Naltrexone Selectively Elevates GABAergic Neuroactive Steroid Levels in Heavy Drinkers With the ASP40 Allele of the OPRM1 Gene: A Pilot Investigation

Lara A. Ray, Kent E. Hutchison, James R. Ashenhurst, and A. Leslie Morrow

Background: Preclinical studies have implicated GABAergic neurosteroids in behavioral responses to alcohol. Naltrexone is thought to blunt the reinforcing effects of alcohol, and a few studies have found that the effects of naltrexone are moderated by the Asn40Asp polymorphisms of the *OPRM1* gene. The present study seeks to integrate these lines of research by testing (i) the moderating role of the functional Asn40Asp polymorphism of the *OPRM1* gene on naltrexone-induced alternations in GABAergic neurosteroid levels, namely (3α,5α)-3-hydroxypregnan-20-one (allopregnanolone, ALLO); and (ii) the combined effects of naltrexone or genotype with alcohol administration on neurosteroid levels in a sample of at-risk drinkers.

Methods: Participants were 32 (9 females) nontreatment-seeking heavy drinkers who completed a placebo-controlled laboratory study of naltrexone (50 mg/d for 3 days) and provided complete sets of serum samples for ALLO assays before and after alcohol administration under both naltrexone and placebo conditions.

Results: Naltrexone treatment raised ALLO levels among carriers of the Asp40 allele, but not homozygotes for the Asn40 allele. The Asn40Asp polymorphism did not moderate effects of naltrexone on cortisol levels. Ethanol infusion modestly reduced ALLO levels in all subjects, independent of genotype or naltrexone exposure.

Conclusions: Naltrexone increased ALLO levels among individuals with the Asn40Asp allele suggesting a potential neurosteroid contribution to the neuropharmacological effects of naltrexone among Asp40 carriers.

Key Words: Naltrexone, Alcohol, Neuroactive Steroid, *OPRM1,* Genetics.

NALTREXONE (NTX) IS an opioid receptor antagonist with empirically supported efficacy for the treatment of alcoholism, when used in combination with behavioral treatments (Anton et al., 1999, 2006; Monti et al., 2001; O'Malley et al., 1992; Volpicelli et al., 1992). Human laboratory studies have shown that naltrexone dampens alcohol's positively reinforcing effects (Drobes et al., 2004; McCaul et al., 2001; Ray and Hutchison, 2007; Swift et al., 1994) and attenuates alcohol craving and self-administration (Anton et al., 2004; Tidey et al., 2008), compared to placebo.

The neurobiological mechanisms of action of naltrexone are less well characterized. It is generally posited that naltrexone works by occupying opioid receptors preventing the binding of such receptors by endogenous opioid peptides released upon alcohol intake. This in turn prevents the γ-aminobutyric acid (GABA)-mediated release of dopamine in the ventral tegmental areas thereby blocking alcohol's reinforcing effects (Johnson and North, 1992). Opioid blockade has been found to increase blood levels of adrenocorticotropic hormone (ACTH), beta-endorphin, and cortisol in humans (Naber et al., 1981; Schluger et al., 1998), although null findings have also been reported (Kemper et al., 1990). Thus, disinhibition of the hypothalamo-pituitary-adrenocortical (HPA) axis may be one of the neuropharmacological effects of naltrexone, as with other opioid blockers. This notion is also consistent with preclinical and clinical data suggesting that HPA-axis stimulation plays an important role in the neurobiology of alcoholism itself (Adinoff et al., 1998, 2005) and findings suggesting that blunted HPA-axis response predicts alcohol preference in rodents (Olive et al., 2003) and an increased risk of early relapse in humans (Junghanns et al., 2003, 2005).

Acute neuroendocrine responses to naltrexone have been examined in a few studies. NTX was found to increase cortisol levels in healthy male volunteers (Volavka et al., 1979) and to raise cortisol and ACTH levels among abstinent alcohol-dependent patients (Farren et al., 1999). King and

From the Department of Psychology (LAR); Brain Research Institute (LAR), University of California Los Angeles (UCLA), Los Angeles, California; The Mind Research Network and Department of Psychology (KEH), University of New Mexico, Albuquerque, New Mexico; Neuroscience Interdepartmental Program (JRA), University of California Los Angeles (UCLA), Los Angeles, California; and Departments of Psychiatry and Pharmacology (ALM), Bowles Center for Alcohol Studies, School of Medicine, University of North Carolina, Chapel Hill, North Carolina.

Received for publication September 24, 2009; accepted March 16, 2010.

Reprint requests: Lara A. Ray, Ph.D., Assistant Professor, University of California, Los Angeles, Psychology Department, 1285 Franz Hall, Box 951563, Los Angeles, CA 90095-1563; Tel: 301-794-5383; Fax: 310-206-5895; E-mail: lararay@psych.ucla.edu

DOI: 10.1111/j.1530-0277.2010.01233.x

colleagues (2002) found that naltrexone increased both ACTH and cortisol levels in healthy participants compared to placebo and that individuals with a family history of alcoholism displayed a heightened ACTH and cortisol response to naltrexone (King et al., 2002). Similarly, O'Malley and colleagues (2002) found that nontreatment-seeking alcohol-dependent individuals treated with naltrexone reported lower levels of cue-induced alcohol craving, higher cortisol levels before drinking, and higher levels of ACTH and cortisol during alcohol intake, when compared to placebo-treated participants. Interestingly, cortisol levels were inversely associated with self-reported alcohol craving (O'Malley et al., 2002). These findings underscore the need to further elucidate the neuropharmacological effects of naltrexone, including HPA-axis-mediated mechanisms, such as neurosteroid involvement.

Stress activation of the HPA-axis increases the GABAergic neuroactive steroids (3α,5α)-3-hydroxypregnan-20-one (allopregnanolone, ALLO), and (3α,5α)-3,21-dihydroxypregnan-20-one (tetrahydrodeoxycorticosteron, THDOC) in rodents (Purdy et al., 1991) and humans (Genazzani et al., 1998; Girdler et al., 2001). These steroids are potent modulators of $GABA_A$ receptors that produce anxiolytic, sedative, and anticonvulsant effects (for a review see Morrow, 2007). Several studies have implicated GABAergic neuroactive steroids in the regulation of the HPA stress response. Both ALLO and THDOC inhibit stress-induced corticotropin releasing factor (CRF) production and release, as well as ACTH release in rodents (Owens et al., 1992; Patchev et al., 1994, 1996). Intriguingly, a more recent animal study showed that ALLO injection in the absence of stress produced transient *increases* in basal CORT, ACTH, and CRF levels (Naert et al., 2007). Thus, ALLO may have a regulatory function such that in the basal state, ALLO increases the stress hormone production and secretion (Naert et al., 2007), while in the stressed state, ALLO decreases the activation to a lower level, contributing to the restoration of homeostasis following stress (Morrow, 2007). These findings underscore the interplay between ALLO and HPA-axis hormones, which in turn have been previously associated with naltrexone pharmacotherapy (King et al., 2002; O'Malley et al., 2002). No studies to date have examined the effects of naltrexone on neurosteroid levels. This study seeks to extend the literature by providing initial data on the effects of naltrexone on ALLO levels.

Ethanol administration (>1 g/kg) has been shown to increase ALLO and THDOC levels in rat brain and plasma (Barbaccia et al., 1999; Morrow et al., 1998, 1999; O'Dell et al., 2004; VanDoren et al., 2000). Preclinical studies have shown that the increase in GABAergic neuroactive steroids contributes to electrophysiological and behavioral effects of ethanol administration (Hirani et al., 2002; VanDoren et al., 2000), including impairment of spatial memory (Matthews et al., 2002; Morrow et al., 2001) and sedation (Khisti et al., 2003). In humans, reduced sensitivity to some of the effects of ethanol is associated with a greater risk of alcohol dependence (Schuckit, 1994; Schuckit and Smith, 1996). As such, GABAergic neurosteroid responses to ethanol may mitigate risk of alcohol dependence. Indeed, the 5α-reductase inhibitor finasteride, which blocks the synthesis of GABAergic neuroactive steroids, produced a reduction in subjective responses to alcohol, providing indirect support for the role of neuroactive steroids in the subjective effects of alcohol in humans (Pierucci-Lagha et al., 2005).

Furthermore, identified polymorphisms in the *OPRM1* gene may be an important factor in the link between naltrexone and neurosteroid levels. Recent studies in this sample (Ray and Hutchison, 2007) and others (Anton et al., 2008; Oslin et al., 2003) have suggested that the Asn40Asp SNP of the *OPRM1* gene may moderate responses to naltrexone; although other studies have failed to replicate these findings (Gelernter et al., 2007; Tidey et al., 2008). Our previous work has also implicated this polymorphism as a factor that increases subjective responses to alcohol in the laboratory (Ray and Hutchison, 2004) and in the natural environment (Ray et al., 2010). Because neurosteroids have also been implicated as a factor that increases alcohol sensitivity in rats (Morrow, 2007) and subjective effects of alcohol in humans (Pierucci-Lagha et al., 2005), we sought to determine whether the Asn40Asp SNP of the *OPRM1* gene moderates neuroactive steroid responses to naltrexone or ethanol.

In summary, this pilot study seeks to integrate previously unrelated lines of research by providing pilot data on the effects of naltrexone, alcohol, and the *OPRM1* gene on neuroactive steroid levels in humans. We examined levels of the neuroactive steroid ALLO because it is the most potent, efficacious, and abundant endogenous neurosteroid studied to date. This report represents a secondary analysis of a laboratory-based study of naltrexone and alcohol administration among at-risk drinkers. The specific aims of this study were to test the moderating role of the Asn40Asp polymorphism of the *OPRM1* gene on (i) naltrexone-induced alterations in ALLO levels, and (ii) the effect of alcohol administration on ALLO levels in humans as well as potential interactions between naltrexone and alcohol. Based on the available literature, it was hypothesized that naltrexone would elevate ALLO levels but that the naltrexone effects would be moderated by the Asn40Asp SNP of the *OPRM1* gene.

METHOD

Participants

Participants were 32 (9 females) nontreatment-seeking hazardous drinkers, all of whom met the following inclusion criteria: (i) age between 21 and 35; (ii) a score of 8 or higher on the Alcohol Use Disorders Identification Test (AUDIT), indicating a hazardous drinking pattern (Allen et al., 1997); (iii) self-reported drinking frequency of 3 or more drinks (2 for women) at least twice per week, indicating a current pattern of regular drinking; (iv) no history of adverse reactions to needle puncture; (v) no history of prior treatment for an alcohol use disorder (AUD) and no current interest in treatment for an AUD; (vi) no history of medical conditions that would contraindicate the study medication; and (vii) successfully completing a physical exam to ensure medical eligibility for the trial. Female participants completed a pregnancy test before each alcohol administration session. All participants were required to have a breath alcohol concentration (BrAC) of zero before each session. There was no upper-limit

to the AUDIT, and alcohol use disorders were not formally diagnosed; however, individuals with treatment history or currently interested in treatment were excluded from the study.

A total of 124 participants (39 women) were screened in the laboratory and 53 completed the physical exam. Because of efforts to oversample for the Asp40 allele of the OPRM1 gene, only 53 individuals were invited to participate in the medical exam and subsequent procedures. Invitations for the physical exam and remainder of the study were based on the results of DNA analysis. A total of 7 participants were ineligible for the study because of a medical reason, and 6 individuals decided not to participate in the trial, resulting in 40 participants (12 women) who completed the trial. Three participants did not provide a complete set of blood samples, and five participants did not provide sufficient blood for the ALLO assays leaving a sample of 32 participants (9 women) who provided complete data for the neurosteroid analyses reported in this study.

Procedures

Upon arrival at the laboratory, eligible participants read and signed an informed consent form, completed a series of individual difference measures (which are not the focus of this manuscript on pilot biological findings), and a DNA sample was obtained. After an individual's genotyping was complete, participants were invited to a physical exam at the General Clinical Research Center (GCRC), taking into account oversampling for the Asp40 allele. The medical exam consisted of a liver panel test and a medical history. The physical took place, on average, 2 weeks prior to the alcohol administrations. The criterion for medical exclusion was taking a medication or having a medical condition that counterindicated the use of naltrexone, or liver panel results indicating elevated liver enzymes, which counterindicated the alcohol administration. Individuals who passed the physical exam were then prescribed naltrexone (or placebo), and each participant completed two alcohol infusion sessions, one after taking naltrexone (50 mg/d) for 3 days, and one after taking a matched placebo for 3 days. Medication was delivered in a counterbalanced and double-blinded fashion. Experimental sessions started between 11:00 AM and 1:00 PM, and each participant's start time was identical between the 2 sessions. The wash-out period between infusions was at least 7 days.

A number of studies have highlighted the importance of effectively controlling blood alcohol levels in alcohol administration studies (Li et al., 2001; O'Connor et al., 1998; Ramchandani et al., 1999); hence, this study employed an intravenous alcohol administration, which produced tightly controlled levels of breath alcohol concentration at each assessment point, hence addressing individual differences in the pharmacokinetics of alcohol. In addition, a recent comparison of intravenous (IV) and oral alcohol administrations revealed that the IV administration produced higher ratings of subjective intoxication, even after controlling for blood alcohol concentration (Ray et al., 2007). Consistent with the procedures developed in our laboratory (Ray and Hutchison, 2004), the alcohol infusion was performed using a 5% ethanol IV solution, and a nomogram was developed taking into account participant's sex and weight. The formulas for determining target infusion rates were: 0.166-ml/min × weight, in kilograms, for males, and 0.126-ml/min × weight, for females. Participants received IV alcohol at their target infusion rate, and BrAC was monitored every 3 to 5 minutes. Sessions lasted an average of 3 to 4 hours, including time required for participants' BrAC to return to ≤0.02 g/dl. Participants were infused alcohol for an average of 75 minutes, such that the collection of blood samples at baseline and at BrAC = 0.06 g/dl occurred approximately 1.5 to 2 hours apart. Information regarding the menstrual cycle of female participants was not collected.

During the experimental sessions, participants were seated in a recliner chair and the IV was placed in their nondominant arm. Participants were told that they would receive IV doses of alcohol but remained blind to their BrAC throughout the trial. Participants were assessed before receiving the IV doses of alcohol (i.e., when BrAC = 0.00 g/dl) and at three target points in the ascending curve of the BrAC (BrAC: 0.02, 0.04, and 0.06 g/dl). After the third assessment point at the ascending limb, the infusion procedure was stopped, and participants were given a meal. All participants stayed in the laboratory until their BrAC was < 0.02 g/dl. Blood samples were collected in both medication conditions (i.e., naltrexone and placebo) at *baseline* (BrAC = 0.00 g/dl) and at the final *target BrAC level* (i.e., 0.06 g/dl).

Medication

Participants were required to take the first medication (naltrexone 50 mg or placebo) once a day for 2 days prior to the first experimental session and on the morning of their appointment. After the first session, participants were given the second medication, which they took in the same fashion prior to their second experimental session. Compliance was examined by packing the medication and placebo into capsules with 50 mg of riboflavin. Urine samples were collected prior to each infusion session and were analyzed for riboflavin content under an ultraviolet light, which makes the riboflavin detectable (Del Boca et al., 1996). All samples tested positive for riboflavin content verifying that the last dose was taken. Participant report and pill minder data were employed to verify compliance with the first two medication doses.

Cortisol Assay

Blood samples for cortisol were collected by the nursing staff into heparinized tubes (0.15 μl heparin) and placed on ice immediately after blood drawing. Within 15 minutes of collection, blood was centrifuged at 4°C and the serum transferred to a microtube stored at −20°C. The cortisol assay was performed at the Neurogenetics Core Laboratory at the Mind Research Network using a cortisol enzyme immunoassay (EIA) provided by Assay Designs (Catalog number 900-071), and protocol instructions were followed exactly as directed. The optical density of the EIA plate was read at 405 nm using a Multiskan Ascent plate reader from Thermo Scientific, and results were extrapolated using standard curves and a 4 parameter logistic curve fitting algorithm. The sensitivity of the cortisol assay is 0.22 μg/dl, the intra-assay coefficient of variation is 2.9%, and the inter assay coefficient of variation is 6.0%.

ALLO Radioimmunoassay

Allopregnanolone immunoreactivity was measured by radioimmunoassay following extraction and purification by column chromatography as previously described (Girdler et al., 2001; Janis et al., 1998). Plasma (1 ml) was thawed, and 4,000 dpm of [3H] ALLO was added to monitor extraction efficiency. ALLO was extracted twice with 8 ml of diethyl ether and dried under N2. Dried extracts were re-dissolved in 50 ml of isopropanol and 150 ml of sodium phosphate/bovine serum albumin (BSA) assay buffer (0.1 mol/l, 0.9%NaCl, pH 7.0, containing 1 g/l BSA). The re-dissolved extract (200 ml) was then used in the RIA determination of ALLO immunoreactivity levels (150 ml) or to measure the extraction efficiency (50 ml) by liquid scintillation spectroscopy. Sample extracts were incubated with ALLO antiserum and [^{3}H]ALLO for a minimum of 90 minutes. Bound steroids were separated from free steroids by the addition of ice-cold dextran-coated charcoal followed by centrifugation for 20 minutes at 2,000 *g*. Supernatants were collected and counted by liquid scintillation spectroscopy. Sample values are compared with a concurrently run standard curve analyzed using a one site competition model (Prism, Graphpad, San Diego, CA). Sample values are adjusted to account for the previously determined extraction efficiency. The radioimmunoassay of ALLO immunoreactivity

employed a sheep polyclonal antibody (1:1,000). The ALLO antiserum has minimal cross-reactivity with other circulating steroids, including THDOC (Janis et al., 1998). Cross-reactivity with progesterone (< 3%), as well as the stereochemical isomers of ALLO is minimal [(3α,5β)-3-hydroxypregnan-20-one 6.6%; (3β,5α)-3-hydroxypregnan-20-one 2.8%; (3β,5β)-3-hydroxypregnan-20-one 0.5%]. In contrast, the steroid (3α)-3-hydroxy-4-pregnen-20-one binds to the antibody to a greater degree than ALLO (169%). This compound, as well as (3α,5β)-3-hydroxypregnan-20-one, may contribute to the measurement of ALLO immunoreactivity. However, because these compounds are both potent agonists of $GABA_A$ receptor-mediated Cl uptake (Morrow et al., 1990), they would be expected to produce similar effects as ALLO and thus provide a more accurate estimate of GABAergic neuroactive steroids in plasma samples.

Tag SNP Selection

Consistent with the study hypotheses, we prioritized the Asn40Asp SNP of the OPRM1 gene. To hedge against the possibility that there might be an unknown functional polymorphism in linkage disequilibrium (LD) with the more widely studied Asn40Asp SNP, we used the bioinformatics resources from the International HapMap Project to identify tag SNPs (tSNPs). The parameters for our search were haplotype r^2 cut-off = 0.8 and a minor allele frequency (MAF) of 0.2, and the results recommended the following three tSNPs: rs510769, rs553202, rs2075572. This approach assumes that because the majority of SNPs are not functional, associations may be because of LD with a risk-increasing allele. Therefore, even at very high LD (range = 0 to 1, where 1 represents total LD), differences in allele frequency between the candidate SNP or tSNP and the risk-increasing locus can have a significant impact on the power to detect associations. Haplotype blocks were built using Haploview v4.1 (Barrett et al., 2005); see Fig. 1. The small sample size did not allow for full haplotype analyses, which would break up the sample into three groups (smaller groups with only 6 participants). However, to probe for the effects of the functional SNP (Asn40Asp), the tag SNP in block 1 (rs510769), where the functional SNP is located, was also examined.

DNA Analysis

DNA was collected following published procedures (Freeman et al., 1997; Walker et al., 1999). Participants swabbed their cheeks with three cotton swabs, followed by a rinse of the mouth with 10 ml of sucrose solution (4% in tap water). Genomic DNA was isolated from buccal cells using a modification of published procedures (Lench et al., 1988; Spitz et al., 1996). An ABI PRISM 7,500 instrument was used to conduct 5′-nuclease (TaqMan) assays of the *OPRM1* SNP and the probe tag SNP (rs510769) using assays commercially available from Applied Biosystems. This method relies on allele-specific hybridization of oligonucleotide probes (Livak, 1999). Genotyping accuracy was determined by replicate genotyping of at least 20% of the DNA samples at each locus and computing discrepancy rates. In this study, the discrepancy rates were < 5%, and discrepant findings were assayed a third time to determine the correct genotype.

Statistical Analysis

To test the hypothesis that naltrexone would raise ALLO levels more strongly among Asp40 carriers, a 2 × 2 repeated measures analyses of variance (ANOVAs) was conducted to examine the effects of *Medication* (naltrexone vs. placebo) and *Genotype* (Asn40Asn vs. Asp40Asn/Asp40Asp) on serum ALLO levels. Medication was a *within-subjects* factor, and genotype was a *between-subjects* factor. Alcohol (pre vs. post) was subsequently added to the model as a *within-subjects* factor. To further probe for the specificity of the genetic effect at the functional locus (Asn40Asp SNP of the OPRM1 gene), the genetic effect of a tag SNP (rs510769) located in the same haplotype block as the functional SNP was also tested.

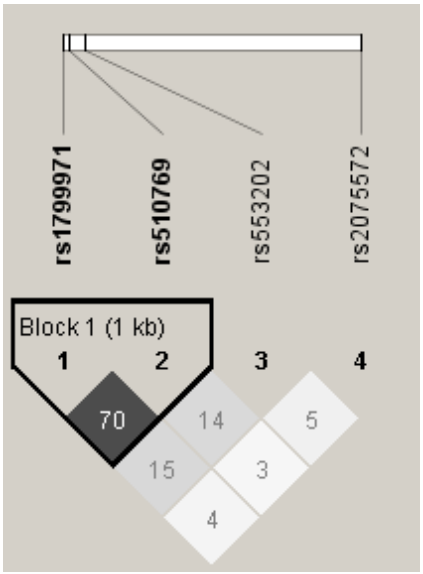

Fig. 1. Linkage disequilibrium plot from Haploview 4.1 based on data obtained from participants in this study for OPRM1 functional (rs1799971) and tag SNPs (rs510769, rs553202, and rs2075572). Pair-wise SNP |D'| values × (100) are shown along with one haplotype blocks identified using the 4-gamete rule. Darkened blocks indicate SNP pairs without evidence of extensive recombination (i.e., 4-gamete rule for haplotype block characterization with at least one 2-SNP haplotype having a frequency <0.02).

RESULTS

Sample Description

The average age of the sample was 22.2 (SD = 2.2; Range = 21 to 32), and 26 (82.3%) of the participants were Caucasian, 4 (12.5%) were Asian, and 2 (6.3%) were Latino. In this sample, the average number of drinks per drinking episode was 4.7 (SD = 2.2; Range = 2 to 12), the average drinking frequency was twice per week, and the average AUDIT score was 12.8 (SD = 4.4; Range = 8 to 21). The study was approved by the appropriate human subjects committee, and all participants provided written informed consent.

Given that this study oversampled for the Asn40Asp allele (Ray and Hutchison, 2007), a total of 21 (65.6%) participants in this report were homozygotes for the major allele (Asn40), 10 (31.3%) had one copy of the Asp40 allele, and 1 participant (3.1%) was homozygous for the Asp40 allele. Comparisons reported below are between Asn40 homozygotes (n = 21) and Asp40 carriers (both heterozygotes and homozygotes) (n = 11). For the OPRM1 tag SNP (rs510769), 14 (46.7%) participants in this report were homozygotes for the C allele, 14 (46.7%) were CT heterozygotes, 2 (6.6%) were homozygotes for the T allele, and 2 participants could not be

assayed for this SNP. Allele frequencies in the unselected screening sample ($n = 124$) were in conformity with Hardy–Weinberg Equilibrium for both the Asn40Asp SNP [$\chi^2(1) = 0.47$, $p > 0.05$] and the OPRM1 tag SNP selected as a probe [$\chi^2(1) = 0.07$, $p > 0.05$].

Baseline Comparisons

Genotype for the Asn40Asp allele of the *OPRM1* gene was unrelated to any of the demographic and baseline drinking measures, including ethnic background and family history. There was no effect of sex on ALLO levels at any of the four assessment points (*ps* > 0.05). There were no significant correlations between ALLO levels and average alcohol use frequency, quantity, or AUDIT scores (*p*s > 0.10). There were no significant differences in BrACs in the NTX vs. Placebo conditions, $F(1, 36) = 1.58$, $p = 0.22$. Therefore, it is highly unlikely that population stratification, sex, or other variables measured at baseline confounded the statistical analyses presented herein. A total of 3 participants in this sample were regular smokers, and smoking status had no effect on ALLO levels ($p = 0.86$). Likewise, sex ($p = 0.42$), ethnicity ($p = 0.83$), and family history of alcoholism ($p = 0.91$) were not associated with ALLO levels in this sample. In addition, sex ($p = 0.37$), ethnicity ($p = 0.48$), and family history of alcoholism ($p = 0.39$) were not associated with *OPRM1* status.

Genotype Effects on ALLO Levels

Naltrexone increased ALLO levels by 49.4% (post hoc test $p < 0.05$), relative to placebo, among individuals with the Asp40 allele of the *OPRM1* gene (AG/GG), but did not alter ALLO levels among homozygotes for the Asn40 allele. Specifically, percent change was calculated as the difference between ALLO after naltrexone versus placebo in the same subjects, given that medication was a within-subject factor. The planned 2 × 2 repeated measures ANOVA revealed a significant main effect of naltrexone medication [$F(1, 30) = 12.55$, $p < 0.01$], a main effect of genotype [$F(1, 30) = 5.47$, $p < 0.05$], and a medication × genotype interaction [$F(1, 30) = 7.74$, $p < 0.01$]; Fig. 2*A* depicts the genotype × medication interaction at baseline. This interaction remained significant even after alcohol administration. Additionally, the Asn40Asp SNP did not moderate the effect of naltrexone on basal cortisol levels in this trial [genotype × medication, $F(1, 30) = 0.80$, $p = 0.38$]; Fig. 2*B*. Mean (SD) ALLO levels across medication and alcohol conditions are presented in Table 1.

Tag SNP Probe

Analyses for the tag SNP (rs510769) located in the same haplotype block as the functional SNP revealed no significant main effect of naltrexone [$F(1, 28) = 3.59$, $p = 0.07$], no genotype main effect [$F(1, 28) = 2.48$, $p = 0.13$], and no genotype × medication interaction [$F(1, 28) = 0.97$, $p = 0.33$] on ALLO levels. Effect size estimates, using eta squared (η^2), indicated that the effect of the genotype × medication interaction on ALLO level was 0.26 for the functional SNP and 0.04 for the tag SNP, suggesting a precipitous decline in power to detect a pharmacogenetic effect.

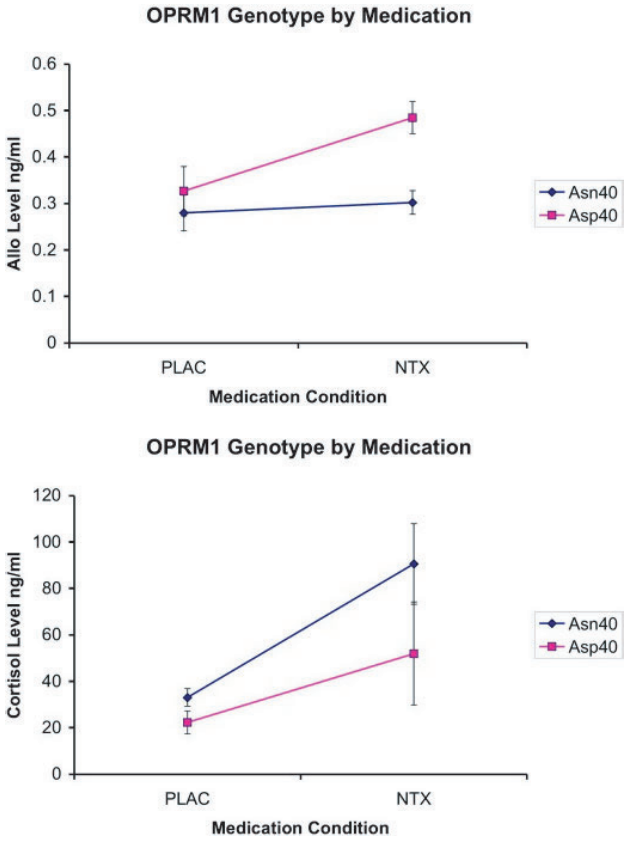

Fig. 2. Average ALLO level (ng/ml) (*A*) and cortisol level (ng/ml) (*B*) on naltrexone and placebo conditions before alcohol administration by *OPRM1* genotype. Analyses revealed a significant genotype × medication interaction for ALLO levels such that naltrexone elevated baseline ALLO levels among carriers of the Asp40 allele of the *OPRM1* gene, when compared to Asn40 homozygotes and placebo. There was no pharmacogenetic effect found for cortisol levels in this sample.

Table 1. Mean (SD) ALLO Levels (ng/ml) Across OPRM1 Genotype, Medication, and Alcohol Administration

	Placebo		Naltrexone	
	Pre alcohol	Post alcohol	Pre alcohol	Post alcohol
Asn40Asn	0.28 (0.11)	0.21 (0.09)	0.30 (0.12)	0.25 (0.11)
Asn40Asp/ Asp40Asp	0.33 (0.12)	0.27 (0.13)	0.49 (0.22)	0.38 (0.17)

Alcohol Effects on ALLO Levels

Alcohol decreased ALLO levels by 20.8% compared to baseline levels (i.e., pre–post alcohol ALLO level difference was compared for each subject), across medication condition,

and this difference was statistically significant, F (1, 27) = 34.81, $p < 0.0001$. The effects of naltrexone, genotype, and their interaction remained significant following alcohol infusion. In addition, there were no significant alcohol × genotype [F (1, 27) = 0.70, $p = 0.41$], alcohol × medication [F (1, 27) = 0.39, $p = 0.54$], or alcohol × genotype × medication [F (1, 27) = 1.53, $p = 0.23$] interactions.

DISCUSSION

Results of this study revealed that naltrexone significantly raised neurosteroid levels, when compared to placebo, and that these effects were moderated by the Asn40Asp SNP of the *OPRM1* gene. Specifically, carriers of the Asp40 allele displayed, on average, a 49% naltrexone-induced increase in ALLO levels. These findings advance previous work suggesting that naltrexone, as well as other opioid blockers, disinhibit the HPA-axis (Hernandez-Avila et al., 2002; Kiefer et al., 2006; King et al., 2002; O'Malley et al., 2002). This is relevant in light of the clinical literature suggesting that higher basal levels of cortisol may contribute to the relapse-preventing effects of naltrexone (Adinoff et al., 2005; Kiefer et al., 2006). Thus, it is plausible that the enhanced therapeutic effect of naltrexone among Asp40 carriers, reported in clinical samples (Anton et al., 2008; Oslin et al., 2003), may be due, at least in part, to its ability to increase ALLO levels among these individuals. The fact that a pharmacogenetic effect was found for ALLO but not for cortisol is not surprising, given that these steroids have different biosynthetic pathways, and as such, may serve different roles in alcohol sensitivity (Morrow et al., 2001). Clearly, further studies of clinical samples and integration of neurosteroids with clinical outcomes are needed to ascertain the therapeutic implications of the initial laboratory findings provided in this pilot study. Likewise, further studies of the role of neuroactive steroids in alcohol sensitivity in humans are clearly warranted. If supported by future research, these results suggest that GABAergic neurosteroids may be useful adjunctive therapy for alcoholism in those that lack the *OPRM1* allele associated with therapeutic efficacy to naltrexone. These findings also suggest that ALLO levels may be useful in elucidating naltrexone studies with null effects.

Interestingly, animal studies have shown that increases in GABAergic neuroactive steroids contribute to the behavioral effects of ethanol administration (Hirani et al., 2002; VanDoren et al., 2000), including enhanced sedation (Khisti et al., 2003). Given the differential subjective responses to alcohol reported for Asp40 carriers (Ray and Hutchison, 2004; Ray et al., 2010), it is plausible that naltrexone-induced increases in ALLO levels may underlie differential subjective intoxication profiles among these individuals. Further studies are necessary to address this hypothesis, including studies of higher alcohol doses and tests of the descending limb of alcohol intoxication.

Analyses of pharmacogenetic effects on cortisol assays suggested that the effects observed for ALLO levels were not seen for cortisol, even though naltrexone increased cortisol levels, across OPRM1 genotypes, in this sample (Ray et al., 2009). A recent study has found that carriers of the Asp40 allele had greater cortisol response to a naloxone challenge, when compared to homozygotes for the Asn40 allele (Hernandez-Avila et al., 2007). Interestingly, the pharmacogenetic effect on HPA-axis activation was population specific, and only found among individuals of European Ancestry and was not seen among individuals of Asian descent (Hernandez-Avila et al., 2007). Although these results are seemingly conflicting with the current study, given the complex interplay between neuroactive steroids and HPA-axis activation, these findings converge in underscoring the need to examine neurobiological markers of naltrexone pharmacogenetics to elucidate the mechanisms underlying their clinical and biobehavioral significance.

The confidence in the genetic findings obtained in this study is enhanced by the addition of a tag SNP probe (rs510769), which suggested no pharmacogenetic effect for the tag SNP despite high LD between the tag and the functional SNPs. Effect size estimates revealed a precipitous drop in genetic effect from the functional to the tag SNP, η^2 of 0.26 and 0.04, respectively. These results suggest an associated decrease in power to detect pharmacogenetic effects and underscore the need to characterize the functional significance of candidate SNPs under study. In addition to probing for the specificity of the genetic effect, analyses of pharmacogenetic effects on cortisol assays suggested that the effects observed herein were not seen for cortisol, even though naltrexone increased cortisol levels in this sample (Ray et al., 2009). Together, these additional analyses support the main finding that the pharmacogenetic effects of naltrexone are specific to the Asn40Asp locus and to neuroactive steroids.

Regarding the effects of alcohol, results revealed a small, but significant decrease in ALLO levels following alcohol consumption. These results are consistent with previous studies in humans showing that similar doses of ethanol reduce or do not alter plasma ALLO levels (Holdstock et al., 2006; Nyberg et al., 2005; Pierucci-Lagha et al., 2005). Notably these studies are somewhat inconsistent with the preclinical literature suggesting that ethanol administration (> 1 g/kg) increases ALLO and THDOC levels in rat brain and plasma (Barbaccia et al., 1999; Morrow et al., 1998, 1999; O'Dell et al., 2004; VanDoren et al., 2000). This inconsistency, however, may be explained by the moderate doses of ethanol administered in this study because equivalent ethanol doses fail to elevate neuroactive steroids in rat studies as well (Boyd et al., 2010; VanDoren et al., 2000). In humans, ethanol doses between 0.04 and 0.08 tend to maximize the stimulant effects of alcohol during the ascending limb of BrAC, while minimizing the sedative and unpleasant effects of alcohol. On balance, and to fully translate the preclinical findings of the effects of neurosteroids on alcohol intoxication, human alcohol administration studies with higher target BrAC are warranted. Importantly, the pharmacogenetic effect of naltrexone on neuroactive steroid levels remained significant even after alcohol administration and was not modulated by alcohol itself.

These initial results should be interpreted in light of the study's strengths and limitations. Study strengths include the double-blinded within-subjects counterbalanced design, an IV alcohol administration that produces high levels of control over BrAC and allows us to focus more directly on the pharmacology of alcohol, and prospective genotyping for the Asn40Asp SNP of the OPRM1 gene. As noted in this manuscript, the vast majority of the neurosteroid literature is preclinical in nature. This pilot study represents an initial examination of the contribution of GABAergic neurosteroids to naltrexone pharmacogenetics and alcohol administration in human samples. Limitations include the lack of a placebo alcohol condition (e.g., using a saline solution), a sample that was relatively small and composed of young nontreatment-seeking at-risk drinkers that may not generalize to clinical samples, and the use of an alcohol infusion administration, which differs from oral alcohol consumption (Ray et al., 2007). While our analyses provide no evidence of population stratification, these effects cannot be completely ruled out given the known differential allele frequency of the Asn40Asp SNP by ethnicity. Although future studies are necessary to further clarify these relationships, on balance, the observation that naltrexone increases GABAergic steroids in individuals with the Asp40 allele of the OPRM1 gene suggests a potential neurosteroid contribution to the neuropharmacological effects of naltrexone among these individuals.

ACKNOWLEDGMENTS

This research was supported by grants from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) to LR (AA14847), KEH (AA012238), and ALM (AA010564), and by a grant from the General Clinical Research Center Program of the National Center for Research Resources, National Institutes of Health (M01-RR00051). The authors wish to thank Amy Audette, Stephanie DiCristophoro, Keira Odell, and the staff at the General Clinical Research Center at the University of Colorado, at Boulder, for their contribution to data collection in this project.

REFERENCES

Adinoff B, Iranmanesh A, Veldhuis J, Fisher L (1998) Disturbances of the stress response: the role of the HPA axis during alcohol withdrawal and abstinence. Alcohol Health Res World 22:67–72.

Adinoff B, Junghanns K, Kiefer F, Krishnan-Sarin S (2005) Suppression of the HPA axis stress-response: implications for relapse. Alcohol Clin Exp Res 29:1351–1355.

Allen JP, Litten RZ, Fertig JB, Babor T (1997) A review of research on the Alcohol Use Disorders Identification Test (AUDIT). Alcohol Clin Exp Res 21:613–619.

Anton RF, Drobes DJ, Voronin K, Durazo-Avizu R, Moak D (2004) Naltrexone effects on alcohol consumption in a clinical laboratory paradigm: temporal effects of drinking. Psychopharmacology (Berl) 173:32–40.

Anton RF, Moak DH, Waid LR, Latham PK, Malcolm RJ, Dias JK (1999) Naltrexone and cognitive behavioral therapy for the treatment of outpatient alcoholics: results of a placebo-controlled trial. Am J Psychiatry 156:1758–1764.

Anton RF, O'Malley SS, Ciraulo DA, Cisler RA, Couper D, Donovan DM, Gastfriend DR, Hosking JD, Johnson BA, LoCastro JS, Longabaugh R, Mason BJ, Mattson ME, Miller WR, Pettinati HM, Randall CL, Swift R, Weiss RD, Williams LD, Zweben A (2006) Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: a randomized controlled trial. JAMA 295:2003–2017.

Anton RF, Oroszi G, O'Malley S, Couper D, Swift R, Pettinati H, Goldman D (2008) An evaluation of mu-opioid receptor (OPRM1) as a predictor of naltrexone response in the treatment of alcohol dependence: results from the Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence (COMBINE) study. Arch Gen Psychiatry 65:135–144.

Barbaccia ML, Affricano D, Trabucchi M, Purdy RH, Colombo G, Agabio R, Gessa GL (1999) Ethanol markedly increases "GABAergic" neurosteroids in alcohol-preferring rats. Eur J Pharmacol 384:R1–R2.

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265.

Boyd KN, Kumar S, O'Buckley TK, Porcu P, Morrow AL (2010) Ethanol induction of steroidogenesis in rat adrenal and brain is dependent upon pituitary ACTH release and de novo adrenal StAR synthesis. J Neurochem 112:784–796.

Del Boca FK, Kranzler HR, Brown J, Korner PF (1996) Assessment of medication compliance in alcoholics through UV light detection of a riboflavin tracer. Alcohol Clin Exp Res 20:1412–1417.

Drobes DJ, Anton RF, Thomas SE, Voronin K (2004) Effects of naltrexone and nalmefene on subjective response to alcohol among non-treatment-seeking alcoholics and social drinkers. Alcohol Clin Exp Res 28:1362–1370.

Farren CK, O'Malley S, Grebski G, Maniar S, Porter M, Kreek MJ (1999) Variable dose naltrexone-induced hypothalamic-pituitary-adrenal stimulation in abstinent alcoholics: a preliminary study. Alcohol Clin Exp Res 23:502–508.

Freeman B, Powell J, Ball D, Hill L, Craig I, Plomin R (1997) DNA by mail: an inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. Behav Genet 27:251–257.

Gelernter J, Gueorguieva R, Kranzler HR, Zhang H, Cramer J, Rosenheck R, Krystal JH (2007) Opioid receptor gene (OPRM1, OPRK1, and OPRD1) variants and response to naltrexone treatment for alcohol dependence: results from the VA Cooperative Study. Alcohol Clin Exp Res 31:555–563.

Genazzani AR, Petraglia F, Bernardi F, Casarosa E, Salvestroni C, Tonetti A, Nappi RE, Luisi S, Palumbo M, Purdy RH, Luisi M (1998) Circulating levels of allopregnanolone in humans: gender, age, and endocrine influences. J Clin Endocrinol Metab 83:2099–2103.

Girdler SS, Straneva PA, Light KC, Pedersen CA, Morrow AL (2001) Allopregnanolone levels and reactivity to mental stress in premenstrual dysphoric disorder. Biol Psychiatry 49:788–797.

Hernandez-Avila CA, Covault J, Wand G, Zhang H, Gelernter J, Kranzler HR (2007) Population-specific effects of the Asn40Asp polymorphism at the mu-opioid receptor gene (OPRM1) on HPA-axis activation. Pharmacogenet Genomics 17:1031–1038.

Hernandez-Avila CA, Oncken C, Van KirkJ, Wand G, Kranzler HR (2002) Adrenocorticotropin and cortisol responses to a naloxone challenge and risk of alcoholism. Biol Psychiatry 51:652–658.

Hirani K, Khisti RT, Chopde CT (2002) Behavioral action of ethanol in Porsolt's forced swim test: modulation by 3 alpha-hydroxy-5 alpha-pregnan-20-one. Neuropharmacology 43:1339–1350.

Holdstock L, Penland SN, Morrow AL, de Wit H (2006) Moderate doses of ethanol fail to increase plasma levels of neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one-like immunoreactivity in healthy men and women. Psychopharmacology (Berl) 186:442–450.

Janis GC, Devaud LL, Mitsuyama H, Morrow AL (1998) Effects of chronic ethanol consumption and withdrawal on the neuroactive steroid 3α-hydroxy-5α-pregnan-20-one in male and female rats. Alcohol Clin Exp Res 22:2055–2061.

Johnson SW, North RA (1992) Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 12:483–488.

Junghanns K, Backhaus J, Tietz U, Lange W, Bernzen J, Wetterling T, Rink L, Driessen M (2003) Impaired serum cortisol stress response is a predictor of early relapse. Alcohol Alcohol 38:189–193.

Junghanns K, Tietz U, Dibbelt L, Kuether M, Jurth R, Ehrenthal D, Blank S, Backhaus J (2005) Attenuated salivary cortisol secretion under cue exposure is associated with early relapse. Alcohol Alcohol 40:80–85.

Kemper A, Koalick F, Thiele H, Retzow A, Rathsack R, Nickel B (1990) Cortisol and beta-endorphin response in alcoholics and alcohol abusers following a high naloxone dosage. Drug Alcohol Depend 25:319–326.

Khisti RT, VanDoren MJ, O'Buckley T, Morrow AL (2003) Neuroactive steroid 3 alpha-hydroxy-5 alpha-pregnan-20-one modulates ethanol-induced loss of righting reflex in rats. Brain Res 980:255–265.

Kiefer F, Jahn H, Otte C, Naber D, Wiedemann K (2006) Hypothalamic-pituitary-adrenocortical axis activity: a target of pharmacological anticraving treatment? Biol Psychiatry 60:74–76.

King AC, Schluger J, Gunduz M, Borg L, Perret G, Ho A, Kreek MJ (2002) Hypothalamic-pituitary-adrenocortical (HPA) axis response and biotransformation of oral naltrexone: preliminary examination of relationship to family history of alcoholism. Neuropsychopharmacology 26:778–788.

Lench N, Stanier P, Williamson R (1988) Simple non-invasive method to obtain DNA for gene analysis. Lancet 1:1356–1358.

Li TK, Yin SJ, Crabb DW, O'Connor S, Ramchandani VA (2001) Genetic and environmental influences on alcohol metabolism in humans. Alcohol Clin Exp Res 25:136–144.

Livak KJ (1999) Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal 14:143–149.

Matthews DB, Morrow AL, Tokunaga S, McDaniel JR (2002) Acute ethanol administration and acute allopregnanolone administration impair spatial memory in the Morris water task. Alcohol Clin Exp Res 26:1747–1751.

McCaul ME, Wand GS, Stauffer R, Lee SM, Rohde CA (2001) Naltrexone dampens ethanol-induced cardiovascular and hypothalamic- pituitary-adrenal axis activation. Neuropsychopharmacology 25:537–547.

Monti PM, Rohsenow DJ, Swift RM, Gulliver SB, Colby SM, Mueller TI, Brown RA, Gordon A, Abrams DB, Niaura RS, Asher MK (2001) Naltrexone and cue exposure with coping and communication skills training for alcoholics: treatment process and 1-year outcomes. Alcohol Clin Exp Res 25:1634–1647.

Morrow AL (2007) Recent developments in the significance and therapeutic relevance of neuroactive steroids – Introduction to the special issue. Pharmacol Ther 116:1–6.

Morrow AL, Janis GC, VanDoren MJ, Matthews DB, Samson HH, Janak PH, Grant KA (1999) Neurosteroids mediate pharmacological effects of ethanol: a new mechanism of ethanol action? Alcohol Clin Exp Res 23:1933–1940.

Morrow AL, Pace JR, Purdy RH, Paul SM (1990) Characterization of steroid interactions with γ-aminobutyric acid receptor-gated chloride ion channels: evidence for multiple steroid recognition sites. Mol Pharmacol 37:263–270.

Morrow AL, VanDoren MJ, Devaud LL (1998) Effects of progesterone or neuroactive steroid? Nature 395:652–653.

Morrow AL, VanDoren MJ, Penland SN, Matthews DB (2001) The role of GABAergic neuroactive steroids in ethanol action, tolerance and dependence. Brain Res Brain Res Rev 37:98–109.

Naber D, Pickar D, Davis GC, Cohen RM, Jimerson DC, Elchisak MA, Defraites EG, Kalin NH, Risch SC, Buchsbaum MS (1981) Naloxone effects on beta-endorphin, cortisol, prolactin, growth hormone, HVA and MHPG in plasma of normal volunteers. Psychopharmacology (Berl) 74:125–128.

Naert G, Maurice T, Tapia-Arancibia L, Givalois L (2007) Neuroactive steroids modulate HPA axis activity and cerebral brain-derived neurotrophic factor (BDNF) protein levels in adult male rats. Psychoneuroendocrinology 32:1062–1078.

Nyberg S, Andersson A, Zingmark E, Wahlstrom G, Backstrom T, Sundstrom-Poromaa I (2005) The effect of a low dose of alcohol on allopregnanolone serum concentrations across the menstrual cycle in women with severe premenstrual syndrome and controls. Psychoneuroendocrinology 30:892–901.

O'Connor S, Morzorati S, Christian J, Li TK (1998) Clamping breath alcohol concentration reduces experimental variance: application to the study of acute tolerance to alcohol and alcohol elimination rate. Alcohol Clin Exp Res 22:202–210.

O'Dell LE, Roberts AJ, Smith RT, Koob GF (2004) Enhanced alcohol self-administration after intermittent versus continuous alcohol vapor exposure. Alcohol Clin Exp Res 28:1676–1682.

Olive MF, Mehmert KK, Koenig HN, Camarini R, Kim JA, Nannini MA, Ou CJ, Hodge CW (2003) A role for corticotropin releasing factor (CRF) in ethanol consumption, sensitivity, and reward as revealed by CRF-deficient mice. Psychopharmacology (Berl) 165:181–187.

O'Malley SS, Jaffe AJ, Chang G, Schottenfeld RS, Meyer RE, Rounsaville B (1992) Naltrexone and coping skills therapy for alcohol dependence. A controlled study. Arch Gen Psychiatry 49:881–887.

O'Malley SS, Krishnan-Sarin S, Farren C, Sinha R, Kreek MJ (2002) Naltrexone decreases craving and alcohol self-administration in alcohol-dependent subjects and activates the hypothalamo-pituitary-adrenocortical axis. Psychopharmacology (Berl) 160:19–29.

Oslin DW, Berrettini W, Kranzler HR, Pettinati H, Gelernter J, Volpicelli JR, O'Brien CP (2003) A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. Neuropsychopharmacology 28:1546–1552.

Owens MJ, Ritchie JC, Nemeroff CB (1992) 5 alpha-pregnane-3 alpha, 21-diol-20-one (THDOC) attenuates mild stress-induced increases in plasma corticosterone via a non-glucocorticoid mechanism: comparison with alprazolam. Brain Res 573:353–355.

Patchev VK, Hassan AH, Holsboer DF, Almeida OF (1996) The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. Neuropsychopharmacology 15:533–540.

Patchev VK, Shoaib M, Holsboer F, Almeida OF (1994) The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. Neuroscience 62:265–271.

Pierucci-Lagha A, Covault J, Feinn R, Nellissery M, Hernandez-Avila C, Oncken C, Morrow AL, Kranzler HR (2005) GABRA2 alleles moderate the subjective effects of alcohol, which are attenuated by finasteride. Neuropsychopharmacology 30:1193–1203.

Purdy RH, Morrow AL, Moore PH Jr, Paul SM (1991) Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc Natl Acad Sci U S A 88:4553–4557.

Ramchandani VA, Bolane J, Li TK, O'Connor S (1999) A physiologically-based pharmacokinetic (PBPK) model for alcohol facilitates rapid BrAC clamping. Alcohol Clin Exp Res 23:617–623.

Ray LA, Hutchison KE (2004) A polymorphism of the mu-opioid receptor gene (OPRM1) and sensitivity to the effects of alcohol in humans. Alcohol Clin Exp Res 28:1789–1795.

Ray LA, Hutchison KE (2007) Effects of naltrexone on alcohol sensitivity and genetic moderators of medication response: a double-blind placebo-controlled study. Arch Gen Psychiatry 64:1069–1077.

Ray LA, Mackillop J, Leggio L, Morgan M, Hutchison KE (2009) Effects of naltrexone on cortisol levels in heavy drinkers. Pharmacol Biochem Behav 91:489–494.

Ray LA, Meskew-Stacer S, Hutchison KE (2007) The relationship between prospective self-rating of alcohol sensitivity and craving and experimental results from two alcohol challenge studies. J Stud Alcohol Drugs 68:379–384.

Ray LA, Miranda R Jr, Tidey JW, McGeary JE, MacKillop J, Gwaltney CJ, Rohsenow DJ, Swift RM, Monti PM (2010) Polymorphisms of the micro-opioid receptor and dopamine D4 receptor genes and subjective responses to alcohol in the natural environment. J Abnorm Psychol 119:115–125.

Schluger JH, Ho A, Borg L, Porter M, Maniar S, Gunduz M, Perret G, King A, Kreek MJ (1998) Nalmefene causes greater hypothalamic-pituitary-adrenal axis activation than naloxone in normal volunteers: implications for the treatment of alcoholism. Alcohol Clin Exp Res 22:1430–1436.

Schuckit MA (1994) Low level of response to alcohol as a predictor of future alcoholism. Am J Psychiatry 151:184–189.

Schuckit MA, Smith TL (1996) An 8-year follow-up of 450 sons of alcoholic and control subjects. Arch Gen Psychiatry 53:202–210.

Spitz E, Moutier R, Reed T, Busnel MC, Marchaland C, Roubertoux PL, Carlier M (1996) Comparative diagnoses of twin zygosity by SSLP variant analysis, questionnaire, and dermatoglyphic analysis. Behav Genet 26:55–63.

Swift RM, Whelihan W, Kuznetsov O, Buongiorno G, Hsuing H (1994) Naltrexone-induced alterations in human ethanol intoxication. Am J Psychiatry 151:1463–1467.

Tidey JW, Monti PM, Rohsenow DJ, Gwaltney CJ, Miranda R Jr, McGeary JE, MacKillop J, Swift RM, Abrams DB, Shiffman S, Paty JA (2008) Moderators of naltrexone's effects on drinking, urge, and alcohol effects in non-treatment-seeking heavy drinkers in the natural environment. Alcohol Clin Exp Res 32:58–66.

VanDoren MJ, Matthews DB, Janis GC, Grobin AC, Devaud LL, Morrow AL (2000) Neuroactive steroid 3 alpha-hydroxy-5 alpha-pregnan-20-one modulate electrophysiological and behavioral actions of ethanol. J Neurosci 20:1982–1999.

Volavka J, Mallya A, Bauman J, Pevnick J, Cho D, Reker D, James B, Dornbush R (1979) Hormonal and other effects of naltrexone in normal men. Adv Exp Med Biol 116:291–305.

Volpicelli JR, Alterman AI, Hayashida M, O'Brien CP (1992) Naltrexone in the treatment of alcohol dependence. Arch Gen Psychiatry 49:876–880.

Walker AH, Najarian D, White DL, Jaffe JF, Kanetsky PA, Rebbeck TR (1999) Collection of genomic DNA by buccal swabs for polymerase chain reaction-based biomarker assays. Environ Health Perspect 107:517–520.