

GABRA2 Alleles Moderate the Subjective Effects of Alcohol, Which are Attenuated by Finasteride

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GABA_A receptors are involved in the subjective effects of alcohol. Endogenous neuroactive steroids interact with GABA_A receptors to mediate several behavioral effects of alcohol in rodents. Based on a haplotypic association of alcohol dependence with the gene encoding the GABA_A receptor α -2 subunit (*GABRA2*), we examined whether *GABRA2* alleles are associated with the subjective response to alcohol. We also examined whether finasteride (a 5- α steroid reductase inhibitor), which blocks the synthesis of some neuroactive steroids, reduces the subjective response to alcohol. In all, 27 healthy social drinkers (15 males) completed a randomized, double-blind, placebo-controlled study of high-dose finasteride. After being pretreated with study drug, subjects consumed three alcoholic drinks. Subjective effects were measured repeatedly over the ascending blood alcohol curve. To examine the moderating role of genetic variation in *GABRA2*, a single-nucleotide polymorphism that was informative in association studies was included as a factor in the analysis. Subjects homozygous for the more common A-allele ($n = 7$) showed more subjective effects of alcohol than did individuals with one or two copies of the alcohol dependence-associated G-allele ($n = 20$, including two homozygotes). Among the A-allele homozygotes, there was a greater reduction in several subjective effects during the finasteride session compared to the placebo session. These findings provide preliminary evidence that the risk of alcoholism associated with *GABRA2* alleles may be related to differences in the subjective response to alcohol. The effects of finasteride provide indirect evidence for a mediating role of neuroactive steroids in some of the subjective effects of alcohol.

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INTRODUCTION

Two genome-wide scans in humans provided evidence of linkage of alcohol dependence to a region of chromosome 4p that includes a cluster of four genes encoding GABA_A receptor subunits (Reich *et al*, 1998; Long *et al*, 1998). Edenberg *et al* (2004), by fine mapping this region, found that numerous single-nucleotide polymorphisms (SNPs) in the gene encoding the GABA_A α -2 subunit (*GABRA2*), but not in other members of the gene cluster, were associated with alcohol dependence. In addition, all 3-SNP haplotypes examined in the 3' region of *GABRA2* were significantly associated with alcohol dependence (Edenberg *et al*, 2004).

Covault *et al* (2004) replicated this haplotypic association in a region of the gene that overlaps the region identified by Edenberg *et al* (2004). These findings, together with evidence that GABA_A receptors mediate several behavioral effects of alcohol (Grobin *et al*, 1998; Davies, 2003), underscore the potential contribution of variation at *GABRA2* to the risk for alcohol dependence.

As alcohol elevates plasma and brain concentrations of GABAergic neuroactive steroids, including 3 α -5 α -THP (allopregnanolone or ALLO), and 3 α -5 α -THDOC (allotetrahydrodeoxycorticosterone), it has been hypothesized that these compounds may contribute to specific behavioral actions of ethanol by modulating GABA_A receptor function (Morrow *et al*, 1999; Barbaccia *et al*, 1999; VanDoren *et al*, 2000). Two recent reports in humans suggest that acute alcohol exposure stimulates neuroactive steroid production (Torres and Ortega, 2003, 2004). Compared to adolescents seen for nonalcohol-related reasons, plasma levels of ALLO and progesterone were elevated in both male and female adolescents presenting to the emergency department with acute alcohol intoxication. The effects were seen among

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females during both the luteal and follicular phases of the ovarian cycle. Studies in chronic drinkers have shown 40–50% lower plasma concentrations of ALLO and THDOC in the first week of abstinence, with normalization during the subsequent 3 weeks (Romeo *et al*, 1996, 2000). These studies suggest a direct link between increased neuroactive steroid levels and acute alcohol intoxication in humans, with chronic intoxication producing the opposite effect, findings that parallel those described in the animal literature (Barbaccia *et al*, 1999; VanDoren *et al*, 2000; Khisti *et al*, 2003; Finn *et al*, 2004).

The present study examined the moderating effects of *GABRA2* alleles and high-dose finasteride on subjective and physiological effects of a standardized dose of alcohol in healthy social drinkers. Finasteride, a 5- α steroid reductase (5AR) inhibitor that limits the conversion of testosterone to dihydrotestosterone, is FDA approved for the treatment of benign prostatic hypertrophy. Finasteride also reduces the metabolism of progesterone to the 5 α -reduced neuroactive steroids ALLO and THDOC. Based on these considerations, we hypothesized that *GABRA2* alleles would moderate the subjective effects of alcohol measured during the ascending limb of the breath alcohol concentration (BrAC) curve and that finasteride would attenuate the subjective response to alcohol. We focused on the ascending limb of the BrAC curve, since it is commonly associated with the stimulating effects of alcohol (de Wit *et al*, 1987), which are in turn associated with alcohol preference (de Wit *et al*, 1987; Chutuaue and de Wit, 1994) and heavy drinking (Holdstock *et al*, 2000).

METHODS

Subjects

In all, 27 subjects (15 men) were recruited from the greater Hartford, CT area by advertisement and were paid for their participation. After an initial telephone interview, a psychiatric history was obtained using the Structured Clinical Interview for DSM-IV (First *et al*, 1995). A 90-day timeline follow-back interview (Sobell and Sobell, 1992) was used to quantify recent alcohol and drug use. Subjects underwent a medical history and physical examination and routine laboratory tests (including complete blood count, liver and renal function tests, blood glucose and electrolyte concentration, and among female subjects, a serum pregnancy test). All subjects gave written informed consent to participate in the protocol, as approved by the University of Connecticut Health Center Institutional Review Board.

Subjects were included in the study if they reported moderate social drinking (ie a minimum of three drinks per week and at least three drinks on one occasion in the past month) and had a body mass index of 18.5–30 kg/m². They were excluded if they ever met the criteria for DSM-IV (American Psychiatric Association, 1994) substance abuse or dependence or another major psychiatric disorder, had evidence of liver dysfunction, were using benzodiazepines or other psychotropic medications, were smokers, or were pregnant or nursing. Women were included only if they reported having regular menstrual cycles and were not using oral contraceptives or other hormones and had no history of endocrine or reproductive abnormalities.

Study Design

The study involved a balanced design, in which each subject served as his or her own control, with two experimental sessions, separated by 1 month. At 24 h prior to each laboratory session, and 2 h before the first drink, subjects were pretreated with high-dose finasteride or matching placebo, under double-blind conditions. The order of the sessions was randomized. Among women, the experimental sessions were held during the follicular phase of two consecutive menstrual cycles (based on a 28-day menstrual cycle). The sessions were scheduled 5–9 days following the onset of menstruation, with the menstrual cycle phase determined by counting the days from the onset of menstruation. To ensure comparability, the laboratory sessions were separated by 1 month for men also.

Subjective Effects

Subjective effects were measured using the following self-report questionnaires:

Alcohol Sensation Scale (SS; Maisto *et al*, 1980) consists of 26 items that are divided into six subscales measuring somatic sensations produced by alcohol, particularly on the ascending limb of the blood alcohol concentration curve: the Central-Stimulant Subscale measures sensations associated with effects on the brain (four items), the Dynamic-Peripheral Subscale measures sensations associated with excitation, including increased breathing and heart rate (three items), the Warmth-Glow Subscale measures blushing sensations (three items), the Anesthetic Subscale measures sensations associated with loss of feeling or decreased sensitivity to feeling (nine items), the Gastro-intestinal Subscale measures sensations felt in the stomach (four items), and the Impaired-Function Subscale measures perceived changes in psychomotor performance (three items).

Biphasic Alcohol Effects Scale (BAES; Martin *et al*, 1993) is a 14-item unipolar adjective rating scale designed to measure both the stimulant and sedative effects of alcohol. Subjects rated the adjectives on a scale of 0 (not at all) to 10 (extremely). We used the items from the Stimulation Subscale (seven items), which has been experimentally associated with increasing BrAC (de Wit *et al*, 1987; Martin *et al*, 1993).

Drug Effects Questionnaire (DEQ; Holdstock and de Wit, 1998) consists of four items that measure current drug effects that were adapted to measure alcohol effects: 'feel alcohol', 'feel high', 'like alcohol', and 'want more alcohol'. Subjects indicated on a 100-mm line the extent to which they endorsed each statement, with anchors from 'not at all' on the left end of the scale to 'extremely' on the right end of the scale.

We also measured BrAC, blood pressure, and heart rate (while seated).

Laboratory Procedures

To maximize the antagonism of 5AR activity (Ohtawa *et al*, 1991), subjects received either finasteride 100 mg or placebo in identical capsules 24 h prior to each laboratory session (see Figure 1) and were instructed to avoid alcohol,

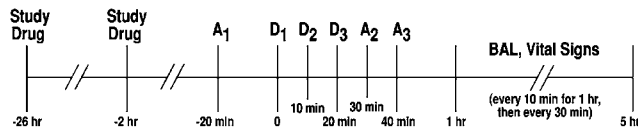


Figure 1 Timeline of events. A1 = 1st Assessment Battery (SS, BAES, and DEQ); A2 = 2nd Assessment Battery; A3 = 3rd Assessment Battery; D1 = 1st Alcoholic Drink; D2 = 2nd Alcoholic Drink; D3 = 3rd Alcoholic Drink; BrAC: Breath Alcohol Concentration. AA ($n = 7$), G-allele carriers ($n = 20$).

nicotine, or other drugs (except caffeine) until after completion of the laboratory session. Subjects arrived at the laboratory at 0800, having been instructed to fast from solid foods for 8 h prior to the session. At 2 h prior to the first dose of alcohol (ie time (t) = -120 min), a second dose of finasteride 100 mg or placebo was administered, followed by a light breakfast. An alcoholic drink, which was divided into three equal portions, was served at $t = 0$, 10, and 20 min, with subjects having 10 min to consume each drink. The dosage of alcohol was chosen to ensure a measurable subjective effect (Holdstock and de Wit, 1998; Swift *et al*, 1994), with an adjustment based on sex differences in alcohol pharmacokinetics (Watson, 1989): men received 0.8 g/kg and women received 0.7 g/kg.

Subjects completed the SS, BAES, and DEQ 20 min before ingesting the first alcoholic drink, and then along the ascending limb of the BrAC curve, that is, at 10 and 20 min after the third drink was consumed (ie 30 and 40 min after the onset of alcohol consumption). BrAC and physiological measures (heart rate and blood pressure) were recorded at consecutive 10-min intervals after consumption of the first drink.

Genotyping

DNA was purified from venous blood samples using the PureGene kit (GentraSystems, Minneapolis, MN). The rs279858 SNP, which is a synonymous A-to-G substitution (K132K) in exon 4 of the *GABRA2* gene, was genotyped using the TaqMan 5' nuclease assay with ABI Assay-on-Demand #2073557. This SNP lies in the middle of the haplotype block in *GABRA2* that was observed to be associated to alcohol dependence by Edenberg *et al* (2004) in a family-based study and by Covault *et al* (2004) in a case-control study. Although the G-allele of this SNP was less common overall, it was over-represented among alcohol-dependent individuals (Covault *et al*, 2004), and may therefore be considered a potential 'risk' allele. Conversely, since the A-allele, although more common overall, was less frequently transmitted (Edenberg *et al*, 2004) among alcoholics or was over-represented among healthy controls (Covault *et al*, 2004), it may be considered a 'protective' allele.

Data Analysis

The data were examined using a full factorial, repeated measures analysis of variance model. Within-subject variables were Genotype (A-allele homozygotes ($n = 7$) vs G-allele carriers (AG, $n = 18$ or GG, $n = 2$)), Study Drug

(finasteride vs placebo), and Time (-20 min and 30 and 40 min after the first alcoholic drink). Significant interactions were decomposed to reveal the specific nature of the effects. Statistical significance was defined as $p < 0.05$.

The dependent measures examined in the analyses were the six SS subscale scores, the BAES Stimulation Subscale score and the four DEQ items. BrAC did not differ by Study Drug or Genotype (Figure 2), nor was it significant as a time-varying covariate, so it was removed from the analysis, with no substantial change in the observed effects. Similarly, when included as a between-subjects factor, the order of laboratory sessions had no effect on the results, and so it too was removed from the analysis. When the assumption of sphericity was not met (as determined by Mauchly's W), we used the Greenhouse-Geisser adjusted F test for within-subjects effects and within-/between-subjects interaction effects.

In view of the potential for population differences to confound the effects of genotype, all analyses were repeated including only non-Hispanic European-American subjects, the most common population subgroup.

RESULTS

Subjects

Of the 27 participants, 15 (56%) were male, 22 (82%) were European-American, and five (18%) were Hispanic (all of whom were heterozygous for the SNP examined). The average age of the subjects was 29.2 years ($SD = 6.7$). The majority of subjects (74%) were college graduates. Most subjects were single (56%), and were either working ($n = 12$) or attending graduate school ($n = 14$). In total, 14 subjects received finasteride (10 G-allele carriers) and 13 subjects (10 G-allele carriers) received placebo prior to the first laboratory session. As shown in Table 1, the genotype groups did not differ on sociodemographic or clinical characteristics, including recent drinking history (all p -values > 0.10).

Laboratory Sessions

Physiological and adverse effects. As shown in Figure 2, there was a main effect of time on BrAC ($F(2,46) = 135.24$, $p < 0.001$), with an increase in BrAC from $t = -20$ to 30 min, followed by a decrease from $t = 30$ to 40 min. There were also main effects of time on heart rate ($F(2,46) = 15.05$, $p < 0.001$), and both systolic ($F(2,46) = 9.53$, $p < 0.001$) and diastolic ($F(2,46) = 7.74$, $p = 0.001$) blood pressure. Alcohol produced increases in all three physiological measures from $t = -20$ to 30 min, followed by a decrease from $t = 30$ to 40 min for the systolic and diastolic blood pressures, while remaining stable for heart rate. There were no statistically significant main or interactive effects of Genotype or Study Drug on any of these measures.

A total of 17 subjects (63%) reported 25 adverse events during or after the laboratory session in which finasteride was administered, and 15 subjects (56%) reported 20 adverse events during or after placebo administration. For both sessions, the adverse event reported most frequently was headache (finasteride = 50.0%, placebo = 47.6%), followed by nausea (finasteride = 4.2%, placebo = 9.5%). There

Table 1 Demographic and Drinking Features by *GABRA2* Genotype^a

	AG or GG (n = 20)	AA (n = 7)
Gender (%)		
Male	55.0	57.1
Female	45.0	42.9
Race (%)		
European-American	75.0	100
Hispanic	25.0	0
Age (mean (SD), years)	28.2 (5.7)	32.3 (8.7)
Annual household income (%)		
<\$20 000	15.0	0
\$20 000–49 999	45.0	42.9
\$50 000–79 000	10.0	14.3
≥\$80 000	30.0	42.9
Education level (%)		
Some high school	5.0	0
Some college	20.0	28.6
College graduate	20.0	14.3
Some postcollege	35.0	14.3
Advanced degree	20.0	42.9
Marital status (%)		
Married	40.0	42.9
Single or divorced	60.0	57.1
Employment status (%)		
Employed (full or part time)	45.0	42.9
Unemployed	5.0	0
Student	50.0	57.1
Recent drinking behavior (mean (SD))		
#Drinking days in past 90 days	15.3 (4.1)	12.6 (2.5)
#Standard drinks in past 90 days	34.6 (9.4)	29.8 (6.0)
#Heavy drinking days in past 90 days	1.7 (0.7)	1.7 (0.5)

^aNo significant differences between genotype groups.

was no Study Drug effect on either the proportion of subjects reporting adverse events (McNemar χ^2 (1 df) = 0.06, $p = 0.80$) or the number of adverse events reported ($t_{(23)} = 0.87$, $p = 0.40$).

Subjective Effects

Alcohol SS. All six of the SS subscale scores showed a significant effect of Time, with increased scores following alcohol administration. There were also Genotype and/or Study Drug effects on four of the subscale scores.

Central-Stimulant Subscale: All main and interactive effects on this subscale score were statistically significant: Time ($F_{(2,50)} = 29.68$, $p < 0.001$), Genotype ($F_{(1,25)} = 10.61$, $p = 0.003$), Study Drug ($F_{(1,25)} = 8.25$, $p = 0.008$), Time \times Genotype ($F_{(2,50)} = 6.97$, $p = 0.002$), Time \times Study Drug ($F_{(2,50)} = 6.34$, $p = 0.004$), Genotype \times Study Drug ($F_{(1,25)} = 6.56$, $p = 0.017$), and Time \times Genotype \times Study Drug ($F_{(2,50)} = 7.11$, $p = 0.002$). As shown in Figure 3, the increase in Central-Stimulant Subscale scores following alcohol administration was greater among the A-allele homozygotes than the G-allele carriers. However, it should be noted that G-allele carriers showed a significant response to alcohol on this measure ($F_{(2,38)} = 8.32$, $p = 0.004$). Overall, pretreatment with finasteride reduced scores on this measure relative to the placebo session. There was no effect of Genotype prior to alcohol administration, but a significantly greater increase in scores among A-allele homozygotes at $t = 30$ min ($F_{(1,25)} = 7.28$, $p = 0.012$), but not at $t = 40$ min ($F_{(1,25)} = 1.63$, $p = 0.213$). Decomposition of the three-way interaction revealed only that finasteride significantly attenuated Central-Stimulant Subscale scores for the A-allele homozygotes compared to the G-allele carriers at $t = 30$ min ($F_{(1,25)} = 10.87$, $p = 0.003$), an effect that was no longer significant at $t = 40$ min ($F_{(1,25)} = 1.27$, $p = 0.27$).

Dynamic-Peripheral Subscale: There were significant effects of Time ($F_{(2,50)} = 9.28$, $p = 0.002$), Study Drug ($F_{(1,25)} = 4.41$, $p = 0.046$), and Time \times Study Drug ($F_{(2,50)} = 3.99$, $p = 0.037$) on this subscale score. As shown in Figure 4, finasteride attenuated the increase in scores on this subscale following alcohol administration. The effect was seen at $t = 30$ min ($F_{(1,25)} = 6.11$, $p = 0.021$), but was no longer significant at $t = 40$ min ($F_{(1,25)} = 3.28$, $p = 0.082$).

Anesthetic Subscale: There were significant effects of Time ($F_{(2,50)} = 9.27$, $p = 0.002$), Study Drug ($F_{(1,25)} = 4.57$, $p = 0.043$), Genotype \times Study Drug ($F_{(1,25)} = 4.27$, $p = 0.049$), and Time \times Genotype \times Study Drug ($F_{(2,50)} = 4.78$, $p = 0.024$) on this subscale score. As shown in Figure 5, finasteride attenuated the increase in scores resulting from alcohol administration. The effects of finasteride were significant among A-allele homozygotes at $t = 30$ min ($F_{(1,25)} = 6.05$, $p = 0.021$), but were reduced to the level of a nonsignificant trend at $t = 40$ min ($F_{(1,25)} = 3.74$, $p = 0.065$).

Gastrointestinal Subscale: There were significant effects of Time ($F_{(2,50)} = 12.76$, $p < 0.001$), Genotype ($F_{(1,25)} = 7.18$, $p = 0.013$), and Time \times Genotype ($F_{(2,50)} = 5.18$, $p = 0.009$) on this subscale score. As shown in Figure 6, the increase in scores following alcohol administration was greater among A-allele homozygotes than among G-allele carriers. At $t = 30$ min A-allele homozygotes had higher scores on this measure ($F_{(1,25)} = 10.94$, $p = 0.003$), an effect that was no longer significant at $t = 40$ min ($F_{(1,25)} = 1.83$, $p = 0.19$).

Stimulation Subscale of the BAES. There was a significant effect of Time ($F_{(2,50)} = 4.97$, $p = 0.018$) and of Time \times Genotype \times Study Drug ($F_{(2,50)} = 3.56$, $p = 0.036$) on this subscale score (Figure 7). Decomposition of the three-way

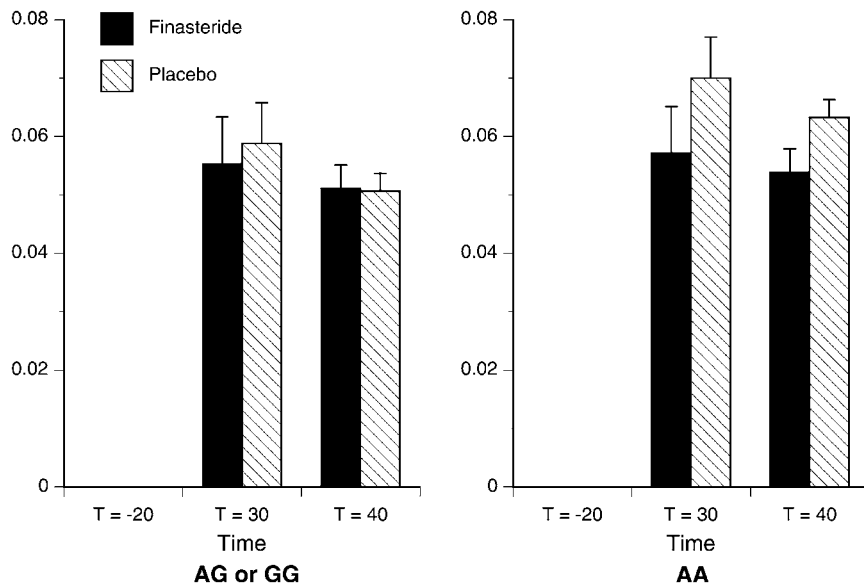


Figure 2 Mean (SEM) BrAC by Study Drug and Genotype. Significant effect of Time ($p < 0.001$), BrAC did not differ by Study Drug or Genotype. AA ($n = 7$), G-allele carriers ($n = 20$).

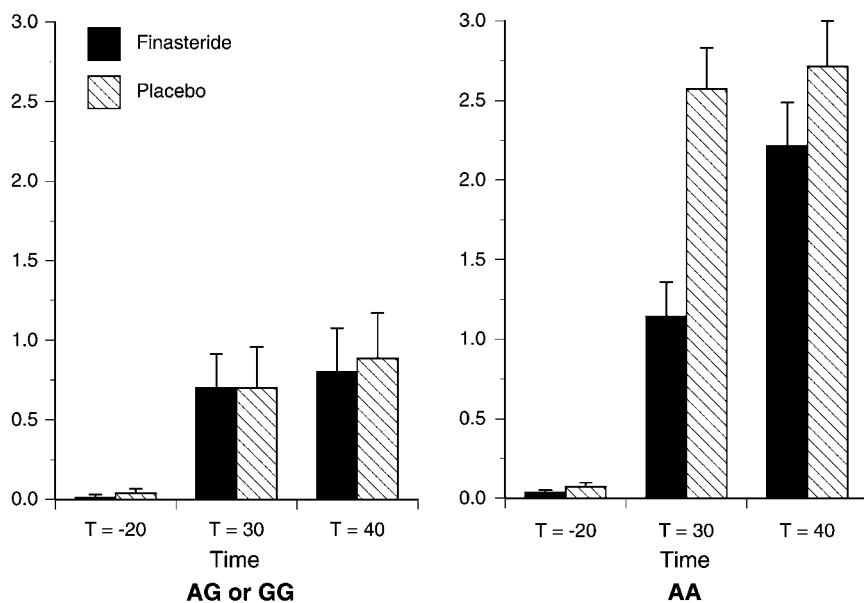


Figure 3 Estimated mean (SEM) for the Central-Stimulant Subscale score by Study Drug and Genotype. There were significant effects of Time ($p < 0.001$), Genotype ($p = 0.003$), Study Drug ($p = 0.008$), Time \times Genotype ($p = 0.002$), Time \times Study Drug ($p = 0.004$), Genotype \times Study Drug ($p = 0.017$), and Time \times Genotype \times Study Drug ($p = 0.002$). Finasteride significantly attenuated Central-Stimulant Subscale scores for the A-allele homozygotes compared to the G-allele carriers at $t = 30$ min ($p = 0.003$). AA ($n = 7$), G-allele carriers ($n = 20$).

interaction showed that, among G-allele carriers, scores remained stable across the three time points, during both the finasteride and placebo sessions. However, among subjects homozygous for the A-allele, the increase from baseline to $t = 40$ min was significantly steeper during the placebo session than during the finasteride session ($F_{(1,25)} = 6.86$, $p = 0.015$), an effect that was due only to the lower score on this measure at -20 min among A-allele carriers during the placebo session. However, analysis of

covariance, in which the baseline placebo value was used as a covariate, revealed a significant main effect of genotype ($F_{(1,24)} = 5.39$, $p = 0.029$), with A-allele homozygotes reporting greater stimulation following alcohol administration.

Drug Effects questionnaire. There was only a main effect of Time for the DEQ items 'feel alcohol effect' ($F_{(2,50)} = 68.95$, $p < 0.001$), 'feel alcohol high' ($F_{(2,50)} = 28.56$, $p < 0.001$), and

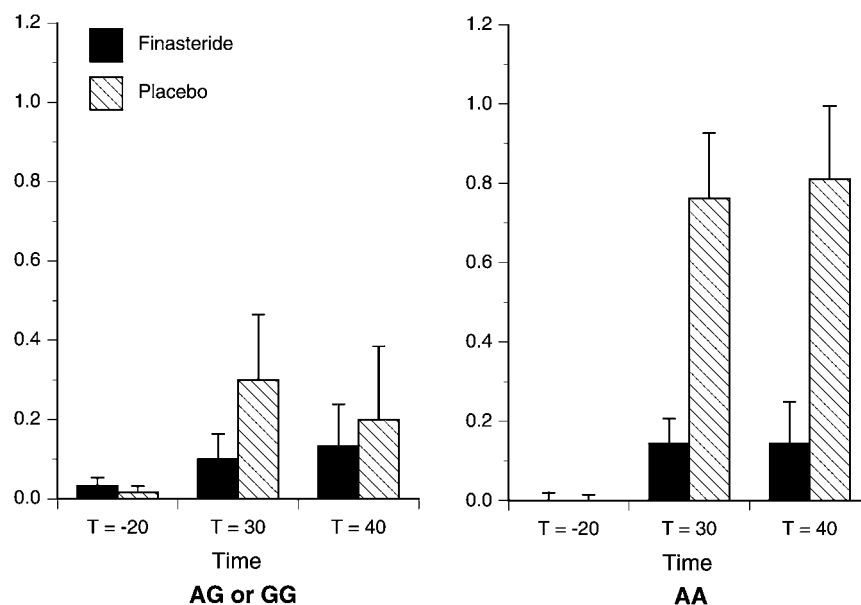


Figure 4 Estimated mean (SEM) for the Dynamic-Peripheral Subscale Score of the SS by Study Drug and Genotype. There were significant effects of Time ($p=0.002$), Study Drug ($p=0.046$), and Time \times Study Drug ($p=0.037$). Finasteride significantly attenuated the increase in Dynamic-Peripheral Subscale scores at $t=30$ min ($p=0.021$). AA ($n=7$), G-allele carriers ($n=20$).

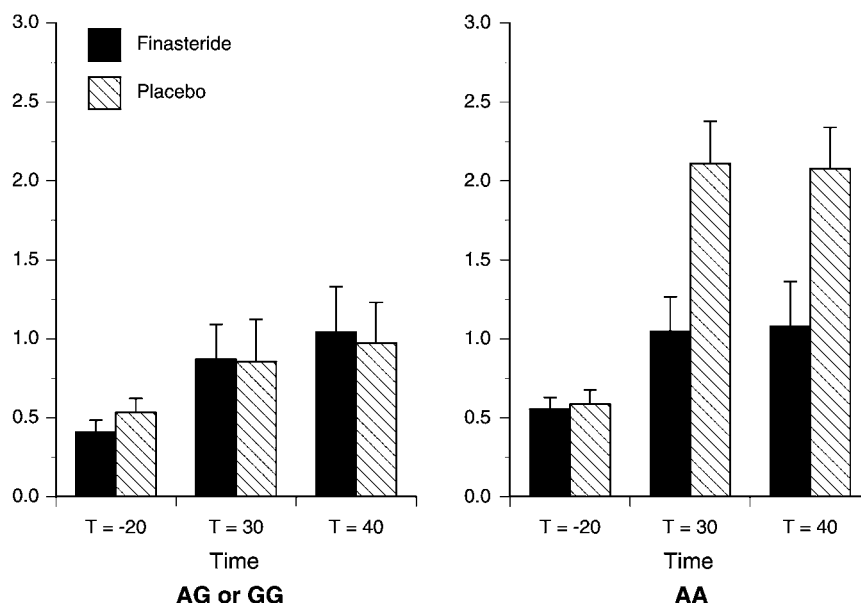


Figure 5 Estimated mean (SEM) for the Anesthetic Subscale Score of the SS by Study Drug and Genotype. There were significant effects of Time ($p=0.002$), Study Drug ($p=0.043$), Genotype \times Study Drug ($p=0.049$), and Time \times Genotype \times Study Drug ($p=0.024$). Finasteride significantly attenuated the increase in Anesthetic Subscale scores for the A-allele homozygotes compared to the G-allele carriers at $t=30$ min ($p=0.021$). AA ($n=7$), G-allele carriers ($n=20$).

'like alcohol' ($F_{(2,50)}=11.64$, $p=0.001$). For the item 'want more alcohol', there was a significant effect of Time ($F_{(2,50)}=6.76$, $p=0.010$), Time \times Study Drug ($F_{(2,50)}=4.03$, $p=0.025$), and Time \times Genotype \times Study Drug ($F_{(2,50)}=4.33$, $p=0.020$). As shown in Figure 8, although the genotype groups were comparable on this measure prior to alcohol administration, there was a trend for higher scores among A-allele homozygotes at $t=30$ min ($F_{(1,25)}=3.56$, $p=0.071$). Decomposition of the

three-way interaction showed that at $t=30$ min, A-allele homozygotes reported a greater desire to drink than did G-allele carriers, but only during the placebo session ($F_{(1,25)}=4.32$, $p=0.047$).

When analyses were repeated including only European-Americans (ie excluding the five Hispanic subjects), the overall pattern of effects was comparable to that seen in the larger sample, but reflected the reduced statistical power attributable to the smaller sample size.

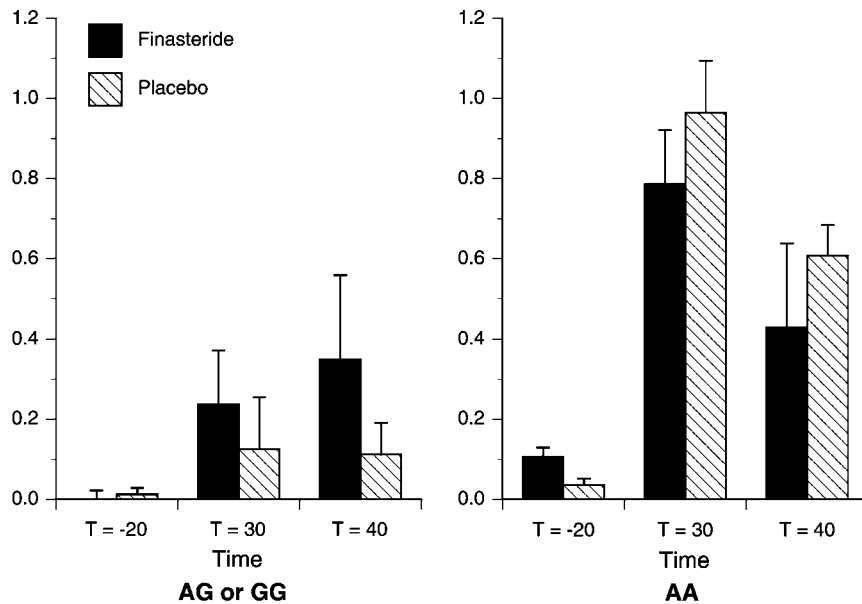


Figure 6 Estimated mean (SEM) for the Gastrointestinal Subscale Score of the SS by Study Drug and Genotype. There were significant effects of Time ($p < 0.001$), Genotype ($p = 0.013$), and Time \times Genotype ($p = 0.009$). Increased scores on the Gastrointestinal Subscale Score following alcohol administration were greater among A-allele homozygotes than among G-allele carriers at $t = 30$ min ($p = 0.003$). AA ($n = 7$), G-allele carriers ($n = 20$).

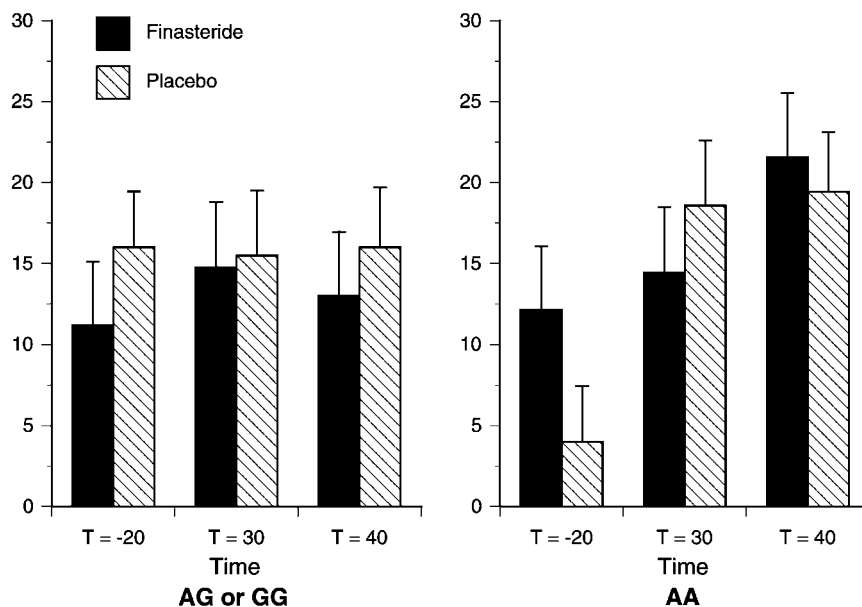


Figure 7 Estimated mean (SEM) for the Stimulation Subscale Score of the BAES as a function of Study Drug and Genotype. There were significant effects of Time ($p = 0.018$) and Time \times Genotype \times Study Drug ($p = 0.036$). Among subjects homozygous for the A-allele, the increase from baseline to $t = 40$ min was significantly steeper during the placebo session than during the finasteride session ($p = 0.015$). AA ($n = 7$), G-allele carriers ($n = 20$).

DISCUSSION

The most consistent finding in this study was an effect of genotype on many of the subjective effects of alcohol. There was also evidence that finasteride antagonized some alcohol effects, particularly among A-allele homozygotes. These findings are interesting in view of the animal and human literature implicating both GABA and neuroactive steroids in alcohol consumption and dependence.

There is consistent evidence that GABAergic systems mediate alcohol self-administration in animal models, probably by stimulating reward circuitry in the mesolimbic system (Chester and Cunningham, 2002). GABA_A receptor agonists increase both the speed of acquisition of alcohol drinking behavior (Petry, 1997; Smith *et al*, 1992) and the volume of alcohol consumed (Boyle *et al*, 1993; Pohorecky and Brick, 1988). Consistent with these findings, GABAergic inverse agonists diminish the reinforcing effects of alcohol

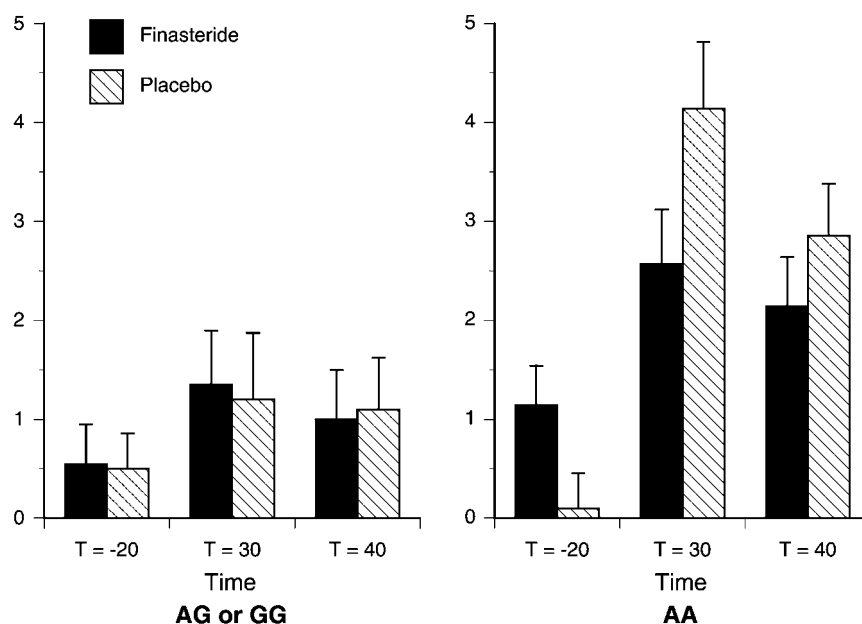


Figure 8 Estimated mean (SEM) for the 'want more alcohol' item of the DEQ by Study Drug and Genotype. There were significant effects of Time ($p = 0.010$), Time \times Study Drug ($p = 0.025$), and Time \times Genotype \times Study Drug ($p = 0.020$). At $t = 30$ min, there was a trend for higher scores among A-allele homozygotes ($p = 0.071$) than G-allele carriers and a greater increase among A-allele homozygotes than among G-allele carriers, but only following placebo treatment ($p = 0.047$). AA ($n = 7$), G-allele carriers ($n = 20$).

(Rassnick *et al*, 1993; Samson *et al*, 1987) and its intake (Balakleevsky *et al*, 1990; McBride *et al*, 1998).

Several GABA_A receptor subunits have been implicated in alcohol's action, so that the specific subunit composition of the receptor may be an important determinant of alcohol's CNS effects. The α -2 subunit of the GABA receptor mediates the anxiolytic effects of benzodiazepines (Harris *et al*, 1998; Rudolph *et al*, 1999; Low *et al*, 2000; Tobler *et al*, 2001), and enhances the hypnotic, but not the sedative, effects of combined exposure to alcohol and benzodiazepines (Tauber *et al*, 2003). In the present study, individuals homozygous for the A-allele at *GABRA2* reported greater stimulant and gastrointestinal subjective effects of alcohol (as measured on the Alcohol SS) compared with subjects having one or more G-alleles.

A recent genetic study has shown the G-allele to be over-represented among alcohol-dependent individuals (Covault *et al*, 2004). Alcoholism risk has been associated with a low level of response to alcohol, as measured by subjective feelings of intoxication following an alcohol challenge (Schuckit, 1984, 1994; Bauer and Hesselbrock, 1993; Schuckit *et al*, 1996). A family history of alcoholism has also been associated with a diminished response to alcohol in nonalcoholics (Moss *et al*, 1989; O'Malley and Maisto, 1985; Pollock *et al*, 1986; Savoie *et al*, 1988; Schuckit, 1984; Schuckit *et al*, 2000; cf. Newlin and Thompson, 1990; see Pollock, 1992 for a meta-analysis). Studies of monozygotic twins show greater similarity in sensitivity to an alcohol challenge than do dizygotic twins (Martin *et al*, 1985; Martin, 1988; Heath and Martin, 1992; Viken *et al*, 2003), providing evidence that alcohol sensitivity is an inherited trait. In view of this literature, one interpretation of the findings reported here is that the risk of alcoholism

associated in a population study with the G-allele (Covault *et al*, 2004) may, in part, be mediated by the decreased subjective response to alcohol of individuals carrying this allele.

The findings reported here indicate that A-allele individuals experience the stimulating effects of a moderate dose of alcohol soon after their BrAC rises. It is possible that, because of these subjective effects, such individuals may be less likely to continue drinking. In contrast, individuals with one or two copies of the G-allele may need to drink more alcohol to achieve a comparable level of alcohol-induced stimulation and may be more likely to drink if given access to alcohol. This interpretation appears to be contradicted by the finding that the A-allele homozygotes reported a greater desire for alcohol as measured by the DEQ item 'want more alcohol'. It is unclear whether self-reported desire for alcohol leads to subsequent drinking, so studies are needed that directly examine whether a priming dose of alcohol leads to greater subsequent alcohol consumption among A-allele homozygotes than among G-allele carriers. Furthermore, studies are needed to determine the effects of *GABRA2* alleles on drinking behavior as it occurs in natural settings.

In this study, we also examined the effects of finasteride, a 5AR inhibitor, on the subjective response to a moderate dose of alcohol in social drinkers. As hypothesized, finasteride reduced a number of the self-reported stimulant effects of alcohol, as measured by two subscales of the Alcohol SS (ie the Central-Stimulant and Dynamic-Peripheral Subscales), as well as the Stimulation Subscale of the BAES. These findings are consistent with the results of studies in rodents, which show that the pregnane neuroactive steroids, especially ALLO, mediate some of

the effects of acute alcohol administration (Morrow *et al*, 1999, 2001). In rats, the time course of the alcohol-induced increase in brain ALLO concentration paralleled the appearance of specific behavioral and electrophysiological effects of alcohol (Finn *et al*, 2004; VanDoren *et al*, 2000), which were reversed by finasteride (VanDoren *et al*, 2000; Khisti *et al*, 2003).

Finasteride also reduced the numbing effects of alcohol, as measured by the SS Anesthetic Subscale. The mechanisms implicated in the numbing effects of alcohol are not as well understood as the general anesthetic effects. A recent study suggests that neuroactive steroids contribute to the hypnotic effects of ethanol in rats (Khisti *et al*, 2003). Moreover, several studies (Mihic *et al*, 1997; Koltchine *et al*, 1999; Mascia *et al*, 2000; Ueno *et al*, 2001) have suggested that general anesthesia can be produced, at least in part, by enhancing neuronal inhibition mediated by the GABA_A α -2 subunit.

The strengths of this study include the choice of an SNP within *GABRA2* to predict the response to alcohol based on findings from two recent association studies that yielded highly convergent results of an allelic association to alcohol dependence. These findings were based on the positional and functional implication of *GABRA2* as a candidate contributing to alcoholism risk. The hypothesis that finasteride would attenuate the effects of alcohol were based on a well-developed animal literature and a developing human literature demonstrating that neuroactive steroids mediate a number of the effects of alcohol. In animal studies, finasteride has been shown to dampen those effects. Other strengths of the study include the fact that the subjects were well characterized and careful attention was paid to avoid potential confounders. In addition, the double-blind, placebo-controlled crossover design involved laboratory sessions separated by a period of time adequate to reduce the risk of a carryover effect.

The findings reported here must nonetheless be viewed in the context of the study's limitations. Although of interest in relation to alcoholism risk, the *GABRA2* polymorphism that was examined in this study as a moderator of alcohol's effects has not been shown to be functional; the variation in this gene that underlies the association with alcohol dependence remains to be determined. Although finasteride is a potent antagonist of type 2 5AR, it has modest antagonist activity at type 1 5AR (Tian *et al*, 1994; Tian, 1996), the predominant isoform in the mature human brain (Poletti *et al*, 1998; Stoffel-Wagner *et al*, 1998). To compensate for the lower antagonist activity of finasteride at the human brain type 1 5AR, we administered a high dose of the drug (ie a total of 200 mg over 24 h, which is 40 times the standard daily dose). Human studies using lower doses of finasteride, which inhibit only peripheral 5AR, would help to define the relative contribution of central or peripheral effects of finasteride on the subjective effects of alcohol in humans. Further, the lack of a placebo alcohol condition limited our capacity to ascertain whether the impact of finasteride on subjective responses was pharmacological or psychological (eg reflecting expectancies), or to determine the subjective effects produced by finasteride alone.

Since the literature on the effects of neuroactive steroids in humans is limited to ALLO (Torres and Ortega, 2003,

2004), we have focused primarily on that compound as a potential mediator of the observed effects of finasteride. However, we did not measure peripheral neuroactive steroid concentrations, so it is possible that the observed effects of finasteride were mediated by changes in other brain steroids. For example, changes in testosterone concentration may have mediated the observed subjective effects, consistent with a recent finding that testosterone concentrations are elevated in both men and women following acute alcohol intake (Sarkola and Eriksson, 2003; Sarkola *et al*, 2000). Although alcohol may also have increased levels of 5 β -reduced steroids, such as pregnanolone, finasteride blocks the conversion of progesterone and deoxycorticosterone to the 5 α -reduced metabolites, with no effect on the concentration of 5 β -reduced metabolites. This makes it unlikely that changes in concentration of the 5 β -reduced steroids provide an adequate explanation for the observed subjective effects.

Although the primary outcome measures used in the study were based on participants' self-report, the instruments that were chosen are used widely to evaluate alcohol-induced subjective effects. In this study, A-allele homozygotes were more sensitive to the subjective effects of alcohol. However, it is possible that the presence of the G-allele changed the threshold at which subjects experienced alcohol's effects, which may also have limited the potential effects of finasteride in the G-allele group. A study design in which the dose of alcohol is varied could be used to test this hypothesis by determining the concentration at which G-allele carriers experience substantial subjective effects. Subsequent studies of the impact of a 5AR inhibitor on a behavioral measure of alcohol's effects in humans should also include a physiological measure that is sensitive to alcohol effects, such as static ataxia, or by using an operant paradigm to measure alcohol self-administration.

The comparatively small number of subjects in this study, in particular the fact that only seven individuals were homozygous for the A-allele, raises the possibility that the findings occurred by chance. In addition, because only two individuals were homozygous for the G-allele, we were able to test only for a dominant effect of the G-allele by comparing carriers (ie AG or GG genotypes) with A-allele homozygotes. A larger sample would make it possible to examine an additive effect of the alleles by using all three genotype groups to examine both the response to alcohol and its modification by finasteride. In addition, the functional effects of the allelic variation at *GABRA2* are not understood (Edenberg *et al*, 2004; Covault *et al*, 2004), so that additional research is required to elucidate the potential mechanism by which the gene influences the response to alcohol.

Despite these limitations, the results of this study provided support for the hypothesis that *GABRA2* alleles moderate the stimulating effects of alcohol, and that these effects can be attenuated by finasteride. The effects of finasteride observed here are consistent with findings from an extensive animal literature and a growing human literature that implicate neuroactive steroids as mediators of some of the effects of alcohol. Further investigations of neuroactive steroid interactions with GABA_A receptor variants and their potential role in determining risk of alcohol dependence are warranted.

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