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

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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; Tidley 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 hypothalmo-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
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-hydroxyprogland-20-one (allo-pregnanolone, ALLO), and (3α,5α)-3,21-dihydroxyprogland-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<sub>γ</sub> receptors that produce anxiolytic, sedative, and anti-convulsant 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 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 administration. 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 the pharmacokinetics of alcohol. In addition, a recent comparison of 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 Hutchinson, 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 ml 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/1 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 [3H]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...
because these compounds are both potent agonists of GABAA receptors to contribute to the measurement of ALLO immunoreactivity. However, the compound, as well as (3b,5b)-3-hydroxy-4-pregnen-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 analysis of variance (ANOVA) 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.

**RESULTS**

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 \((p > 0.05)\). There were no significant correlations between ALLO levels and average alcohol use frequency, quantity, or AUDIT scores \((p > 0.10)\). 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 and placebo in the same subjects, given that medication was a within-subject factor. The planned 2 x 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 x genotype interaction \([F(1, 30) = 7.74, p < 0.01]\); Fig. 2A depicts the genotype x 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 x medication, \(F(1, 30) = 0.80, p = 0.38\)]; Fig. 2B. 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 x medication interaction \([F(1, 28) = 0.97, p = 0.33]\) on ALLO levels. Effect size estimates, using eta squared \((\eta^2)\), indicated that the effect of the genotype x medication interaction on ALLO 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.

**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 Asp40Asp 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, $\eta^2$ 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.

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