebre et al., 1987). Consequently, it may be that the differential sensitivity of the LS and SS mice is due to differences in neuronal sensitivity to nicotine. However, modest gender differences in nicotine disposition were found in both the LS and SS mice, which may explain why females of both lines are more sensitive to nicotine than are the males (de Fiebre et al., 1987).

It is well established that the actions of nicotine are initiated by binding to nicotinic cholinergic receptors. Nicotinic receptors are found at the skeletal neuromuscular junction, in autonomic ganglia, and in the brain and spinal cord. Ten genes have been cloned and sequenced that encode for neuronal nicotinic receptors (reviewed in Lindstrom, 1997). In situ hybridization studies indicate that some of the subunits are likely to be expressed in only a few brain regions (e.g., α2, α5, α6, β3, and β4), whereas others are found throughout the brain (α4, α7, and β2).

In the results reported here, the LS and SS mice were screened for polymorphisms in all of the nicotinic receptor subunit genes, except α9 and β3, using the restriction fragment length polymorphism (RFLP) approach. Polymorphisms were found in several of the genes. The potential role of these polymorphisms in regulating sensitivity to the seizure-inducing effects of nicotine was evaluated by comparing the segregation pattern of the RFLP with sensitivity to nicotine-induced seizures using the recombinant inbred (RI) strains that were derived from the LS and SS mice (de Fries et al., 1989).

Materials and Methods

Animals. LS and SS mice and the 26 surviving RI strains derived from them were maintained at the specific pathogen-free mouse colony at the Institute for Behavioral Genetics. The RI strains were derived by crossing the LS and SS mice to yield the F1 generation of animals, which were then crossed to yield the F2 generation. Forty sibling pairs from the F2 population were chosen at random to serve as the progenitors for the RI strains. Only 26 of the strains were available at the time this project was started. Mice were maintained on a 12-h light/dark cycle (lights on between 7:00 AM and 7:00 PM) and were tested between 60 and 90 days of age, and approximately equal numbers of each gender were tested.

Behavioral Testing. For seizure testing, the RI animals were injected i.p. with nicotine. Nicotine (base) was dissolved in isotonic saline. Concentrations were adjusted so that each dose was injected in a volume of 0.01 ml/g. Each animal was tested only once. The mean ED50 values of the RI strains that have the LS-like RFLP with the samples were genotyped by using the Southern Transfer and Hybridization. Genomic DNA was digested with 10 to 20 U of the appropriate restriction endonuclease and electrophoresed on an 8% agarose gel. The restriction enzymes that were tested include AvaI, BamHI, BglII, BgIII, DraI, EcoRI, EcoRV, HaeIII, HincII, HindIII, Hinfl, KpnI, MboI, NcoI, NotI, PstI, PvuII, RsaI, SacI, SfiI, StuI, TaqI, and XbaI. The gel was subsequently transferred to a nylon membrane (Gene Screen Plus; New England Nuclear, Boston, MA) 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 covalently link the DNA to the membrane. Membranes were prehybridized at 35 min at 65°C in Rapid Hyb (Amersham Corp, Arlington Heights, IL) hybridization solution (0.2 ml/cm²). A radiolabeled [α-32P]dCTP (New England Nuclear) full-length probe was generated by a random priming method (Feinberg and Vogelstein, 1983) for each of the subunits tested, using a commercially available kit (Decaprise II; Ambion, Austin, TX), and subsequently added (2 ng/ml) to the solution for α2, α3, α4, α5, α6, and β4 probes, rat cDNAs, generously provided by Dr. Jim Boulter (University of California at Los Angeles), were cut from their respective vectors and gel purified before use. The α7 and β2 probes were derived from gel-purified mouse cDNA clones. After a 3-h hybridization at 65°C, membranes were washed at increasing stringencies until the background was not detectable with a Geiger counter. For most experiments, the final wash was at 65°C and consisted of 0.5 X SSC (1 X SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0) and 0.1% SDS. Membranes were then exposed to X-ray film (Kodak XAR-5) at −70°C with an exposure screen for 1 to 7 days. After exposure, films were evaluated by two independent observers for RFLPs. Each nicotinic receptor subunit cDNA probe produced a unique hybridization pattern. This indicates that the probes did not cross-hybridize with the other nicotinic receptor subunits. All potential RFLPs were confirmed by repeating the Southern analysis.

Data Analysis. The effective dose that produced seizures in 50% of the animals (ED50 value) was calculated for each strain using a regression line comparison program (Diem and Lentner, 1970). The ED50 and latency to seizure data were analyzed initially using a 3-way ANOVA that tested for significant overall effects of strain, gender, and dose. The potential effect of genotype (RFLP status) on seizure sensitivity was assessed by comparing the mean ED50 values of the RI strains that have the LS-like RFLP with the mean ED50 values of the RI strains that have the SS-like RFLP using a t test. Only 22 of the 26 RI strains were genotyped because four of the strains were lost between the time of behavioral testing and time of genotyping. The strains were lost because of reduced fertility, and unfortunately, we did not retain DNA samples so that the genotypic status of the RI animals was not evaluated initially using a 3-way ANOVA that tested for significant overall effects of strain, gender, and dose. The potential effect of genotype (RFLP status) on seizure sensitivity was assessed by comparing the mean ED50 values of the RI strains that have the LS-like RFLP with the mean ED50 values of the RI strains that have the SS-like RFLP using a t test. Only 22 of the 26 RI strains were genotyped because four of the strains were lost between the time of behavioral testing and time of genotyping. The strains were lost because of reduced fertility, and unfortunately, we did not retain DNA samples so that the genotypic status was used in a variety of the method of Falconer (1981). The number of genes is conventionally calculated using the equation: gene number = V/Va. Under normal circumstances, the difference between the values for the parental strains is used to calculate the R value. However, one of the assumptions that is made when this equation is used is clearly violated in the current study. Specifically, the assumption that one parental strain should have all of the genes that promote a high phenotypic value and the other parental strain should have all of the
genes that promote a low phenotypic value is violated because the LS and SS mice were not at the extremes for seizure sensitivity. Consequently, the RI strains that are at the extremes for seizure sensitivity, as measured by ED50 values, were used to calculate the R value. An estimate of the additive variance (V_A) was made using the method of DeFries et al. (1989). Specifically, V_A was taken as half of the variance of the RI strain means.

Results

Figure 1 presents dose-response curves for nicotine-induced seizures obtained from the parental LS and SS lines and four of the RI strains (genders combined). The RI strains selected represent the phenotypic extremes. A dose-dependent increase in the fraction of animals that showed a clonic seizure after i.p. nicotine injection was observed in all of the strains. ED50 values for nicotine-induced seizures were calculated for both genders in each of the strains and are listed in Table 1. No significant difference in seizure sensitivity, as measured by ED50 value, was observed between the LS and SS mice. However, a significant overall effect of strain (F25,51 = 2.36, P < .05) was observed in the RI strains. In addition, an overall effect of gender (F1,51 = 7.70, P < .01) was observed; the females were more sensitive to the seizure-inducing effects of nicotine. As depicted in Fig. 2, the RI strains showed a wide range in seizure sensitivity, as measured by ED50 values, for both females (left) and males (right). A nearly 3-fold difference in ED50 values was observed between the most sensitive and the least sensitive strains. The strain distribution pattern for the ED50 values also approximates a normal distribution for both sexes. The gene estimate obtained (gender combined), using the methods described in Data Analysis, is 5.04. Thus, sensitivity to the seizure-inducing effects of nicotine is a polygenically regulated trait.

The time between nicotine injection and emergence of a clonic seizure (seizure latency) was also measured in the LS, SS, and RI animals. The seizure latencies in the LS and SS mice were 229.4 ± 16.9 (LS) and 268.5 ± 14.6 (SS) after 3.0 mg/kg. LS–SS results obtained with 3.5 and 4.0 mg/kg are presented in Table 1 along with results obtained for the RI strains after the 3.5 and 4.0 mg/kg doses (the only doses that were used in all of the strains). A significant LS–SS difference in seizure latency (LS more sensitive than SS) was observed when the data obtained using the 3.0, 3.5, and 4.0 mg/kg doses (the three doses that were tested in both mouse lines) were analyzed (F1,162 = 4.54, P < .05).

Figure 3 presents an analysis of the relationship between seizure latency after a nicotine injection of 3.5 or 4.0 mg/kg and ED50 values for the RI strains. The 3.5 and 4.0 mg/kg doses were used for this analysis because these were the only test doses that were used in all of the RI strains. Seizure latency was significantly affected by strain (F24,1188 = 9.59, P < .001), gender (F1,1188 = 22.15, P < .001), and dose (F1,1188 = 87.45, P < .001). The relationship between seizure latency and ED50 in females is presented in the left panel of Fig. 3, whereas the right panel depicts the data obtained with males. Females had a shorter latency to seize than males, and the latency was shorter after treatment with the higher (4.0 mg/kg) nicotine dose than it was after injection with 3.5 mg/kg. A significant strain × gender interaction was also detected (F24,1188 = 1.82, P < .01). Significant correlations between seizure latencies and ED50 values were obtained in both genders at both doses (all P values <.001). Thus, ED50 values and seizure latencies provide similar measures of seizure sensitivity. The number of genes that regulate latency to seizures (gender combined) was estimated as 2.64 for the 3.5 mg/kg dose and as 3.57 for the 4.0 mg/kg dose.

To determine whether the parental LS and SS lines exhibit RFLPs for nicotinic receptor genes, DNA isolated from the LS and SS mice was screened for RFLPs in the α2, α3, α4, α5, α6, α7, β2, and β4 using a panel of 24 restriction endonucleases. Table 2 presents these results. Although RFLPs were detected between the LS and SS for the α2, α3, α4, α5, and α6 nicotinic receptor subunit genes, only the α4 and α6 RFLPs were found in the RI strains. The genotype of the α4 and α6 subunit genes for each of the RI strains was designated as either LS-like or SS-like depending on its RFLP status. Figure 4 provides an illustration of the RFLPs detected in the LS and SS mice for the α4 and α6 genes.

The relationships between the α4 RFLP and ED50 values for nicotine-induced seizures are presented in Fig. 5. Figure 5 (top) depicts the strain distribution pattern for the α4 RFLP contrasted against the ED50 value for nicotine-induced seizures in females, and the bottom panel presents the results obtained with males. In both genders, most of the strains with a low ED50 value for nicotine-induced seizures have the LS α4 RFLP, whereas most of the RI strains with a high ED50 value have the SS α4 RFLP. Figure 5 (insets) presents a comparison of the mean seizure ED50 across α4 genotype. The mean ED50 value in males of those strains with the LS α4 RFLP (3.84 ± 0.27 mg/kg) is significantly lower than the mean ED50 value of those strains with the SS α4 (4.64 ± 0.27 mg/kg) (t22 = 2.10, P < .05). In females, those strains with the LS α4 genotype have a mean ED50 value of 3.17 ± 0.21 mg/kg, whereas the mean ED50 obtained in those strains with the SS α4 genotype is 3.73 ± 0.26 mg/kg. This apparent difference is not significant (t22 = 1.65, P = .12).

The effect of α4 RFLP on seizure latency is presented in Fig. 6. A significant effect of genotype (α4 RFLP) was observed at both the 3.5 mg/kg (F1,609 = 9.79, P < .01) and 4.0 mg/kg (F1,598 = 5.84, P < .02) doses of nicotine. The LS α4 RFLP led to a shorter seizure latency in both sexes at both nicotine doses. In addition, gender also affected seizure latency at both doses (3.5 mg/kg: F1,609 = 14.69, P < .001; 4.0

Fig. 1. Dose-response analysis of nicotine-induced seizure sensitivity showing the percentage of animals that exhibited seizures at various nicotine doses. Seizure sensitivity for the parental LS and SS mouse lines as well as the four RI strains that represent the phenotypic extremes (strains 5, 10, 20, and 23) are shown. On average, 26 animals (13 males and 13 females) were tested per strain per dose (n = 3449).
TABLE 1

Measures of LS – SS and RI strain seizure sensitivity

ED₅₀ values and latency to seizure were determined as described in the text. If an animal failed to convulse within 300 s after injection, a latency of 300 was assigned to that animal. Each value represents the mean ± S.E. In most cases, 13 animals of each sex were tested at each nicotine dose.

<table>
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<tr>
<th>Strain</th>
<th>ED₅₀ Latency (3.5 mg/kg)</th>
<th>Male</th>
<th>Female</th>
<th>ED₅₀ Latency (4.0 mg/kg)</th>
<th>Male</th>
<th>Female</th>
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<td>mg kg</td>
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<td></td>
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<td>204 ± 33</td>
<td>207 ± 28</td>
<td>209 ± 35</td>
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<td>231 ± 27</td>
<td>119 ± 16</td>
<td>184 ± 27</td>
<td>112 ± 15</td>
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<td>120 ± 25</td>
<td>84 ± 19</td>
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<td>224 ± 24</td>
<td>207 ± 27</td>
<td>172 ± 25</td>
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<td>300 ± 0</td>
<td>271 ± 16</td>
<td>243 ± 57</td>
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<tr>
<td>6</td>
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<td></td>
<td>282 ± 19</td>
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<td>143 ± 28</td>
<td>66 ± 8</td>
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<td>247 ± 24</td>
<td>169 ± 23</td>
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<tr>
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<td>128 ± 23</td>
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<td>165 ± 28</td>
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<td>SS</td>
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<td>221 ± 26</td>
<td>192 ± 25</td>
<td>143 ± 19</td>
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Fig. 2. Distribution pattern of the ED₅₀ values for nicotine-induced seizures for the RI strains and the parental LS and SS mouse lines. Nicotine-induced seizure sensitivity as measured by ED₅₀ values for the fraction of animals exhibiting clonic seizures is depicted for both females and males of the 26 RI strains as well as the LS and SS lines. ED₅₀ values were subdivided into 0.5 mg/kg ranges. The numbers plotted within each range are the RI strain numbers. Note that only 26 of the original 40 strains were tested; the other strains had died out generally due to reduced fertility.

mg/kg: $F_{1,586} = 7.77$, $P < .01$; the latency to seize was less in females.

Figure 7 provides the strain distribution pattern for the α6 RFLP contrasted against ED₅₀ value. The top panel of this figure presents the data obtained with the females, and the bottom panel presents the data obtained with the males. In females, the LS α6 RFLP was found primarily in those RI strains that were less sensitive (higher ED₅₀ values) to nicotine-induced seizures. This is demonstrated in Fig. 7 (top inset), where the mean ED₅₀ values for the LS and SS α6 genotypes are presented. Females with the LS α6 genotype had a mean ED₅₀ value of 3.84 ± 0.22 mg/kg, whereas females of those strains with the SS α6 genotype had a mean ED₅₀ value of 2.89 ± 0.20 mg/kg. This difference is significant ($t_{21} = 3.22$, $P < .01$). The effect of the α6 genotype was not significant in males. The mean ED₅₀ value in males with the LS α6 genotype was 4.39 ± 0.29 mg/kg, whereas the mean of males with the SS genotype is 4.03 ± 0.29 mg/kg ($t_{21} = 0.90$, $P = 0.38$).

The influence of α6 RFLP on seizure latency is shown in Fig. 8. A significant overall effect of genotype was detected at the 3.5 mg/kg dose ($F_{1,586} = 11.36$, $P < 0.001$). A main effect of gender was also observed ($F_{1,586} = 15.51$, $P < 0.001$). In addition, a significant genotype × gender interaction was detected ($F_{1,586} = 5.88$, $P < .05$). This interaction arises because no effect of α6 genotype was seen in males after injection with the 3.5 mg/kg nicotine dose. Significant effects of both genotype ($F_{1,582} = 23.16$, $P < .001$) and gender ($F_{1,582} = 9.42$, $P < .01$) were seen in those animals that were treated with the 4.0 mg/kg nicotine dose. Those RI strains that have the LS α6 RFLP tended to have a longer latency to seizures after nicotine injection than did those RI strains that have the SS α6 RFLP.

**Discussion**

Several earlier studies demonstrated that the LS mice are slightly more sensitive than the SS mice to nicotine-induced seizures, as measured by ED₅₀ values (de Fiebre et al., 1987; de Fiebre and Collins, 1992). A significant LS – SS difference in ED₅₀ values was not seen in the study reported here, but when seizure latency was used as the measure, the LS mice were more sensitive than the SS to the seizure-inducing effects of nicotine. The seizure sensitivities of the parental LS and SS lines were in the midrange of seizure sensitivity compared with the RI strains. This could occur only if the LS...
mice do not have all of the genes that result in a high sensitivity to nicotine-induced seizures and the SS mice do not have all of the genes that result in reduced sensitivity. The findings that the LS α4 RFLP is associated with increased sensitivity to seizures and the LS α6 RFLP is associated with reduced sensitivity to seizures are consistent with this conclusion.

Estimates of the number of genes that regulate seizure sensitivity yielded values that ranged from three to five. It must be recognized that these estimates are dependent on the validity of several assumptions (Falconer, 1981). One of these assumptions is that the genes act in an additive fashion without epistasis (gene-gene interaction). However, epistasis was detected by de Fiebre and Collins (1992) in a study that evaluated the seizure sensitivity of F1, F2, and backcross generations derived from the LS and SS mice. Consequently, the gene estimates reported here should not be taken literally and are likely to be an underestimate of the number of genes that regulate nicotine-induced seizures.

The mRNA for the α4 nicotinic receptor subunit is widely distributed in mouse brain (Marks et al., 1992; Marubio et al., 1999) and, in combination with the β2 subunit, makes up the vast majority of high-affinity agonist (e.g., [3H]nicotine) binding sites in brain (Whiting and Lindstrom, 1988; Picciotto et al., 1995; Marubio et al., 1999). Many, if not nearly all, of the α4β2-type nicotinic receptors are found on presynaptic nerve terminals (Wonnacott, 1997). They apparently modulate the release of the inhibitory neurotransmitter γ-aminobutyric acid (GABA; Léna and Changeux, 1997; Lu et al., 1998; Alkondon et al., 1999). As noted by Alkondon et al. (1997), desensitization of this receptor could result in a decrease in GABA release and cause convulsions by producing disinhibition. This postulate is supported by the observation that injection with the α4β2-selective nicotinic antagonist dihydro-β-erythroidine elicits seizures in the rat (Felix and Levin, 1997) and the mouse (Marubio et al., 1999). Moreover, a genetically determined form of epilepsy found in humans seems to be due to a mutation of the α4 nicotinic receptor (Steinlein et al., 1995). The mutation results in a receptor that desensitizes faster than does the wild-type receptor (Weiland et al., 1996). Thus, it may be that the association between the α4 RFLP and nicotine-induced seizure sensitivity is due to a difference in the α4 gene that leads to differences in GABAergic activity.

Females were slightly more sensitive to the seizure-inducing effects of nicotine as determined by both ED50 value and seizure latency. This might be due to differences in nicotine metabolism or distribution because both LS and SS females have higher brain levels of nicotine, after i.p. injection, than do the males (de Fiebre et al., 1987). However, two studies (Ke and Lukas, 1996; Bullock et al., 1997) have demonstrated that progesterone and some of its metabolites are allosteric inhibitors of several neuronal nicotinic receptors. If desensitization of the α4β2-type nicotinic receptor promotes nicotine-induced seizures, it may be that inhibition of this receptor by progesterone or one of its metabolites results in a functional equivalent of desensitization, thereby promoting seizures.

The α6 subunit is found principally in nuclei where dopaminergic neurons are found (Le Novère et al., 1996). Because nicotine stimulates the release of dopamine (Rowell et al., 1987; Rapier et al., 1988; Grady et al., 1997), it has been suggested, but not yet been demonstrated, that the α6 subunit is a member of the nicotinic receptor or receptors that modulate dopamine release (Le Novère et al., 1996). Dopamine plays a role in regulating the potency and efficacy of a broad array of seizure-inducing drugs (e.g., see Hoffman et al., 1997; Lindsey et al., 1998). Thus, it may be that a polymorphism associated with the α6 gene regulates nicotine-induced seizures by influencing the activity of dopaminergic neurons.

It is also possible that one or more genes that are tightly linked to these nicotinic receptor subunit genes is involved in
regulating the seizure response. Although it seems logical that variability in seizure sensitivity to nicotine-induced seizures might be due to variability in nicotinic receptors, RFLP analysis does not prove that a mutation associated with the marker gene (in this case, \( \alpha \) nicotinic receptor subunit genes) causes a difference in the amount or activity of protein derived from the gene. Further studies must be performed to

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**Fig. 4.** RFLPs for the nAChR \( \alpha \)4 and \( \alpha \)6 subunit genes between LS and SS mice. Autoradiograms of a Southern blot of LS and SS DNA digested with NciI (\( \alpha \)4), XbaI (\( \alpha \)6), or HincII (\( \alpha \)6) and probed with either a full-length \( \alpha \)4 cDNA fragment or a full-length \( \alpha \)6 cDNA fragment are shown.

**Fig. 5.** Association between the \( \alpha \)4 RFLP and nicotine-induced seizure \( ED_{50} \) values across the RI strains. The distribution pattern of seizure \( ED_{50} \) values for both females and males of the RI strains are shown along with their \( \alpha \)4 genotype. Thirteen strains carried the LS variant of \( \alpha \)4 (\( \bullet \)), whereas 9 strains carried the SS variant of the \( \alpha \)4 subunit gene (\( \triangle \)). Inset, effect of \( \alpha \)4 genotype on mean seizure \( ED_{50} \) values in the RI strains. LS, animal with the LS allele of the \( \alpha \)4 subunit gene as detected by NciI; SS, animals carrying the SS allele of the \( \alpha \)4 subunit gene as detected by NciI.

**Fig. 6.** Association between \( \alpha \)4 genotype and latency to nicotine-induced seizures. The RI strains were examined for latency to clonic seizures at 3.5 and 4.0 mg/kg, and the \( \alpha \)4 genotype of each strain was determined. The relationship between \( \alpha \)4 genotype and seizure latency was subsequently determined for each dose of nicotine for both male animals (M) and female animals (F). LS, LS allele of the \( \alpha \)4 subunit gene; SS, SS allele of the \( \alpha \)4 subunit gene.
sort out whether polymorphisms in the $\alpha 4$ or $\alpha 6$ genes or genes closely linked to them regulate the genetic influences in seizure sensitivity seen in the study reported here.

The LS and SS mice were selectively bred for differences in duration of loss of the righting response (sleep time) after i.p. injection of high doses of ethanol. The major cause of the LS – SS difference in response to the sleep-time inducing (anesthetic) effects of ethanol arises because of differential central nervous system sensitivity to ethanol, as demonstrated by the fact that the LS mice lose and regain the righting response at lower blood and brain concentrations of ethanol than do the SS mice (e.g., see Erwin et al., 1988; Finn et al., 1991). At least in theory, selective breeding should have led to a situation where the LS mice are isogenic (usually homozygous) for all of the genes that promote an increase in ethanol-induced sleep time and the SS mice should be isogenic for the genes that result in a decreased sensitivity to ethanol. Thus, the finding that the LS and SS mouse lines are isogenic for RFLPs associated with the $\alpha 4$ and $\alpha 6$ nicotinic receptor genes implicates a role for these nicotinic receptor subunits in regulating the anesthetic actions of ethanol. This suggestion should be accepted cautiously, however, because homozygosity might have been obtained as a consequence of unwanted inbreeding. If inbreeding resulted in fixation of either the $\alpha 4$ or $\alpha 6$ genes, these nicotinic receptor subunits would not play a role in regulating the many effects of alcohol.

Evidence obtained by others suggests that the $\alpha 4\beta 2$-type nicotinic receptor may be a critical site of action of alcohol. Ethanol, at concentrations less than 100 mM, enhances the nicotinic activation of $\alpha 4\beta 2$ receptors expressed in oocytes (Cardoso et al., 1999). Similarly, ethanol enhances electrical currents in rat cortical cells in culture (Aistrup et al., 1998; Marszalec et al., 1999). The currents affected by ethanol in rat cortical cells most probably arise as a consequence of activation of $\alpha 4\beta 2$-containing receptors. These provocative findings suggest that further studies of the role of the $\alpha 4$ nicotinic receptor subunit in regulating behavioral responses to both nicotine and ethanol are warranted.

Virtually nothing is known about the function of the $\alpha 6$ nicotinic receptor subunit, but it could be that the same property of the $\alpha 6$ receptor that makes an animal more sensitive to the seizure-inducing effects of nicotine makes the animal less sensitive to the behavioral effects of alcohol. The finding that the $\alpha 6$ nicotinic receptor subunit may be different in the LS and SS mice indicates that the potential role of this subunit in regulating the actions of alcohol should be evaluated.

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