GABAergic Systems Modulate Nicotinic Receptor-Mediated Seizures in Mice

PETER DOBELIS, SCOTT HUTTON, YING LU, and ALLAN C. COLLINS

Department of Pharmacology, University of Colorado Health Sciences Center, Denver, Colorado (P.D.); and Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado (S.H., Y.L., A.C.C.)

Received April 14, 2003; accepted June 10, 2003

ABSTRACT

The pharmacology of nicotinic receptor-mediated seizures was investigated in C3H mice. Eleven nicotinic agonists and six antagonists were administered centrally (i.c.v.). Epibatidine and ephedrine were the most potent agonists tested, whereas acetylecholine and the α7*-selective compounds 3-(2,4-dimethoxybenzylidene)-anabaseine (GTS-21) and anabaseine, were the least potent. Nicotine-induced seizures were blocked by co-treatment with either the nonselective antagonist mecamylamine or the α7*-selective antagonist methyllycaconitine. The α4β2*-selective antagonist dihydro-β-erythroidine was ineffective at blocking seizures. However, high doses of all six antagonists tested were fully efficacious in producing seizures, with d-tubocurarine being the most potent and mecamylamine the least potent. Potential relationships between nicotinic receptor-mediated seizures and drug effects on GABA function were also investigated. No correlation was seen between potencies of the agonists in producing seizures and stimulating [3H]GABA release or between potencies of the antagonists in producing seizures and antagonist inhibition of nicotine-stimulated [3H]GABA release. However, a robust correlation was detected between potencies of the agonists in producing seizures and the IC50 values for inhibition of nicotine-stimulated [3H]GABA release produced by agonist-induced receptor desensitization. We also compared inbred mouse strain sensitivity to nicotine, picrotoxin, bicuculline, and kainate-induced seizures. Robust positive correlations were revealed for nicotine-induced seizures and seizures induced by either picrotoxin or bicuculline, both GABA receptor antagonists. No correlation was found between nicotine-induced seizures and those induced by the excitatory amino acid receptor agonist kainate. Based on these findings, we present a model for nicotinic receptor-mediated seizures mediated through GABAergic systems.

Nicotine produces a variety of behavioral effects, some stimulant and others depressant. For example, high doses of nicotine cause seizures (Miner and Collins, 1989; Damaj et al., 1999), whereas lower doses of nicotine decrease or reduce anxiety (for review, see Picciotto et al., 2002). It is highly likely that most, if not all, of the effects of nicotine are mediated by nicotinic cholinergic receptors (nAChRs) because nAChR antagonists block many of nicotine’s effects. Mammalian brain expresses nine distinct nAChR subunit genes, designated α2 to α7 and β2 to β4, which are expressed in a regionally specific manner in the brain and spinal cord (for review, see Itier and Bertrand, 2001). This finding suggests that many different nAChR subtypes might exist, assuming that nAChRs expressed in the brain and spinal cord are, like the muscle-type nAChRs, composed of five subunits.

In recent years, many studies have been geared toward identifying the subunit compositions of the naturally occurring nAChRs. The two most abundant nAChRs are the α4β2* and α7* types, which bind nicotine and α-Bungarotoxin with high affinity, respectively (Buisson and Bertrand, 2002). Note that the * indicates the possibility that other subunits may be included in the native receptor as recommended by the International Union of Pharmacology nomenclature committee. The finding that multiple subtypes of nAChRs probably exist raises the question, Are specific nAChR subtypes involved in mediating each of the various effects of nicotine?

We have been especially interested in identifying which nAChRs modulate brain excitability, as measured by nicotine-induced seizures. In vivo electrophysiological recordings revealed that high doses of nicotine elicit seizures that originate in the hippocampus (Cohen et al., 1981). However, the possibility exists that nicotinic compounds elicit seizures in areas other than the hippocampus such as the thalamocortical pathways that may be involved in autosomal dominant nocturnal frontal lobe epilepsy, which is associated with poly-

ABBREVIATIONS. nAChR, nicotinic acetylcholine receptor; MLA, methyllycaconitine; ABT-418, (S)-(3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole; dTC, d-tubocurarine; GTS-21, 3-(2,4-dimethoxybenzylidene)-anabaseine; DHβE, dihydro-β-erythroidine; Ach, acetylcholine; TMA, tetramethylammonium; DMPP, dimethylphenylpiperazinium iodide.
morphisms in the α4 and β2 nAChR subunits (for review, see Raggenbass and Bertrand, 2002). Two strategies, genetic and pharmacological, have been used to identify the nAChR subtypes that mediate this response to nicotine. Early genetic data indicated the involvement of α7* receptors based on the finding that seizure sensitivity is significantly correlated with levels of α-bungarotoxin binding in the hippocampus of multiple inbred mouse strains (Miner and Collins, 1989). Subsequent studies found that this correlation persisted in F2 hybrids derived from two of the inbred strains (DBA/2 and C5H) that differ maximally in sensitivity to nicotine-induced seizures (Stitzel et al., 1998). However, studies done with mice with genetically modified α7 receptors have yielded confounding results: α7-null mutant mice show no difference in susceptibility to nicotine seizures (Franceschini et al., 2002), whereas mice engineered to express an α7 “gain of function” receptor exhibit an increased sensitivity to nicotine-induced seizures (Broido et al., 2002).

A recent report presented pharmacological data suggesting that nicotine-induced seizures result from increased glutamatergic synaptic transmission due to α7* receptor stimulation (Damaj et al., 1999). This interpretation may be correct, but it neglects the fact that α7* receptors are located on GABAergic interneuron cell bodies and nerve terminals (Frazier et al., 1998a,b; Alkondon and Albuquerque, 2001) in addition to the nerve terminals and distal dendrites of glutamatergic neurons (Ji et al., 2001). Furthermore, more recent genetic studies argue that nicotine can elicit seizures via effects on other nAChR subtypes. For example, we (Stitzel et al., 1998, 2000) found that polymorphisms associated with the α4, α5, and α6 subunits are associated with variability among mouse strains in nicotine-induced seizure sensitivity, and Fonck et al. (2003) recently reported that mice designed to express α4 gain of function nAChRs are exquisitely sensitive to nicotine-induced seizures. These reports suggest that nicotine may elicit seizures by modulating the activities of more than one nAChR subtype.

The following report extends the pharmacological analysis of nAChR-mediated seizures reported previously (Damaj et al., 1999) by determining the ability of additional nicotinic agonists to elicit seizures and by determining the ability of nicotinic antagonists to both block and induce seizures. We also compare the seizure-inducing ability of nicotinic agents to their effects on GABAergic function. Finally, we propose alternate explanations for the mechanism of these seizures.

Materials and Methods

Materials. Methyllycaconitine (MLA) citrate, (+)-anatoxin-α hydrochloride, and methylcarbachol chloride were purchased from Sigma/RBI (Natick, MA). ABT-418 was a gift from Abbott Diagnostics (Abbott Park, IL). Mecamylamine was a gift from Merck (Whitehouse Station, NJ). The following compounds were purchased from Sigma-Aldrich (St. Louis, MO): nicotine hydrogén (−)-tartrate (L-nicotine), d-tubocurarine (dTc), dihydro-β-erythroidine (DHβE) HBr, acetylcholine chloride (ACh), cytisine, (±)-epibatidine-L-tartrate, epiboxidine-chloride, tetramethylammonium (TMA) iodide, (±)-anabasine, dimethylphenylpiperazinium iodide (DMPP), decamethonium, and hexamethonium. All drug solutions were made fresh in 0.9% sterile saline the day of the experiment.

Animals. Male and female C3H/2 mice were used in the study. The animals were bred at the Institute for Behavioral Genetics and were housed five per cage with free access to food and water. The

Results

Agonist Pharmacology of Nicotinic Receptor-Mediated Seizures. The ability of 11 nicotinic receptor agonists to elicit seizures in adult (60–90-day-old) C3H/2 mice was tested. Figure 1 shows the seizure dose-response curves for these agonists. The data are presented as the percentage of mice experiencing clonic seizures on the right abscissa, and mean seizure scores for each dose are presented on the left abscissa. Drug doses are given on the ordinate. All 11 agonists induced seizures in at least 90% of the mice with the exception of cytisine. (±)-Epibatidine, and its analog epiboxidine, were the most potent, whereas ACh was the least potent of the agonists exhibiting full efficacy. Because anticho-
linesterases were not coadministered with ACh, its potency was likely to be underestimated due to hydrolysis decreasing the amount of ACh that reached the receptors. The $\alpha_7^*$-selective compound GTS-21 and its parent compound anabasine were also low in potency. ABT-418, a compound with high affinity for $\alpha_4\beta_2^*$ nAChRs and low affinity for $\alpha_7^*$ nAChRs (Arneric et al., 1994), was fully efficacious as a seizure-inducing agent, whereas cytisine was only partially effective in eliciting seizures with only 25% of the mice displaying clonic seizures. The pharmacological potency for agonist-induced seizures is listed in Table 1, along with the fatality rates (percentage of mice that died) at doses that induced clonic and or tonic seizures.

Nicotinic Antagonist Blockade of Nicotine-Induced Seizures. The ability of nicotinic agonists to elicit seizures suggests that activation of nicotinic receptors by high doses of agonists is proconvulsant. To test this assertion, we determined the ability of nicotinic antagonists to block nicotine-induced seizures. Figure 2 shows the results of these experiments. The nonselective nicotinic antagonist mecamylamine produced a dose-dependent blockade of nicotine-induced seizures with a dose of 100 $\mu$g/mouse blocking 100% of the seizures (Fig. 2A). The $\alpha_7^*$-selective antagonist MLA was ineffective at blocking nicotine-induced seizures when administered (i.c.v.) simultaneously with nicotine (Fig. 2B). However, when MLA was administered 3 min before (i.c.v.) to nicotine (5 mg/kg i.p.), it was partially effective at blocking seizures (Fig. 2B, inset). This difference may indicate that MLA and nicotine differ significantly in their ability to diffuse from the cerebral ventricles to the receptor sites. The $\alpha_4\beta_2^*$-selective antagonist DH$\beta$E was ineffective at blocking nicotine-induced seizures (Fig. 2C). These results confirm those obtained by Damaj et al. (1999).

Antagonist-Induced Seizures. Previous studies have shown that curare (dTC) and $\alpha$-bungarotoxin, when administered i.c.v., elicit seizures (Cohen et al., 1981; Stevens et al., 1995). Figure 3 shows the results of those experiments that assessed the potential seizure-inducing effects of six antagonists. All six antagonists that were tested were fully efficacious for inducing seizures. dTC was the most potent for eliciting seizures, whereas mecamylamine was the least potent. Except for dTC and mecamylamine, the ED$_{50}$ values for antagonist-induced seizures were very similar (Table 1). Mice given MLA began to exhibit seizure behavior at a dose (30 $\mu$g/mouse) just above that which produced partial blockade of nicotine-induced seizures. The $\alpha_4\beta_2^*$-selective antagonist DH$\beta$E, although ineffective at blocking nicotine-induced seizures, was fully efficacious in eliciting seizures. A major difference was observed between agonist- and antagonist-induced seizures: death was the usual outcome after
antagonist-induced seizures, whereas death was rare after agonist-induced seizures with the exception of ACh and anabasine.

**Table 1**

<table>
<thead>
<tr>
<th>Drug</th>
<th>ED₅₀ (µg/mouse)</th>
<th>Fatality Rate (Clonic and Tonic Seizures) %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agonists</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-Nicotine</td>
<td>3.23 (2.55, 4.08)</td>
<td>21</td>
</tr>
<tr>
<td>Epibatidine</td>
<td>0.026 (0.021, 0.032)</td>
<td>0</td>
</tr>
<tr>
<td>Epiboxide</td>
<td>0.11 (0.004, 0.3)</td>
<td>9</td>
</tr>
<tr>
<td>ABT-418</td>
<td>3.81 (1.67, 8.66)</td>
<td>18</td>
</tr>
<tr>
<td>ACh</td>
<td>330 (136, 798)</td>
<td>40</td>
</tr>
<tr>
<td>Methylcarbachol</td>
<td>4.22 (3.48, 5.13)</td>
<td>6</td>
</tr>
<tr>
<td>Cytisine</td>
<td>8.45 (6.84, 10.4)</td>
<td>0</td>
</tr>
<tr>
<td>(±)-Anabasine</td>
<td>55.7 (47.2, 65.7)</td>
<td>60</td>
</tr>
<tr>
<td>TMA</td>
<td>5.55 (4.40, 7.01)</td>
<td>6</td>
</tr>
<tr>
<td>DMPP</td>
<td>7.96 (5.84, 8.54)</td>
<td>0</td>
</tr>
<tr>
<td>GTS-21</td>
<td>60.6 (29.7, 122)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Antagonists</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLA</td>
<td>54.0 (49.9, 58.5)</td>
<td>100</td>
</tr>
<tr>
<td>Hexamethonium</td>
<td>41.5 (17.6, 98.2)</td>
<td>63</td>
</tr>
<tr>
<td>d-Tubocurarine</td>
<td>1.71 (1.38, 2.12)</td>
<td>100</td>
</tr>
<tr>
<td>Mecamylamine</td>
<td>631 (544, 733)</td>
<td>71</td>
</tr>
<tr>
<td>Decamethonium</td>
<td>49.9 (42.1, 59.1)</td>
<td>94</td>
</tr>
<tr>
<td>DHRE</td>
<td>63.7 (52.9, 76.7)</td>
<td>86</td>
</tr>
</tbody>
</table>

**Fig. 2.** Nicotinic antagonist blockade of nicotine-induced seizures. The ability of mecamylamine (A), DHβE (B), and MLA (C) to block seizures induced by an ED₅₀ dose of nicotine. Mecamylamine, coadministered with nicotine, blocked seizures, with 100 µg of mecamylamine/mouse producing 100% blockade. Coadministration of DHβE with nicotine was ineffective at blocking seizures (B). Pretreatment with DHβE was also ineffective at blocking seizures (data not shown). Coadministration of MLA with nicotine was ineffective at blocking seizures (C). However, when MLA was administered before nicotine, partial blockade was achieved (C, inset). Each point represents the average of eight mice.

aptosomes with agonist concentrations that desensitize nicotine-stimulated [³H]GABA release (Lu et al., 1999) and potencies of the agonists in producing seizures yielded a strong positive correlation (r = 0.93, p < 0.01; Fig. 4C).

**Fig. 5C.**

**Genetic Relationship between Nicotine-Induced Seizures and Seizures Induced by Other Convulsants.** Previous studies from our laboratory demonstrated that inbred mouse strains differ in sensitivity to nicotine-induced seizures (Miner and Collins, 1989). Inbred mouse strains also differ in sensitivity to seizures induced by convulsants acting through several different receptor systems (Kosobud and Crabbe, 1990; Kosobud et al., 1992). The relationship between nicotine-induced seizures and seizures induced by agents acting on GABAergic, and glutamatergic systems was determined by regression analysis of seizure sensitivity for the inbred mouse strains common to both studies. Data for nicotine-induced seizures were obtained from Miner and Collins (1989), and data for bicuculline-, picrotoxin- and kainate-induced seizures were obtained from Kosobud and Crabbe (1990). Comparison of nicotine-induced seizures with seizures induced by GABA₃ receptor antagonists revealed a significant positive correlation (bicuculline, r = 0.80, p < 0.01; picrotoxin, r = 0.68, p < 0.01; Fig. 5, A and B, respectively). However, comparison of nicotine-induced seizures with seizures induced by kainate (an excitatory amino acid agonist) detected no significant correlation (r = 0.39, p > 0.1; Fig. 5C).
Fig. 3. Dose-response relationships for nicotinic antagonist-induced seizures. All antagonists were administered i.c.v. The open symbols represent the seizure scores and are quantified on the left abscissa. The filled symbols represent the percentage of mice that displayed clonic seizures and are quantified on the right abscissa. Mice were observed for up to 5 min after injection, and the behavior was scored as described under Materials and Methods. Each point on the dose-response curves represents the average of eight mice.

Fig. 4. Relationship of nicotinic receptor-mediated seizures to nicotinic ligand effects on [3H]GABA release. The ED<sub>50</sub> values for agonist-induced seizures obtained in the present study were compared with the median values for nicotinic ligand effects on [3H]GABA release from synaptosomal preparations. The values for nicotinic ligand effects on [3H]GABA release were obtained from Lu et al. (1998, 1999). A, no significant relationship ($r = 0.48, p > 0.05$) exists between agonist potency for eliciting seizures and agonist potency for eliciting [3H]GABA release. Analysis of the relationship between antagonist-induced seizures and antagonist effects on [3H]GABA release also reveal no significant correlation ($r = -0.38, p > 0.05$). A robust positive relationship ($r = 0.93, p < 0.01$; C) was revealed in the comparison of agonist potency for desensitizing nicotine-stimulated [3H]GABA release with agonist potency of eliciting seizures.

Fig. 5. Genetic relationship between nicotine-induced seizures and seizures elicited by other convulsants. The sensitivity of several inbred mouse strains to seizures induced by nicotine was compared with their sensitivity to seizures elicited by the convulsants picrotoxin, bicuculline, and kainate. A and B, comparisons of seizures induced by nicotine with those induced by GABA<sub>A</sub> receptor antagonists picrotoxin and bicuculline, respectively. C, comparison of nicotine-induced seizures to seizures induced by the glutamate receptor agonist kainate. The data for nicotine-induced seizures were obtained from Miner and Collins (1989). The data for picrotoxin-, bicuculline-, and kainate-induced seizures was obtained from Kosobud and Crabbe (1990). The correlation coefficient for each relationship is given in the corresponding panel.
Discussion

All of the agonists tested, with the exception of cytisine, were fully efficacious in eliciting seizures. These findings, coupled with the antagonist results (MLA and mecamylamine blocked nicotine-induced seizures, whereas pretreatment with DHβE did not), extend the findings of Damaj et al. (1999) who concluded that nicotinic agonists induce seizures via an effect on α7* nAChRs. The conclusion that α7* nAChRs modulate seizures is consistent with the observations that nicotine-induced seizure sensitivity is correlated with levels of hippocampal α-bungarotoxin binding in genetically segregating mouse populations (Miner et al., 1984; Miner and Collins, 1989; Stitzel et al., 1998), and mice engineered to express an α7* gain of function mutation (L250T) are supersensitive to nicotine-induced seizures (Broide et al., 2002).

Damaj et al. (1999) proposed that the activation of α7* receptors located on glutamatergic nerve terminals is the major stimulus that results in nicotine-induced seizures. It has been shown that nicotine enhances glutamate release when applied to embryonic chicken medial habenula-interpeduncular nucleus cocultures (Mc Gee et al., 1995) and CA1 pyramidal neurons obtained from 14- to 24-day-old mice (Ji et al., 2001). However, the finding (Aramakis and Metherate, 1998) that α7* receptor enhancement of N-methyl-D-aspartate receptor-mediated synaptic transmission in rat sensory neocortex is seen only in tissue from p8-p19 postnatal rats suggests that an α7-glutamate hypothesis should be accepted with caution. Moreover, our finding that higher doses of MLA induced seizures, coupled with the observation that the α7*-selective antagonist α-bungarotoxin also elicits seizures (Cohen et al., 1981), suggests other, or additional mechanisms, may exist. The results obtained with ABT-418 support a role for α4β2-type receptors in modulating seizures. ABT-418 has a Kᵢ value of 3 nM for α4β2* nAChRs and a Kᵢ value of >10,000 nM for α7* nAChRs (Arneric et al., 1994). The finding that cytisine, a partial agonist for α4β2* receptors and a full agonist at α7* nAChRs ( Chavez-Noriega et al., 1997; Houlihan et al., 2001), was a partial agonist for seizures also argues that seizures may be generated via effects on α4β2* nAChRs. The cytisine findings suggest that a minimum level of α4β2* function may be required for seizures to occur. Alternatively, the rate of α4β2* desensitization is much slower for cytisine than other agonists (Lu et al., 1999). Thus, the resulting current through α4β2* receptors, although lower in peak amplitude, will be of greater duration and result in a greater total charge movement. This would most likely result in increasing the efficacy of GABAergic synaptic transmission by either increasing the probability of GABA release via increased α4β2* activity (increased depolarization and Ca²⁺ influx) on GABAergic nerve terminals, or by increasing the probability of action potential generation via increased α4β2* activity (depolarization) on GABAergic somata, or both.

The fact that GTS-21 produced seizures may implicate α7* receptors because it is a potent (although partial) agonist at these receptors; however, it also blocks α4β2* receptors at the same concentrations (de Fiebre et al., 1985). This fact suggests that blocking α4β2* receptors may elicit seizures and is supported by our finding that the α4β2*-selective antagonist DHβE, although ineffective in blocking nicotine-induced seizures, is fully efficacious in eliciting seizures.

Genetic evidence also argues for a role for α4β2* nAChRs in generating seizures. Stitzel et al. (2000) detected an association between a polymorphism in the mouse α4 nAChR gene and sensitivity to nicotine-induced seizures in a panel of recombinant inbred mouse strains, and Fonck et al., (2003) found that mice that express an α4 gain of function mutation are supersensitive to nicotinic agonist-induced seizures. The finding that α4 null-mutant mice have a lower threshold for pentylenetetrazol- and bicuculline-induced seizures (Wong et al., 2002), and the finding that a heritable seizure disorder in humans (autosomal dominant nocturnal frontal lobe epilepsy) is associated with polymorphisms in the α4 and β2 genes (Raggenbass and Bertrand, 2002) also suggest that seizures may result from altered activity of α4β2* nAChRs.

α7* receptors are expressed on the cell bodies and dendrites of many, but not all, GABAergic interneurons in the hippocampus (Frazier et al., 1998a,b). Stimulation of these interneurons results in somatic and dendritic inhibition of pyramidal cells (Buhler and Dunwiddie, 2002), as well as inhibition of other interneurons (Alkondon et al., 1998, 2000; Ji and Dani, 2000). Nicotine-stimulated [3H]GABA release from synaptosomes is mediated by αβ2* receptors as demonstrated by the findings that nicotine-stimulated [3H]GABA release is abolished in both β2 (Lu et al., 1998) and α4 null mutant mice (S. McCallum and A. C. Collins, unpublished observations). These findings support the hypothesis that altering the nicotinic modulation of GABAergic neurons can elicit seizures.

Three potential mechanisms for nicotine-induced seizures, two of these involving the GABAergic system, are illustrated in Fig. 6. The α7-glutamate mechanism proposed by Damaj et al. (1999) is also included, but will not be discussed here.

Disinhibition Model. Freund et al. (1988), Chiordini et al. (1999), and Alkondon et al. (2000) speculated that seizures induced by high doses of nicotine are the result of a decrease in GABAergic function. In this model, nicotinic cholinergic fibers provide excitatory input to GABAergic neurons, which in turn inhibit excitatory pyramidal cells. Blockade of the nicotinic cholinergic input to GABAergic neurons (either by desensitization or antagonism) reduces GABAergic input to pyramidal cells, resulting in increased excitability and seizures. Freund et al. (1988) showed that bath application of high concentrations of nicotine induced epileptiform activity in CA1 pyramidal cells in mouse hippocampal slices. This effect was similar to that produced by bath application of bicuculline and was blocked by bath application of GABA or compounds that increase GABAergic function. Chiordini et al. (1999) demonstrated, in rat hippocampal slices, that high doses of nicotine increased hippocampal excitability as measured by the EPSP slope, and presynaptic fiber volley. Application of nicotinic antagonists produced a similar effect. The authors speculated that the increased hippocampal excitability was due to reduced function of nicotinic receptors located on GABAergic interneurons. Alkondon et al. (2000) showed, in human cortical tissue, that nicotinic receptors located on GABAergic neurons modulate the release of GABA onto pyramidal cells as well as other GABAergic neurons. Based on this finding, the authors speculated that reduced activity of nicotinic receptors on GABAergic neurons could result in disinhibition of pyramidal cells and seizures. Evi-
Agonists or antagonists → Desensitization or blockade of α4β2* receptors on GABA terminals → Decreased GABA release → Increased glutamate release via disinhibition → Seizure

Nicotine → Low dose MLA

Activation of α7* receptors on glutamate terminals → Entrainment of GABAergic systems → Synchronization of glutamatergic systems

GABA Entrainment Model. An important function of interneurons is to generate synchronous oscillations in populations of neurons in the central nervous system. In this model, high doses of nicotine produce a widespread synchronous activation (i.e., entrainment) of hippocampal interneurons, which in turn leads to synchronous activity in large populations of hippocampal pyramidal neurons and seizures. Avoli et al. (1996) demonstrated that application of nicotine, as well as nicotinic antagonists, reduced the threshold for long-term potential in hippocampal slices by reducing GABAergic function. Together, these findings indicate that nicotinic effects on brain excitability are mediated primarily through GABAergic systems. Furthermore, these effects seem to be due to a nicotinic receptor-mediated disinhibition of excitatory systems. This conclusion is supported by the current results which detected a robust correlation between nicotinic receptor-mediated seizures and the inhibition of nicotine-stimulated GABA release produced by nicotinic receptor desensitization (Fig. 4C), and the genetic correlation between nicotine-induced seizures and seizures induced by GABA_A receptor antagonists (Fig. 5, A and B). Also, a recent report (Wong et al., 2002) demonstrated that pentylentetrazol- and bicuculline-induced seizure thresholds were decreased in α4 null mutant mice, suggesting that nicotinic-cholinergic input drives GABAergic function.

In conclusion, we present evidence that implicates the involvement of α4β2* receptors, as well as α7* receptors, in the generation of nicotine-induced seizures. We present two pathways for the generation of nicotine-induced seizures that work primarily through the hippocampal GABAergic system, one involving the activation of α7* receptors and one in which α4β2* receptors (and possibly α7* receptors) are rendered inactive, either through antagonism or desensitization. Both of these models are consistent with known mechanisms of epileptiform activity. The contribution of direct α7* receptor-mediated effects on glutamatergic function cannot be discounted, but the finding that α7*-glutamate interactions may be restricted to early developmental stages raises questions about the role of this system in modulating seizures in adult mice.

References


Address correspondence to: Dr. Allan C. Collins, Institute for Behavioral Genetics, University of Colorado/Boulder, 447 UCB, Boulder, CO 80309-0447. E-mail: acollins@colorado.edu.