Interaction of the Nicotinic Cholinergic System with Ethanol Withdrawal

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ABSTRACT

The observation that alcohol and nicotine are commonly abused together suggests that the two drugs have common sites of action. In vitro studies indicate that nicotinic acetylcholine receptor (nAChR) function is enhanced by ethanol. Furthermore, some ethanol-related behaviors are associated with a region of mouse chromosome 2 that contains the gene encoding the α4 subunit of the nAChR (Chrna4). We have identified a polymorphism in Chrna4 that results in an alanine (A) or threonine (T) residue at position 529 in the second intracellular loop of the protein. Nicotinic receptors expressing the A variant have greater responses to nicotine and ethanol than receptors with the T variant when measured in vitro, but the possible effects of the polymorphism on the severity of ethanol withdrawal have not been assessed. The handling-induced convolution (HIC) assay is an established method for studying drug withdrawal in vivo. We monitored the HIC responses of mice for 8 h after an injection of ethanol (4 g/kg). A survey of 16 mouse strains, as well as previously published data, indicated an association of the A/T polymorphism with ethanol withdrawal. This association was also found in wild-type animals from an F2 intercross of the A/J (A529-genotype) strain with C57BL/6J (T529-genotype) mice that also lack expression of the β2 nAChR subunit. β2 −/− animals, which do not express α4β2 nAChRs in the brain, exhibited significantly lower HIC responses and no effect of the polymorphism. These results suggest that the nicotinic cholinergic system and the A/T polymorphism modulate ethanol withdrawal.

Alcohol is frequently coabused with other addictive drugs. For example, most alcoholics are heavy tobacco users, and, among nonalcoholics, those who smoke consume more alcohol than those who do not (Batel et al., 1995). These findings suggest that alcohol and nicotine may have common sites of action. This conclusion is supported by the observations that common genes influence alcohol and tobacco abuse (Madden and Heath, 2002).

Nicotine acts in the central nervous system through the nicotinic acetylcholine receptor (nAChR). The nAChR is a pentameric ligand-gated ion channel that is formed from a variety of α (α2–10) and β (β2–4) subunits. The most frequently found nAChRs contain both α4 and β2 subunits (Role and Berg, 1996; Guo and Chiappinelli, 2002). These two subunits are critical components of the high-affinity nicotine binding site, as demonstrated by the finding that this binding site is totally absent in all brain regions of β2 (Picciotto et al., 1995) and α4 (Marubio et al., 1999) null mutant mice. In vitro studies have demonstrated that ethanol enhances maximal agonist-induced ion flux through the α4β2 nAChR (Aistrup et al., 1999; Cardoso et al., 1999). It is therefore possible that the α4β2 nAChR may be a site at which nicotine and ethanol interact.

Genetic studies in both humans and mice have implicated the nAChR in ethanol-related behaviors. In humans, a low level of initial response to alcohol early in life has been genetically correlated with alcoholism as an adult. This trait has also been associated with a marker (D20S94) on human chromosome 20 (Schuckit et al., 2001). D20S94 maps to the same chromosomal region that contains the gene encoding the α4 nAChR subunit (20q13.13–20q13.32; NIH Genome Database). In mice, a polymorphism in the α4 nAChR subunit gene (Chrna4) seems to regulate some responses to alcohol and nicotine. This polymorphism occurs at position 529 in the second intracellular loop of the protein and results in the expression of either an alanine (A) or threonine (T) residue. Mice carrying the A529 variant have greater nicotinic agonist-stimulated ion flux through the nAChR (Dobelis et al., 2002; Butt et al., 2003a) and exhibit less locomotor

ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; Chrna4, gene encoding the α4 nAChR subunit; Chrm2, gene encoding the β2 nAChR subunit; HIC, handling-induced convolution; QTL, quantitative trait loci.

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activation in the Y-maze after ethanol administration (Tritto et al., 2001) than mice carrying the T529 variant. Similarly, mouse strains that express the A529 variant of α4 are more sensitive to ethanol-induced depression of the acoustic startle response (Owens et al., 2003). Moreover, A/T genotype seems to determine whether nAChR function is enhanced by ethanol, i.e., the enhancement of agonist-induced ion flux is greater when the A529 variant is present (Butt et al., 2003a).

The A529T polymorphism is associated with variability in sensitivity to nicotine-induced seizures (Stitzel et al., 2000), perhaps as a consequence of altering GABAergic activity (Dobelis et al., 2003). Withdrawal from ethanol has a number of common symptoms in humans, one of which is seizure. Mice can also experience seizures during ethanol withdrawal (Kalant, 1977). This hyperexcitability can be assayed in mice with the handling-induced convolution (HIC) assay (Goldstein and Pal, 1971).

In this study, we tested the hypothesis that the A529 variant of α4 results in increased severity of withdrawal from ethanol, as measured by HIC. This postulate is based upon the observations that ethanol enhancement of α2β2 nAChR function is greater in A529-containing receptors (Butt et al., 2003a), and several behavioral responses to ethanol are greater in A529 expressing mouse strains (Tritto et al., 2001; Owens et al., 2003). We tested this hypothesis by using 16 inbred strains, by analyzing data from previous studies (Crabbe et al., 1983; Belknap et al., 1993; Metten and Crabbe, 1994; Buck et al., 1997), and by testing an F2 cross derived by breeding A/J mice (an A529 strain) with a C57BL/6 strain (a T529 strain) that was engineered to express a null mutation for the β2 nAChR subunit gene (β2/−/− × C57BL/6J). Virtually all α4-containing nAChRs include the β2 subunit as demonstrated by the finding that β2 null mutants do not express α4-containing nAChRs (Picciotto et al., 1995; Marubio et al., 1999). Consequently, these mice provide a powerful tool to evaluate whether the nAChRs that contain the α4 subunit, or a gene that is linked to Chnna4, is responsible for the associations between the A/T polymorphism and HIC. The results from this F2 intercross demonstrate that both α4β2-containing nAChRs and the A/T polymorphism play a role in the hyperexcitability seen during ethanol withdrawal.

**Materials and Methods**

**Mice.** Male and female mice from 16 strains (129SvEv, A/J, AKR/J, BALB/cByJ, BUB/ByJ, C3H/HeJ, C57Bl/6J, C57Bl/10J, C57Bl/129Sc, C58/J, CBA/J, DBA/2J, DBA/2J, DBA/2J, LS/1J, SS/1J, and RIj/J) were used in these studies. The strains were selected in an attempt to obtain nearly equal numbers of strains that express the A529 and T529 variants of α4 as identified by Dobelis et al. (2002). Most of the animals used were produced from breeder mice maintained at the Institute for Behavioral Genetics (University of Colorado, Boulder, CO). The Ibg strains have been maintained at IBG for over 20 generations. The AKR/J, C57Bl/10J, C57Bl/129Sc, C58/J, CBA/J, DBA/1J, and RIj/J strains were purchased directly from The Jackson Laboratories (Bar Harbor, ME). C57Bl/6J mice containing a null mutation (“knockout”) of the β2 nAChR subunit gene (Chnrb2; Picciotto et al., 1995) were also used. An F2 intercross was also generated for this study (see details below).

All mice were weaned and separated by sex when they were 25 days old. They were then housed in groups of five to a cage and maintained on a 12-h light/12-h dark cycle (lights on 7:00 AM–7:00 PM). All animals were given unlimited access to food (Teklad Rodent Diet) and water. Animals were 60 to 120 days old when used. All animal care and experimental procedures were approved by and performed in accordance with the guidelines of the Animal Care and Utilization Committee of the University of Colorado, Boulder.

**Development of F2 Animals.** An F2 intercross was generated by crossing the A/J strain with C57Bl/6J mice containing a null mutation for Chnrb2. A/J mice express the A529 variant of the α4 polymorphism, whereas C57Bl/6J mice express the T529 variant. The β2 subunit gene deletion had been bred for eight generations on to the C57Bl/6J background. Six different parental matings were made by crossing A/J mice with β2/−/− × C57Bl/6J mice. Six families of F1 animals, which were made by crossing F1 animals from different parental matings, were then used to produce the F2 progeny.

**Genotyping.** Tail clippings (~1 cm) were used for genotyping every β2 or F2 animal. DNA was extracted from the clippings with a QIAxEN (Valencia, CA) DNaseasy tissue kit. β2 genotypes were determined by polymerase chain reaction with oligonucleotide probes specific for the Chnrb2 sequence. The Chnna A/T genotype was determined by polymerase chain reaction amplification with oligonucleotide probes specific for a region of Chnna4 that flanks the A/T polymorphism followed by digestion with Stul (New England Biolabs, Beverly, MA; Dobelis et al., 2002). The gene products were electrophoresed on 1.5% agarose gels and stained with ethidium bromide. Two independent observers then scored the genotypes.

**Ethanol Withdrawal Severity.** Mice were scored for baseline handling-induced convulsions before administration of ethanol (4 g/kg body weight). Scoring was based upon an established scale of 0 (no convulsion) to 7 (full tonic-clonic convulsion; Crabbe et al., 1991). Each mouse was picked up by the tail and observed for signs of convulsion for 2 s. If no initial convulsion occurred, the mouse was spun through a 180 to 360° arc and observed again. The HIC score was then recorded based upon the degree of convulsion and whether spinning was required. The HIC scores in this study rarely exceeded a value of 4.

Experiments began at 9:00 AM. The testing was staggered so that 10 min after the baseline HIC score was established the animals were given an intraperitoneal injection of ethanol [20% (w/v) in 0.9% saline]. Handling-induced convulsions were then scored every hour after injection. The time course of HIC scores after ethanol injection (4 g/kg i.p.) indicated that maximal scores were reached by 8 h (Metten and Crabbe, 1994). We therefore adapted the assay to this shorter time frame for practicality. An initial study of the HIC response under these conditions was conducted in three inbred strains (A/J, C57Bl/10J, and DBA/2J) and a commercial strain. The HIC scores acquired in these strains in our laboratory are highly correlated (r² = 0.94) with those published by the Crabbe laboratory (Metten and Crabbe, 1994).

**Data Analysis.** All HIC scores acquired after ethanol administration were normalized to the baseline HIC score for each animal. Normalization was performed by subtracting the basal, predrug scores from those obtained after ethanol administration. Using Sigma Plot 2001 (SPSS Science, Inc., San Raphael, CA), the normalized scores for each animal were plotted over an 8-h time scale, and the area under the plotted curve was calculated (Crabbe et al., 1991; Metten and Crabbe, 1994). The areas under the curves were then averaged and plotted according to strain or genotype with GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA). Significant differences (p < 0.05) between data sets were determined with unpaired t tests or one-way analysis of variance.

Data from previously published ethanol HIC studies (Crabbe et al., 1983; Belknap et al., 1993; Metten and Crabbe, 1994; Buck et al., 1997) were also analyzed with regard to the A/T polymorphism. The average HIC scores of 13 inbred strains (Crabbe et al., 1983; Metten and Crabbe, 1994) and of the BXD recombinant inbred strains (Belknap et al., 1993; Buck et al., 1997) were grouped according to their A/T genotype and analyzed with unpaired t tests. The A/T genotypes of the inbred strains are reported in Dobelis et al. (2002). BXD recombinant inbred strain genotypes were determined with the
methods described above on DNA samples supplied by Dr. John Crabbe (Oregon Health & Science University, Portland, OR). Previous analyses of nicotinic function indicate that, in general, A529 variants have larger responses to nicotine and ethanol than T529 variants (Dobelis et al., 2002; Butt et al., 2003a). Therefore, all A/T comparisons of the HIC response were analyzed with one-tailed, unpaired t tests. Because the genetic marker D2Mit200 is in proximity to the gene for the α4 nAChR (Bessis et al., 1990; Mouse Genome Database, July 2003), we also performed a similar analysis of the previously published BXD recombinant inbred strain data with regard to genotypes at D2Mit200. The average HIC scores for each strain were grouped according to their genotype [homozygous C57BL/6 (B6) or homozygous DBA/2J (D2)] at D2Mit200 and analyzed with unpaired t tests.

**Results**

**Strain Differences in Ethanol Withdrawal.** Figs. 1 and 2 illustrate that differences in the HIC response after an injection of ethanol (4 g/kg) were detected among the 16 mouse strains that we tested. Strain differences in the HIC response before ethanol administration were also detected (data not shown), as reported previously (Metten and Crabbe, 1994). Therefore, an accurate comparison of the strains during ethanol withdrawal required that the HIC scores be normalized to their respective baseline scores (Fig. 1). After

![Fig. 1. Survey of the ethanol HIC response over an 8-h time course in 16 mouse strains. A and B, average HIC responses above baseline in seven strains that carry the A529 variant of the α4 nAChR polymorphism. C and D, normalized HIC scores in nine strains that carry the T529 polymorphism. Error bars represent the S.E.M. in all graphs. n = 10 for all strains except Balb/CbyJ (5), Bub/BrJ (6), C57/BrJ (6), C58/J (4), DBA/1J (3), DBA/2J (9), and RIII/SJ (5).](image)

![Fig. 2. Areas under the curves for the data presented in Fig. 1. Error bars represent the S.E.M. One-way analysis of variance indicated that there were significant differences in the HIC scores (p < 0.0001).](image)
normalization, the strain responses were analyzed for areas below the curves (Fig. 2). These data were grouped according to the genotype of the strains for the A/T polymorphism using data reported in Dobelis et al. (2002).

**Potential Association of Ethanol Withdrawal with the A/T Polymorphism.** Fig. 3 shows that when the HIC scores illustrated in Figs. 1 and 2 were averaged according to their A/T genotype an association was found between ethanol withdrawal and the polymorphism. A529 variants had higher HIC responses than the T529 variants (Fig. 3A). The differences in the areas under the curves were statistically significant (Fig. 3B). Baseline scores were not correlated with the A/T polymorphism (data not shown).

Figure 4 shows that similar results were obtained when previously reported data (Crabbe et al., 1983; Belknap et al., 1993; Metten and Crabbe, 1994; Buck et al., 1997) were averaged according to their A/T genotype (Dobelis et al., 2002). Inbred strains with the A529 genotype had higher HIC scores than T529 strains after an acute injection of ethanol (4 g/kg; Metten and Crabbe, 1994; Fig. 4A) and after removal from ethanol vapors (Crabbe et al., 1983; Fig. 4B). These differences were significant in the chronic vapor inhalation experiments. Similarly, BXD recombinant inbred strains that were genotyped as homozygous A529 had higher HIC scores than T529 animals after ethanol administration than A529 animals. A, analysis of the areas under the curves by a one-tailed, unpaired t test indicated that the effect suggested in A was significant (\( t < 0.05 \)). Error bars represent the S.E.M. in both graphs.

![Fig. 3.](image)

**Fig. 3.** Average HIC scores as they pertain to A/T genotype of the 16 strains used in this study. A, average HIC scores of the A529-containing strains (A/J, AKR/J, C3H/HeJg, DBA/1J, DBA/2J, RIIIS/J, and SS) and the T529-containing strains (Balb/CbyJ, Bub/BrJ, C57BL/6J, C57BL/10J, C57Br/J, C58/J, CBA/J, LS, and 129/SvEv; n = 8–16 for each strain; Metten and Crabbe, 1994). Inbred strain data were obtained from five A529 inbred strains (A/HeJ, AKR/J, C3H/HeJg, DBA/1J, and DBA/2J) and eight T529 inbred strains (129/J, Balb/CbyJ, CBA/J, C57BL/6J, C57Bl/cdJ, C57L/J, SJL/J, and SWR/J; n = 8–16 for each strain; Metten and Crabbe, 1994). The BXD recombinant inbred strain data were obtained from 12 A529 strains and 9 T529 strains (n = 6–17 for each strain; Belknap et al., 1993). B, data obtained from animals that were exposed to ethanol vapors for 3 days (5.8–8.4 mg ethanol/l air), and their HIC responses were monitored for 25 h after the ethanol was removed from their air supply. The longer evaluation period resulted in the larger areas under the curves. The inbred strain data include five strains with the A529 genotype (DBA/2N, C3H/HeNMTV, A/HeN, DBA/1J, and AKR/J), and eight strains represent the T529 group (SWR/J, SJL/J, CBA/J, C57BL/6N, C57BL/10N, Balb/cAnN, and C58/J, C57Bl/cdJ; n = 3–5 for each strain; Crabbe et al., 1983). The BXD recombinant inbred strain data are the averages of 14 A529 strains and nine T529 strains (total n = 288; Buck et al., 1997). *p < 0.05 compared with appropriate A529 data in a one-tailed unpaired \( t \) test. Error bars represent the S.E.M. in both graphs.

![Fig. 4.](image)

**Fig. 4.** Association of the A/T polymorphism with ethanol HIC in previously published data. A, data obtained from animals that were observed for 12 h after an intraperitoneal injection of ethanol (4 g/kg). The inbred strain data were obtained from five A529 inbred strains (A/HeJ, AKR/J, C3H/HeJg, DBA/1J, and DBA/2J) and eight T529 inbred strains (129/J, Balb/CbyJ, CBA/J, C57BL/6J, C57Bl/cdJ, C57L/J, SJL/J, and SWR/J; n = 8–16 for each strain; Metten and Crabbe, 1994). The BXD recombinant inbred strain data were obtained from 12 A529 strains and 9 T529 strains (n = 6–17 for each strain; Belknap et al., 1993). B, data obtained from animals that were exposed to ethanol vapors for 3 days (5.8–8.4 mg ethanol/l air), and their HIC responses were monitored for 25 h after the ethanol was removed from their air supply. The longer evaluation period resulted in the larger areas under the curves. The inbred strain data include five strains with the A529 genotype (DBA/2N, C3H/HeNMTV, A/HeN, DBA/1J, and AKR/J), and eight strains represent the T529 group (SWR/J, SJL/J, CBA/J, C57BL/6N, C57BL/10N, Balb/cAnN, and C58/J, C57Bl/cdJ; n = 3–5 for each strain; Crabbe et al., 1983). The BXD recombinant inbred strain data are the averages of 14 A529 strains and nine T529 strains (total n = 288; Buck et al., 1997). *p < 0.05 compared with appropriate A529 data in a one-tailed unpaired \( t \) test. Error bars represent the S.E.M. in both graphs.
scores than recombinant inbred strains that were genotyped as homozygous T529 (Belknap et al., 1993; Buck et al., 1997). The differences were statistically significant in both types of experiments. When the BXD recombinant inbred data were segregated according to genotypes (homozygous B6 or homozygous D2) at D2Mit200, a genetic marker very near to the gene for the α4 nAChR (Mouse Genome Database, July 2003), the results were essentially the same (data not shown). The genotypes for each BXD strain are provided in Table 1.

**F2 Analysis of Ethanol Withdrawal.** Fig. 5 shows the HIC results after ethanol administration in the (B2/−/− X C57BL/6J) × AJ F2 intercross. This intercross allowed for the variation of A/T genotypes on a mixed genetic background. A/A +/+ animals had HIC scores that were markedly higher than those of T/T +/+ animals (Fig. 5, A and D). The differences were statistically significant. Data obtained from both B2 +/− (Fig. 5, B and E) and B2 −/− (Fig. 5, C and F) animals showed that the A/T association was diminished or absent with loss of B2 expression. B2 −/− animals also seemed to have lower ethanol withdrawal scores than their +/+ and +/− littermates.

**Interactions of B2 nAChR Expression with Ethanol Withdrawal.** Fig. 6 shows that null-mutation of the gene encoding the B2 nAChR subunit decreased the HIC response after ethanol administration. These results were obtained in animals in which the B2 mutation had been bred on to the C57BL/6J background for eight generations (B2 × C57BL/6; Fig. 6, A and B). Wild-type animals had significantly higher HIC scores than their −/− littermates. B2 genotype had no effect on baseline HIC scores (data not shown).

**Discussion**

The results presented in this study clearly implicate the nicotinic cholinergic system in ethanol withdrawal. Three different genetic strategies (inbred strain comparisons, meta-analysis of recombinant inbred strain data, and an F2 analysis) yielded data that support the assertion that α4-containing nAChRs modulate ethanol withdrawal as measured by the HIC assay.

The data obtained in our inbred strain survey are very similar (r² = 0.94) to those acquired in previously published studies (Crabbe et al., 1983; Metten and Crabbe, 1994). This finding increases our confidence in the reliability of the results. The strains that express the A529 variant of Chrna4 had greater ethanol withdrawal than those strains that expressed the T529 variant. This result suggests that α4-containing nAChRs may play an important role in regulating the severity of HIC measured after ethanol administration. However, this association could be the result of a gene that is closely linked to Chrna4.

Recombinant inbred strains are derived by inbreeding F2 hybrids that are obtained from two progenitor inbred strains. The generation of F2 hybrids results in a scrambling of alleles and the subsequent inbreeding serves to fix the alleles in new combinations. Thus, a study using recombinant inbred strains is of value in addressing the linkage question. Belknap et al. (1993) and Buck et al. (1997) measured ethanol HIC in the BXD recombinant inbred strains that were made by crossing C57BL/6J and DBA/2J progenitors. C57BL/6J mice carry the T529 polymorphism, whereas DBA/2J mice carry the A529 polymorphism (Dobelis et al., 2002). A significant association was found when we examined the potential relationship between the α4 polymorphisms and ethanol HIC using the published BXD recombinant inbred data (Belknap et al., 1993; Buck et al., 1997). This finding provides further support for the conclusion drawn from the inbred strain analysis.

An association between ethanol withdrawal and Chrna4 was not found by previous quantitative trait loci (QTL) studies (Belknap et al., 1993; Buck et al., 1997). One reason for this lack of association is that QTL analyses rely on multiple comparisons and multiple genetic markers. We reexamined the data reported by Belknap et al. (1993) and Buck et al. (1997) focusing on D2Mit200 because this marker is in proximity to Chrna4. D2Mit200 occurs at 107 centimorgans on mouse chromosome 2 (Mouse Genome Database, July 2003), whereas Chrna4 is located in essentially the same region (108 centimorgans; Bessis et al., 1990). Neither of the QTL studies of HIC detected a QTL near D2Mit200 because this marker is in proximity to Chrna4. D2Mit200 occurs at 107 centimorgans on mouse chromosome 2 (Mouse Genome Database, July 2003). We detected significant associations between HIC and both the A/T polymorphism and D2Mit200, most probably because multiple comparison corrections were unnecessary with these single, candidate gene approaches.

The QTL studies of HIC have detected at least six chromosomal regions that influence ethanol withdrawal (Belknap et al., 1993; Buck et al., 1997, 2002). Buck et al. (1997) also identified a possible QTL that is located in the middle of chromosome 2, but they did not list this QTL as being significant. Roughly one-half of the BXD recombinant inbred strains, whose HIC data were analyzed for this study, have alleles at this locus that are the opposite genotype of those

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**TABLE 1**

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<th>D2Mit200 Genotype</th>
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expressed at D2Mit200 (Mouse Genome Database, July 2003) and the α4 polymorphism indicating that a crossover between the C57BL/6 (B) and DBA/2 (D) chromosomes occurred somewhere between these markers. The effects of the provisional chromosome 2 QTL on HIC are inversely correlated with those of the D2Mit200 and Chrna4 markers (Buck et al.,

Fig. 5. Normalized HIC scores and area under the curve analysis of the (C57BL/6J × β2⁻/⁻) × A/J F2 intercross. Both the normalized HIC scores (A) and the area analyses (D) indicated that A/A animals had higher HIC responses than T/T animals that were also β2⁺/+. The A/T effect in β2⁺/+ and β2⁻/+ (B and E) animals was statistically significant (*, p < 0.05 compared with A/A results) in a one-tailed, unpaired t test. The A/T effect could not be detected in β2⁻/+ (C and F) animals. β2⁻/+ animals (C and F) had generally lower HIC scores than their β2⁺/+ (A and D) and β2⁻/+ (B and E) littermates. Error bars represent the S.E.M. in all graphs.
Ethanol HIC has been inversely correlated with ethanol consumption in a survey of inbred strains (Metten et al., 1998). Interestingly, ethanol preference has been associated with D2Mit200 in a survey of a recombinant inbred mice derived from a cross between C57BL/6J and A/J mice (Gill et al., 1998). Meta-analysis of the data reported by Metten et al. (1998) indicates that, in general, animals that carry the T529 variant of Chrna4 consume more ethanol than animals that carry the A529 variant. Meta-analysis of the data reported by Gill et al. (1998) produces similar results, but the effect is only seen in male mice (J. A. Stitzel and A. C. Collins, unpublished observations). These findings match well with the inverse correlation between ethanol consumption and ethanol HIC reported by Metten et al. (1998). An ethanol preference study in the $(\beta 2^{-/-} \times C57BL/6J) \times A/J$ F2 mice is currently underway in our laboratory.

It is well established that ethanol enhances the function of $\alpha 4\beta 2$-containing nAChRs, when measured in vitro (Aistrup et al., 1999; Cardoso et al., 1999). Recently, we reported that the enhancing effect of ethanol is modulated by the A/T polymorphism (Butt et al., 2003a). Ethanol is more effective (greater enhancement) in modulating the activity of $\alpha 4\beta 2$ nAChRs that contain the A529 variant of the $\alpha 4$ gene. These $\alpha 4\beta 2$-containing nAChRs are found throughout the central nervous system (Role and Berg, 1996; Guo and Chiappinelli, 2002) and can act as presynaptic modulators of GABA release (Lu et al., 1999; MacDermott et al., 1999; Alkondon and Albuquerque, 2001). Thus, when ethanol is present it seems likely that these nAChRs enhance GABA release and contribute to the increases in GABAergic tone that are associated with ethanol administration.

It may be that the hyperexcitability seen during ethanol withdrawal arises as a consequence of a rebound decrease in GABAergic tone. Cholinergic nuclei in the basal forebrain project to the amygdala, hippocampus, and cortex (Mesulam et al., 1983). These brain regions have been implicated in epileptiform brain activity (Mark and Finn, 2002). Cholinergic input to these regions increases the likelihood that inhibitory interneurons will fire. It may be that $\alpha 4\beta 2$ nAChRs enhance the release of GABA, which then inhibits excitatory neurons (Freund et al., 1988; Alkondon et al., 2001). Thus, when ethanol is present it seems likely that these nAChRs enhance GABA release and contribute to the increases in GABAergic tone that are associated with ethanol administration.

An F2 analysis of the association between the $\alpha 4$ polymorphism and ethanol HIC yielded additional support for the A/T effect. The $(\beta 2^{-/-} \times C57BL/6J) \times A/J$ F2 mice were used because most, if not all, $\alpha 4$-containing nAChRs contain $\beta 2$ nAChR subunits; $\beta 2^{-/-}$ animals do not express the major $\alpha 4$-containing nAChR (Picciotto et al., 1995; Marubio et al., 1999). We detected an association with the A/T polymorphism in the F2 hybrids that were $\beta 2^{-/+}$. However, when $\beta 2^{-/-}$ animals were studied no association was found. This finding in the $\beta 2^{-/-}$ animals argues against the possibility that a gene that is linked to Chrna4 causes the A/T effect because the $\beta 2^{-/-}$ mice do not express $\alpha 4\beta 2$ nAChRs. Further evidence for the role of nAChRs in ethanol HIC is evident from the finding that the severity of ethanol withdrawal decreases in a gene dose-dependent manner in $\beta 2^{-/+}$, $\beta 2^{-/-}$ animals.

Ethanol HIC has been inversely correlated with ethanol consumption in a survey of inbred strains (Metten et al., 1998). Interestingly, ethanol preference has been associated with D2Mit200 in a survey of a recombinant inbred mice derived from a cross between C57BL/6J and A/J mice (Gill et al., 1998). Meta-analysis of the data reported by Metten et al. (1998) indicates that, in general, animals that carry the T529 variant of Chrna4 consume more ethanol than animals that carry the A529 variant. Meta-analysis of the data reported by Gill et al. (1998) produces similar results, but the effect is only seen in male mice (J. A. Stitzel and A. C. Collins, unpublished observations). These findings match well with the inverse correlation between ethanol consumption and ethanol HIC reported by Metten et al. (1998). An ethanol preference study in the $(\beta 2^{-/-} \times C57BL/6J) \times A/J$ F2 mice is currently underway in our laboratory.

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TR29 animals may be protected from GABA depletion by increased rates of nAChR desensitization in the presence of ethanol (Butt et al., 2003b). $\beta_2$−/− animals have less severe symptoms of ethanol withdrawal through a similar mechanism. Null-mutation of the $\beta_2$ nAChR subunit abolishes nAChR-mediated [3H]GABA release (Lu et al., 1999). This effect leaves more GABA available for inhibiting epileptiform activity. Although this model is conceivable it does not account for the altered function of other systems during withdrawal (Little, 1991; Morrow, 1995; Hoffman and Tabakoff, 1996). Nonetheless, our data provide strong support for the notion that nicotinic mechanisms contribute to the ethanol withdrawal syndrome.

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References


Fig. 7. Schematic representation of a model that links nAChR genotype to the severity of ethanol withdrawal. The genotypes of nAChR subunits affect overall receptor activation (Marks et al., 1999; Dobelis et al., 2002) and whether ethanol changes the degree of activation (Butt et al., 2003a). These differences probably result in changes in acetylcholine-stimulated GABA release (Lu et al., 1999), and it is known that extended ethanol exposure decreases GABA availability (Coffman and Petty, 1985; Buck et al., 1997). Therefore, differences in initial GABA release could result in varying degrees of GABA depletion. The extent of GABA depletion might then influence the severity of ethanol withdrawal. Up arrow, increase; down arrow, decrease; nc, no change.


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