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Pregnenolone and ganaxolone reduce operant ethanol self-administration in alcohol-preferring P rats

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Abstract

Background—Neuroactive steroids modulate ethanol intake in several self-administration models with variable effects. The purpose of this work was to examine the effects of the long-acting synthetic GABAergic neurosteroid ganaxalone and the endogenous neurosteroid pregnenolone, a precursor of all GABAergic neuroactive steroids, on the maintenance of ethanol self-administration in an animal model of elevated drinking – the alcohol preferring (P) rats.

Methods—P rats were trained to self-administer ethanol (15% v/v) vs. water on a concurrent schedule of reinforcement, and the effects of ganaxolone (0 – 30 mg/kg, SC) and pregnenolone (0 – 75 mg/kg, IP) were evaluated on the maintenance of ethanol self-administration. After completion of self-administration testing, doses of the neuroactive steroids that altered ethanol self-administration were assessed on spontaneous locomotor activity. Finally, the effect of pregnenolone administration on cerebral cortical levels of the GABAergic neuroactive steroid (3α, 5α)-3-hydroxypregnan-20-one (allopregnanolone, 3α,5α-THP) was determined in both ethanol experienced and inexperienced P rats since pregnenolone is a precursor of these steroids.

Results—Ganaxolone produced a dose-dependent biphasic effect on ethanol reinforcement, as the lowest dose (1 mg/kg) increased and the highest dose (30 mg/kg) decreased ethanol-reinforced responding. However, the highest ganaxolone dose also produced a nonspecific reduction in locomotor activity. Pregnenolone treatment significantly reduced ethanol self-administration (50 and 75 mg/kg), without altering locomotor activity. Pregnenolone (50 mg/kg) produced a significant increase in cerebral cortical allopregnanolone levels. This increase was observed in the self-administration trained animals, but not in ethanol naïve P rats.

Conclusions—These results indicate that pregnenolone dose-dependently reduces operant ethanol self-administration in P rats without locomotor impairment, suggesting it may have potential as a novel therapeutic for reducing chronic alcohol drinking in individuals that abuse alcohol.

Keywords

ethanol self-administration; GABA; neuroactive steroids; ganaxolone; pregnenolone

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Introduction

GABAergic neuroactive steroids are endogenous neuromodulators derived primarily from progesterone ((3α,5α)-3-hydroxypregnan-20-one, allopregnanolone) and deoxycorticosterone ((3α,5α)-3,21-dihydroxypregnan-20-one, tetrahydrodeoxycorticosterone) that have nanomolar potency at $GABA_A$ receptors ((Morrow et al., 1987), see (Paul and Purdy, 1992), for review). These steroids produce anxiolytic, sedative-hypnotic, anticonvulsant and cognitive impairing effects when administered to rodents (see (Kumar et al., 2009), for review), and may also contribute to the behavioral effects of ethanol in rats, since ethanol increases plasma and brain levels of these steroids (Barbaccia et al., 1999; Morrow et al., 1999; Porcu et al., 2009; VanDoren et al., 2000). Indeed, blockade of ethanol-induced increases in neuroactive steroids using the steroid 5α-reductase inhibitor finasteride attenuates behavioral effects of ethanol in rodents (Hirani et al., 2002; Hirani et al., 2005; Khisti et al., 2003; Matthews et al., 2002; Morrow et al., 2001; VanDoren et al., 2000) and subjective effects of ethanol in humans (Pierucci-Lagha et al., 2005).

Several previous studies have explored whether systemic administration of the endogenous or synthetic GABAergic neuroactive steroids alters ethanol drinking behavior in rodents. Studies in non-dependent rats have shown that pretreatment with a 3 mg/kg dose of allopregnanolone, but not a 1 or 10 mg/kg dose, increases oral self-administration of ethanol (Janak et al., 1998). Similar studies in male C57BL/6J mice showed that allopregnanolone dose-dependently modulated ethanol intake during a two hour session, with low doses (3.2 mg/kg) increasing ethanol consumption and high doses (24 mg/kg) decreasing ethanol consumption (Ford et al., 2005b). The presence of ethanol dependence may influence these effects, since an identical dose of allopregnanolone (5mg/kg) can increase ethanol self-administration in non-dependent ethanol-preferring P rats while decreasing ethanol administration in ethanol-dependent P rats (Morrow et al., 2001). This may indicate a complex relationship where low dose neuroactive steroids promote drinking in non-dependent animals consuming small amounts of ethanol, while preventing heavy drinking by dependent P rats. Moreover, high doses of allopregnanolone suppress drinking in non-dependent animals (Ford et al., 2005b).

Allopregnanolone administered systemically is rapidly metabolized *in vivo* (Purdy et al., 1990), therefore it may be important to use longer acting neuroactive steroids for studies of ethanol self-administration sessions lasting longer than 20 minutes. Short acting compounds may produce a withdrawal state during the testing period that could confound the interpretation of the data. Indeed, the non-hydrolysable, long-acting GABAergic neuroactive steroid 3α,5β-20- oxo-pregnane-3-carboxylic acid dose-dependently reduces operant ethanol-self administration in rats (O'Dell et al., 2005). However, these prior studies did not control for potential effects on locomotor activity that could have influenced the results. Thus, further studies are needed to determine if GABAergic neuroactive steroids reduce excessive drinking in animal models of alcoholism.

Another approach to investigating the role of neuroactive steroids in alcohol drinking involves the administration of the 5α-reductase inhibitor finasteride. Finasteride has been shown to reduce basal and ethanol-induced increases in GABAergic neuroactive steroids (Moran and Smith, 1998; VanDoren et al., 2000). Finasteride administration can reduce ethanol consumption when administered 24 hrs prior to testing of ethanol consumption (Ford et al., 2005a; Ford et al., 2008b). However, the interpretation of these results is complex because finasteride increases pregnenolone and progesterone levels, while reducing allopregnanolone levels (Ford et al., 2008b). In addition, progesterone can be converted to another GABAergic neuroactive steroid, (3α)-3-hydroxy-4-pregnen-20-one, that is present

in rat brain (Griffin and Mellon, 2001) and has potent actions on $GABA_A$ receptors (Morrow et al., 1990).

The purpose of this study was to further clarify the role of neuroactive steroids in ethanol drinking by testing the effects of ganaxolone and pregnenolone on operant ethanol self-administration and locomotor activity in non-dependent alcohol-preferring (P) rats. Ganaxolone ((3α,3β,5α)-3-hydroxy-3-methyl-5-pregnan-20-one) is a synthetic GABAergic neuroactive steroid that differs from allopregnanolone only by the addition of the 3β-methyl group on C3 that prevents the metabolism of the compound, increasing the half life by hours (Carter et al., 1997). Investigation of the effect of ganaxolone addresses whether the biphasic effects of allopregnanolone on ethanol drinking are related to the rapid metabolism of this compound *in vivo*. Pregnenolone was investigated since it is the precursor of several endogenous GABAergic neuroactive steroids, including allopregnanolone (3α,5α-THP), pregnanolone (3α,5β-THP), tetrahydrodeoxycorticosterone (3α,5α-THDOC) and (3α3-hydroxy-4-pregnen-20- one, and has previously been shown to increase the concentrations of allopregnanolone and pregnanolone in rats and humans (Marx et al., 2009; Porcu et al., 2009). We examined the effects of ganaxolone and pregnenolone on ethanol reinforcement in P rats, a prominent genetic model of high alcohol intake (Lumeng et al., 1977; Murphy et al., 2002). The P rat line has been found to fulfill the requirements of an animal model of alcoholism (Lester and Freed, 1973), as these rats voluntarily consume alcohol in quantities that produce significant blood alcohol concentrations, develop tolerance and dependence through voluntary drinking (Kampov-Polevoy et al., 2000; Lumeng and Li, 1986; Murphy et al., 2002) and maintain preference for ethanol when palatable solutions are presented as an alternative (Lankford et al., 1991).

Methods

Subjects

Male ethanol-preferring inbred P (P) rats (n=16) were derived from a line provided by Indiana University. This stock of P rats (5B substrain) was derived from breeders of the selected line of P rats originally provided in 1999 to the Bowles Center for Alcohol Studies (courtesy of Dr. T.K. Li) and has been bred on-site at the University of North Carolina at Chapel Hill. These rats weighed 505.8 ±12.6 g (mean ± S.E.M.) at the beginning of testing and had access to food and water *ad libitum* unless specified otherwise. The rats were pair-housed in Plexiglas cages in a colony room maintained on a 12-h light/dark cycle. All experiments were performed during the light portion of this cycle. All procedures were carried out in accordance with the “Guide for the Care and Use of Laboratory Animals” (National Research Council, National Academy Press, 1996) and institutional guidelines.

Apparatus

The self-administration chambers (30.5 × 24.1 × 21.0 cm; Med Associates, Georgia, VT) were kept within sound attenuating cubicles, each equipped with its own exhaust fan to mask external sound and provide ventilation. The left and right wall of each chamber contained one liquid receptacle and a response lever (Med Associates). Lever press responses activated a syringe pump (Med Associates) that delivered 0.1 ml of solution into the receptacle across a 1.66-s period. Upon and during pump activation, a stimulus light located above each response lever was illuminated. Any lever press responses during reinforcer delivery were recorded but produced no programmed consequence. The self-administration chambers were interfaced (Med Associates) to a computer that was programmed to control sessions and record data.

Clear Plexiglas chambers measuring 43.2 × 43.2 cm (Med Associates) were utilized to assess locomotor activity. The chambers were interfaced to a computer that was programmed to record the number of photo beam breaks collected over 30 minutes.

Procedure

Prior to the first day of training, water was removed from the home cages for 24 hours. On the first training session, rats were placed in the self-administration chambers for a 16 hr shaping session in which the reinforcement schedule increased from a concurrent fixed ratio one (CONC FR1 FR1) to a CONC FR4 FR4. That is, once 4 reinforcers were delivered on a lever, the schedule on that lever increased to FR2. Once 10 total reinforcers were received on that lever, the schedule increased to FR4 (i.e., four lever responses resulted in the presentation of 0.1 ml of the solution paired with that lever) and that response requirement remained in effect for the duration of the session. At the end of this session, the rats were removed from the chambers and returned to their home cage. From this point forward, rats had access to water *ad libitum*. After this initial training session, rats were exposed to 30-minute sessions Monday-Friday on a CONC FR4 schedule of reinforcement in which they were trained to self-administer ethanol (15% v/v) versus water via a sucrose fading method (Samson, 1986). This method involved gradually introducing ethanol to the 10% sucrose (w/v) solution while gradually fading out the sucrose until a 15% (v/v) ethanol solution maintained responding. The schedule for this method started with 10% sucrose alone then proceeded as follows: 10% sucrose/2% ethanol (10S/2E), 10S/5E, 10S/10E, 5S/10E, 5S/15E, 2S/15E. Rats experienced each concentration for two sessions. Following the second 2S/15E session, 15% (v/v) ethanol was maintained for the remainder of the study. Ethanol was paired with the left lever for half of the rats and with the right lever for the other half of the rats. After 28 days of baseline training at 15% ethanol vs. water, testing of the neuroactive steroid compounds began. To habituate the rats to injections, prior to the start of testing, rats received 3 homecage vehicle injections (SC or IP; depending on group) approximately 3–4 h after the self-administration sessions.

Testing the effects of ganaxolone and pregnenolone on ethanol self-administration and locomotor activity

Two groups of rats were administered ganaxolone (0, 1, 3, 10, 20, 30 mg/kg, SC; n=8) or pregnenolone (0, 10, 20, 50, 75 mg/kg, IP; n=8) and returned to their home cage for 45 minutes. After this interval, the rats were placed in the self-administration chambers for a 30-minute testing session. Doses were administered using a Latin-square design. Each testing session was preceded by at least two days in which no drug was administered to allow for baseline level recovery. No tests occurred on Mondays as we usually observe a trend for higher self-administration on these days. Baseline responding remained stable between tests.

After completion of the self-administration assessments, the doses found to significantly alter ethanol self-administration were tested to determine nonspecific motor effects. Rats from each group were administered ganaxolone (0, 1, 20, 30 mg/kg; n=8) or pregnenolone (0, 50, 75 mg/kg; n=8) and returned to the home cage for 45 minutes. The rats were then placed in the locomotor chambers and spontaneous locomotor activity was monitored for 30-minute sessions. Dose order was randomly assigned and each rat experienced three locomotor sessions. These locomotor sessions were 30 minute in duration to parallel the self-administration study and were interspersed with self-administration sessions with at least 2 days between tests. Self-administration sessions were withheld on the locomotor test days.

Evaluating the effects of pregnenolone on cortical allopregnanolone levels in ethanol-experienced and -naïve P rats

The effects of pregnenolone administration on cerebral cortical levels of allopregnanolone were measured since pregnenolone is a precursor that is known to increase endogenous levels of GABAergic neuroactive steroids. After the conclusion of the locomotor assessments, rats experienced standard self-administration sessions for at least 1 month and then the effects of pregnenolone (50 mg/kg) on allopregnanolone levels in cerebral cortex were assessed. Rats were injected IP with pregnenolone (n=6) or vehicle (n=5) 45 minutes prior to brain tissue collection. Rats were not exposed to ethanol self-administration training on this day. Rats were decapitated and cortices were immediately dissected and placed on dry ice before storing at −80°C. To determine the effects of pregnenolone in ethanol naïve animals, a group of naïve P rats was administered pregnenolone (0 or 50 mg/kg, IP; n=7/dose) 45 minutes prior to sacrifice, and brain tissue was collected as described. A radioimmunoassay was then used to determine cerebral cortical allopregnanolone levels.

The assay was carried out as previously described in Janis et al. (1998). Briefly, brain samples were weighed and suspended in 2.5 ml of 0.3N NaOH, homogenized with a sonic dismembrator and extracted three times with 3 ml aliquots of 10% ethyl acetate in heptane (v/v). Extraction recovery was monitored by the addition of 2000 cpm of [^{3}H]allopregnanolone. The brain extracts were purified using solid phase silica columns (Burdick and Jackson, Muskegon, MI) and subsequently dried. Samples were reconstituted and assayed in duplicate by the addition of [^{3}H]allopregnanolone and anti-allopregnanolone polyclonal sheep antibody (Obtained from Dr. Robert Purdy, Scripps Research Institute). Total binding was determined in the absence of unlabeled allopregnanolone and nonspecific binding was determined in the absence of antibody. The antibody binding reaction was allowed to equilibrate for 2 hours and cold dextran-coated charcoal was used to separate bound from unbound steroid. Bound radioactivity was determined by liquid scintillation spectroscopy. Steroid levels in the samples were extrapolated from a concurrently run standard curve and corrected for their respective extraction efficiencies. The inter-assay coefficient of variation was 9.1% and the intra-assay coefficient of variation is 2.2%. The allopregnanolone antibody has minimal cross reactivity with other circulating steroids (Janis et al., 1998), including progesterone < 3%, 3α,5β-THP 6.6%, 3β,5α-THP 2.8%, 3β,5β-THP 0.5%, 5α-pregnan-3α,20α-diol 0.1% and 5α-pregnan-3,20-dione 3.5%. However, the antiserum cross-reacts with (3α3-hydroxy-4-pregnen-20-one > 100%. This steroid is also a potent modulator of $GABA_A$ receptors (Morrow et al., 1990) and has been found at comparable levels to allopregnanolone in serum (Wiebe et al., 1994), but at markedly lower levels than allopregnanolone in cerebral cortex (Griffin and Mellon, 2001).

Drugs

Ethanol (95%) was diluted in distilled water (15% v/v). Ganaxolone (Tocris, Ellisville MO), and pregnenolone (Steraloids, Newport, RI) were dissolved in 45% w/v 2-hydroxypropyl-β-cyclodextrin. Ganaxolone and pregnenolone were injected at 2 ml/kg (SC and IP, respectively) for the self-administration and locomotor experiments. Given that ganaxolone is identical in structure to allopregnanolone, except for the addition of a 3β-methyl group at C3 (Carter et al., 1997), the ganaxalone dose range was chosen based on previously published assessments of allopregnanolone (Finn et al., 2008; Nie and Janak, 2003). The pregnenolone dose range was selected based on prior work showing an increase in GABAergic neurosteroids at 50 mg/kg (Porcu et al., 2009). For the experiments measuring cortical allopregnanolone, pregnenolone was injected at a volume of 1 ml/kg.

Data analysis

For the self-administration studies, total responses on the ethanol and water levers and cumulative ethanol and water responses during the 30-minute sessions were analyzed by a two-way repeated measures analysis of variance (RM ANOVA). Ethanol intake (g/kg) was estimated from body weight and the number of reinforcers delivered and analyzed by a one-way RM ANOVA. For the locomotor assessments, total distance traveled in the 30-minute session was analyzed by a RM ANOVA. Student Newman Keuls' post hoc comparisons were used to extract significant main effects and interactions. Statistical significance was declared at $p \leq 0.05$.

Results

Baseline data (mean ± S.E.M.) were obtained the 2 days prior to the initiation of ganaxolone testing. Average ethanol lever responses were 149.5 ± 20.1, and average water lever responses were 6.1 ± 1.8. The baseline total ethanol intake was 0.79 ± 0.08 g/kg. Ganaxolone produced a biphasic effect on operant responding for ethanol (Figure 1A). The highest dose of ganaxolone (30 mg/kg) significantly reduced ethanol lever responding ($p<0.001$), while the lowest dose of ganaxolone (1 mg/kg) significantly increased ethanol lever responding ($p=0.04$). The two-way repeated measures ANOVA showed a significant main effect of ganaxolone dose ($F(4,24)=7.40$, $p<0.001$), a significant main effect of responses on the ethanol lever ($F(1,6)=93.38$, $p<0.001$) and a significant interaction between dose and ethanol lever responses ($F(4,24)=6.69$, $p<0.001$). In contrast, no dose of ganaxolone significantly affected water lever responding. Ethanol intake (g/kg) was significantly reduced by ganaxolone pretreatment (Table 1), and there was a trend for an increase at the lowest ganaxolone dose (1 mg/kg; $p=0.08$). Although not significant, there was a trend for reduced ethanol intake (g/kg) after vehicle administration relative to baseline levels ($p=0.09$).

Cumulative responses were examined to determine the pattern of ethanol responding across time (Figure 1B). There was a significant main effect of time ($F(5,30)=17.85$, $p<0.001$), a significant effect of ganaxolone dose ($F(4,24)=7.09$, $p<0.001$) and a dose and time interaction ($F(20,120)=3.59$, $p<0.001$). The highest ganaxolone dose (30 mg/kg) significantly reduced ethanol responding relative to vehicle after 5 minutes of the 30-minute self-administration session and this reduction continued for the remainder of the session ($ps<0.05$). Cumulative water responses were also examined (data not shown). There was a significant main effect of time on water responding ($F(5,30)=5.15$, $p=0.002$), but there was no significant main effect of ganaxolone dose. A significant interaction between dose and time existed ($F(20,120)=2.28$, $p=0.003$), but there was no significant difference between dose and vehicle across time according to post hoc tests. Analysis of the locomotor assessment showed a significant reduction in activity with ganaxolone (30 mg/kg) relative to vehicle ($p<0.001$; Figure 1C). Thus, the dose that significantly decreased ethanol self-administration (30 mg/kg) also produced a motor impairment in treated rats.

Baseline (2 days prior to the initiation of pregnenolone testing) ethanol lever responses were 147.8 ± 12.8, and average water lever responses were 14.2 ± 2.1. The baseline total ethanol intake was 0.84 ± 0.07 g/kg. Pregnenolone significantly reduced ethanol self-administration (Figure 2A). The two-way RM ANOVA showed a significant main effect of pregnenolone dose ($F(4,20)=5.05$, $p=0.006$), a significant main effect of responses on the ethanol lever ($F(1,5)=46.24$, $p=0.001$), and a significant interaction ($F(4,20)=3.79$, $p=0.019$). The post hoc comparisons indicated that 75 mg/kg ($p<0.001$) and 50 mg/kg ($p=0.003$) pregnenolone reduced total session ethanol (15%, v/v) reinforced responding relative to vehicle. Ethanol intake (g/kg) was significantly reduced by pregnenolone administration (75 mg/kg; Table 1). Ethanol intake after vehicle administration did not differ from the baseline value.

Examination of the cumulative ethanol responses showed a significant effect of time ($F(5,25)=24.12$, $p<0.001$), a significant effect of pregnanolone dose ($F(4,20)=5.57$, $p=0.004$), and a significant interaction ($F(20,100)=2.03$, $p=0.011$). Post hoc analysis indicated a significant reduction of ethanol responses after pregnenolone (50 and 75 mg/kg) pretreatment relative to vehicle after the first 5 minutes of the session and continuing for the remainder of the session (ps<0.05; Figure 2B). Cumulative water responding was not significantly affected by pregnenolone dose ($F(4,20)=2.63$, $p=0.07$), but was affected by time ($F(5,25)=9.14$, $p<0.001$). However, a significant interaction did exist between dose and time ($F(20,100)=1.79$, $p=0.03$). Water responding after administration of pregnenolone (75 mg/kg) differed from vehicle at the end of the 30-minute session ($p=0.03$). Analysis of the locomotor data showed there were no significant nonspecific motor effects following pregnenolone pretreatment (0, 50, 75 mg/kg; Figure 2C).

In ethanol-naïve P rats, pregnenolone treatment did not produce a significant increase in cortical allopregnanolone levels ($p=0.16$). In contrast, in ethanol-experienced P rats (i.e., the self-administration trained animals) pregnenolone administration significantly increased cortical allopregnanolone levels as compared to vehicle ($p=0.004$; Fig. 3).

Discussion

The goal of the present work was to examine the effects of the neuroactive steroids ganaxalone and pregnenolone on ethanol reinforcement in the alcohol-preferring P rat line, a genetic model of excessive alcohol drinking (Murphy et al., 2002). Indeed, ethanol intake in this study (after vehicle administration) ranged from 0.4 – 1.1 g/kg during the 30-minute sessions, which overlaps with the range we have previously reported corresponds to blood ethanol levels of approximately 80 mg/dl (Besheer et al., 2008). The results from this study show that ganaxalone administration produced a dose-dependent biphasic effect on operant ethanol self-administration. The lowest dose of 1 mg/kg increased ethanol reinforced responding, but the dose of 30 mg/kg significantly reduced ethanol reinforced responding. However the ganaxalone-induced reductions in ethanol self-administration may have been related to nonspecific reductions in motor activity. In contrast, pregnenolone administration reduced operant ethanol self-administration in the absence of nonspecific motor effects on motor activity. These results indicate that elevating pregnenolone may be an effective mechanism by which to reduce ethanol self-administration.

Previous work has shown neuroactive steroid modulation of ethanol drinking using two-bottle choice procedures and operant ethanol self-administration, with both increases and decreases in drinking reported (Ford et al., 2008a; Janak and Michael Gill, 2003; Janak et al., 1998; Morrow et al., 2001; O'Dell et al., 2005; Sinnott et al., 2002b). In the present study, pretreatment with the long-acting synthetic neuroactive steroid ganaxalone produced a bidirectional effect on ethanol-reinforced responding. At the lowest ganaxolone dose tested (1 mg/kg) total session responding on the ethanol lever was increased but ethanol intake (g/kg) showed only a trend, perhaps due to minor variations in body weight and number of ethanol reinforcers. The increase in responding was unrelated to motor activity as shown by the lack of effect of this ganaxalone dose on water responding and in the locomotor assessment. This finding of increased ethanol-reinforced responding has also been demonstrated after pretreatment with allopregnanolone, and like ganaxolone, allopregnanolone had biphasic effects (Janak et al., 1998) and reduced ethanol responding at higher doses (Ford et al., 2007). This result is also consistent with previous work showing that the long-acting non-hydrolyzable GABAergic neuroactive steroid 3α,5β-20-oxo-pregnane-3-carboxylic acid (PCA) reduces total session ethanol self-administration in Wistar rats (O'Dell et al., 2005). Thus, it appears that both short and long acting neuroactive steroids have similar biphasic effects on ethanol-reinforced responding. However, given that

ganaxolone also significantly suppressed spontaneous motor activity at the dose which reduced ethanol intake, it is possible that the reductions in self-administration were associated with nonspecific motor reductions. Indeed, high doses of allopregnanolone have been previously shown to reduce spontaneous locomotor activity (Wieland et al., 1995). Thus, given the associated motor impairment, it is premature to conclude that ganaxolone specifically reduces ethanol reinforcement. Ganaxolone has previously been shown to produce sedation that diminishes with chronic exposure (Nohria and Giller, 2007). Additional studies are needed to determine if the ganaxolone-induced reduction in ethanol responding and intake would show tolerance after chronic exposure.

The increase in ethanol-reinforced responding after administration of the low dose of ganaxolone suggests an increase in the reinforcing function of ethanol. Indeed, allopregnanolone has been shown to have reinforcing effects as rats display preference for orally consumed allopregnanolone over water in a two-bottle choice procedure (Sinnott et al., 2002a) and allopregnanolone can condition a place preference (Finn et al., 1997). A possible behavioral mechanism underlying the ganaxolone-induced increase in responding for ethanol in the present work may be related to drug priming. That is, a priming injection of the self-administered drug can increase drug-seeking behavior (de Wit and Stewart, 1981). An interpretation of this effect is that the stimulus properties of the self-administered drug have acquired control over behavior during self-administration training, and thus a drug with similar pharmacological properties would also promote drug-seeking behavior (de Wit and Stewart, 1981). Consistent with this explanation, is the finding that allopregnanolone can reinstate ethanol-seeking behavior (Finn et al., 2008; Nie and Janak, 2003), and that allopregnanolone has ethanol-like discriminative stimulus effects (Bowen et al., 1999; Grant et al., 1996; Hodge et al., 2001). Thus, it is possible that the low ganaxolone dose has ethanol-like effects that served as a priming cue to increase operant responding for ethanol, although ethanol intake was not enhanced. Interestingly, an increase in ethanol-reinforced responding was not evident after pregnenolone treatment in the present work. It is possible that a lower pregnenolone dose range than the one tested in the present study may enhance self-administration. This will be an interesting possibility to address in future work.

Ethanol self-administration was also significantly reduced by pregnenolone treatment. To assess the effects of pregnenolone over time, the pattern of responding was examined. Pregnenolone treatment at the two highest doses (50 and 75 mg/kg) suppressed ethanol responding throughout the 30-minute session, suggesting that pregnenolone effects were sustained for the duration of the session. This data pattern also suggests that the pregnenolone-induced reductions were likely not due to a pharmacological interaction with the consumed ethanol given that this pattern of responding resulted in little ethanol delivery. In contrast to the motor-impairing effects of the highest dose of ganaxolone, the locomotor assessment following pregnenolone administration showed no significant reduction in motor activity at the same doses that reduced ethanol self-administration, suggesting that suppression of self-administration was not related to nonspecific effects on motor activity. However, given that the highest pregnenolone dose (75 mg/kg) produced a significant reduction in cumulative water responses by the end of the self-administration session, a non-specific motor impairment at this dose cannot be entirely dismissed. Together these results suggest a selective role of the neuroactive steroid pregnenolone, but not ganaxolone in the reduction of ethanol self-administration.

Pregnenolone is the precursor of numerous steroid hormones. It is converted into progesterone and then deoxycorticosterone, precursors of the potent $GABA_A$ receptor positive modulators, such as allopregnanolone, 3α,5β-THP, and 3α,5α-THDOC. Previous work has determined a role for GABAergic systems in the modulation of ethanol reinforcement and drinking behavior (Besheer et al., 2006; Boyle et al., 1993; Hodge et al.,

1995; Hodge et al., 1996; McBride et al., 1988; Rassnick et al., 1993; Roberts et al., 1996), and the pregnenolone-induced reductions in self-administration may be related to increased activity of the GABAergic neuroactive steroids. However, pregnenolone is also the precursor of glucocorticoids (cortisol in humans and corticosterone in rodents), which have the potential to affect ethanol drinking (Chester et al., 2006; Lynch et al., 1999; Vengeliene et al., 2003; Volpicelli et al., 1990). Finally, the sulfated derivative of pregnenolone inhibits $GABA_A$ receptor function (Carette and Poulain, 1984; Majewska and Schwartz, 1987) and positively modulates NMDA receptors (Bowlby, 1993; Irwin et al., 1994; Wu et al., 1991), albeit at high micromolar concentrations and only when applied *in vitro*. Given its widespread actions, it will be interesting to determine the specific mechanism(s) modulating the pregnenolone-induced reduction in ethanol self-administration in future studies.

Previous studies have shown that GABAergic neuroactive steroids decrease ethanol self-administration and drinking (Janak et al., 1998; Martin-Garcia et al., 2007; Morrow et al., 2001; O'Dell et al., 2005), consistent with the decreased self-administration observed after pregnenolone administration in the present work. Further, given that allopregnanolone is a metabolite of pregnenolone, we sought to determine whether pregnenolone elevates allopregnanolone levels in P rats. Indeed, cerebral cortical allopregnanolone levels after pregnenolone administration showed a significant increase in the ethanol-experienced P rats (i.e., self-administration trained), but not in the ethanol naïve P rats. This increased sensitivity to pregnenolone administration in animals with a history of ethanol self-administration suggests the possibility of adaptations in neuroactive steroid biosynthesis. This result also suggests that the decrease in ethanol self-administration after pregnenolone administration may be related, in part, to increased levels of allopregnanolone.

In conclusion, results from the present work suggest that pregnenolone may be a novel therapeutic for reducing chronic ethanol drinking. An advantage to the utilization of this compound is that it has been used in various clinical populations and appears to be generally well tolerated with a positive safety profile (Freeman et al., 1950; Guest et al., 1950; Marx et al., 2009; Meieran et al., 2004). Further, recent work in schizophrenic patients showed that pregnenolone serum levels after pregnenolone administration were positively correlated with cognitive improvements (Marx et al., 2009). Evidence for cognitive improvements is significant given that cognitive deficits are common in alcohol dependent individuals (Fein et al., 1990; Pitel et al., 2007) and may interfere with effective therapy (Bates et al., 2006). Clearly, future work will need to elucidate the mechanism(s) by which pregnenolone affects alcohol drinking; however, the novel findings of this work suggest that pregnenolone may be a viable compound for reducing chronic alcohol drinking.

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References

Barbaccia ML, Affricano D, Trabucchi M, Purdy RH, Colombo G, Agabio R, Gessa GL. Ethanol markedly increases "GABAergic" neurosteroids in alcohol-preferring rats. Eur J Pharmacol. 1999; 384:R1–R2. [PubMed: 10611449]

Bates ME, Pawlak AP, Tonigan JS, Buckman JF. Cognitive impairment influences drinking outcome by altering therapeutic mechanisms of change. Psychol Addict Behav. 2006; 20:241–253. [PubMed: 16938062]

Besheer J, Faccidomo S, Grondin JJ, Hodge CW. Regulation of motivation to self-administer ethanol by mGluR5 in alcohol-preferring (P) rats. Alcohol Clin Exp Res. 2008; 32:209–221. [PubMed: 18162077]

Besheer J, Lepoutre V, Mole B, Hodge CW. $GABA_A$ receptor regulation of voluntary ethanol drinking requires PKCepsilon. Synapse. 2006; 60:411–419. [PubMed: 16881070]

Bowen CA, Purdy RH, Grant KA. An investigation of endogenous neuroactive steroid-induced modulation of ethanol's discriminative stimulus effects. Behavioral Pharmacology. 1999; 10:297–311.

Bowlby MR. Pregnenolone sulfate potentiation of N-methyl-D-aspartate receptor channels in hippocampal neurons. Molecular Pharmacology. 1993; 43:813–819. [PubMed: 7684817]

Boyle AE, Segal R, Smith BR, Amit Z. Bidirectional effects of GABAergic agonists and antagonists on maintenance of voluntary ethanol intake in rats. Pharmacol Biochem Behav. 1993; 46:179–182. [PubMed: 8255910]

Carette B, Poulain P. Excitatory effect of dehydroepiandrosterone, its sulphate ester and pregnenolone sulphate, applied by iontophoresis and pressure, on single neurones in the septo-preoptic area of the guinea pig. Neurosci Lett. 1984; 45:205–210. [PubMed: 6328376]

Carter RB, Wood PL, Wieland S, Hawkinson JE, Belelli D, Lambert JJ, White HS, Wolf HH, Mirsadeghi S, Tahir SH, Bolger MB, Lan NC, Gee KW. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3alpha-hydroxy-3beta-methyl-5alpha-pregnan-20-one), a selective, high-affinity, steroid modulator of the gamma- aminobutyric $acid_A$ receptor. J Pharmacol Exp Ther. 1997; 280:1284–1295. [PubMed: 9067315]

Chester JA, de Paula Barrenha G, DeMaria A, Finegan A. Different effects of stress on alcohol drinking behaviour in male and female mice selectively bred for high alcohol preference. Alcohol Alcohol. 2006; 41:44–53. [PubMed: 16299106]

de Wit H, Stewart J. Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology (Berl). 1981; 75:134–143. [PubMed: 6798603]

Fein G, Bachman L, Fisher S, Davenport L. Cognitive impairments in abstinent alcoholics. West J Med. 1990; 152:531–537. [PubMed: 2190421]

Finn DA, Mark GP, Fretwell AM, Gililland-Kaufman KR, Strong MN, Ford MM. Reinstatement of ethanol and sucrose seeking by the neurosteroid allopregnanolone in C57BL/6 mice. Psychopharmacology (Berl). 2008; 201:423–433. [PubMed: 18758755]

Finn DA, Phillips TJ, Okorn DM, Chester JA, Cunningham CL. Rewarding effect of the neuroactive steroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice. Pharmacol Biochem Behav. 1997; 56:261–264. [PubMed: 9050083]

Ford MM, Beckley EH, Nickel JD, Eddy S, Finn DA. Ethanol intake patterns in female mice: influence of allopregnanolone and the inhibition of its synthesis. Drug Alcohol Depend. 2008a; 97:73–85. [PubMed: 18486362]

Ford MM, Mark GP, Nickel JD, Phillips TJ, Finn DA. Allopregnanolone influences the consummatory processes that govern ethanol drinking in C57BL/6J mice. Behav Brain Res. 2007; 179:265–272. [PubMed: 17376546]

Ford MM, Nickel JD, Finn DA. Treatment with and withdrawal from finasteride alter ethanol intake patterns in male C57BL/6J mice: potential role of endogenous neurosteroids? Alcohol. 2005a; 37:23–33. [PubMed: 16472716]

Ford MM, Nickel JD, Phillips TJ, Finn DA. Neurosteroid modulators of $GABA_A$ receptors differentially modulate ethanol intake patterns in male C57BL/6J mice. Alcohol Clin Exp Res. 2005b; 29:1630–1640. [PubMed: 16205363]

Ford MM, Yoneyama N, Strong MN, Fretwell A, Tanchuck M, Finn DA. Inhibition of 5alpha-Reduced Steroid Biosynthesis Impedes Acquisition of Ethanol Drinking in Male C57BL/6J Mice. Alcohol Clin Exp Res. 2008b; 32:1408–1416. [PubMed: 18565155]

Freeman H, Pincus G, Bachrach S, Johnson CW, Mc CG, Mac GH Jr. Oral steroid medication in rheumatoid arthritis. J Clin Endocrinol Metab. 1950; 10:1523–1532. [PubMed: 14803541]

Grant KA, Azarov A, Bowen CA, Mirkis S, Purdy RH. Ethanol-like discriminative stimulus effects of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in female *Macaca fascicularis* monkeys. Psychopharmacology. 1996; 124:340–346. [PubMed: 8739549]

Griffin LD, Mellon SH. Biosynthesis of the neurosteroid 3 alpha-hydroxy-4-pregnen-20- one (3 alpha hp), a specific inhibitor of FSH release. Endocrinology. 2001; 142:4617–4622. [PubMed: 11606426]

Guest CM, Kammerer WH, Cecil RL, Berson SA. Epinephrine, pregnenolone and testosterone in the treatment of rheumatoid arthritis. J Am Med Assoc. 1950; 143:338–344. [PubMed: 15415262]

Hirani K, Khisti RT, Chopde CT. Behavioral action of ethanol in Porsolt's forced swim test: modulation by 3alpha-hydroxy-5alpha-pregnan-20-one. Neuropharmacology. 2002; 43:1339–1350. [PubMed: 12527484]

Hirani K, Sharma AN, Jain NS, Ugale RR, Chopde CT. Evaluation of GABAergic neuroactive steroid 3alpha-hydroxy-5alpha-pregnane-20-one as a neurobiological substrate for the anti-anxiety effect of ethanol in rats. Psychopharmacology. 2005; 180:267–278. [PubMed: 15719223]

Hodge CW, Chappelle AM, Samson HH. GABAergic transmission in the nucleus accumbens is involved in the termination of ethanol self-administration in rats. Alcohol Clin Exp Res. 1995; 19:1486–1493. [PubMed: 8749815]

Hodge CW, Haraguchi M, Chappelle AM, Samson HH. Effects of ventral tegmental microinjections of the $GABA_A$ agonist muscimol on self-administration of ethanol and sucrose. Pharmacol Biochem Behav. 1996; 53:971–977. [PubMed: 8801605]

Hodge CW, Nannini MA, Olive MF, Kelley SP, Mehmert KK. Allopregnanolone and pentobarbital infused into the nucleus accumbens substitute for the discriminative stimulus effects of ethanol. Alcohol Clin Exp Res. 2001; 25:1441–1447. [PubMed: 11696663]

Irwin RP, Lin S-Z, Rogawski MA, Purdy RH, Paul SM. Steroid potentiation and inhibition of N-methyl-D-aspartate receptor-mediated intracellular Ca^{++} responses: Structure activity studies. J Pharmacol Exp Ther. 1994; 271:677–682. [PubMed: 7965782]

Janak PH, Michael Gill T. Comparison of the effects of allopregnanolone with direct GABAergic agonists on ethanol self-administration with and without concurrently available sucrose. Alcohol. 2003; 30:1–7. [PubMed: 12878269]

Janak PH, Redfern JEM, Samson HH. The reinforcing effects of ethanol are altered by the endogenous neurosteroid, allopregnanolone. Alcohol Clin Exp Res. 1998; 22:1106–1112. [PubMed: 9726282]

Janis GC, Devaud LL, Mitsuyama H, Morrow AL. Effects of chronic ethanol consumption and withdrawal on the neuroactive steroid 3alpha-hydroxy-5alpha-pregnan-20-one in male and female rats. Alcohol Clin Exp Res. 1998; 22:2055–2061. [PubMed: 9884151]

Kampov-Polevoy AB, Matthews DB, Gause L, Morrow AL, Overstreet DH. P rats develop physical dependence on alcohol via voluntary drinking: changes in seizure thresholds, anxiety, and patterns of alcohol drinking. Alcohol Clin Exp Res. 2000; 24:278–284. [PubMed: 10776663]

Khisti RT, VanDoren MJ, O'Buckley TK, Morrow AL. Neuroactive steroid 3 -hydroxy-5 -pregnan-20-one modulates ethanol-induced loss of righting reflex in rats. Brain Res. 2003; 980:255–265. [PubMed: 12867266]

Kumar S, Porcu P, Werner DF, Matthews DB, Diaz-Granados JL, Helfand RS, Morrow AL. The role of GABA(A) receptors in the acute and chronic effects of ethanol: a decade of progress. Psychopharmacology (Berl). 2009; 205:529–564. [PubMed: 19455309]

Lankford MF, Roscoe AK, Pennington SN, Myers RD. Drinking of high concentrations of ethanol versus palatable fluids in alcohol-preferring (P) rats: valid animal model of alcoholism. Alcohol. 1991; 8:293–299. [PubMed: 1908249]

Lester D, Freed EX. Criteria for an animal model of alcoholism. Pharmacol Biochem Behav. 1973; 1:103–107. [PubMed: 4204511]

Lumeng, L.; Hawkins, TD.; Li, TK. New strains of rats with alcohol preference and non preference. In: Thurman, RG.; Williamson, JR.; Drott, H., editors. Alcohol and Aldehyde Metabolizing Systems. Academic Press; New York: 1977. p. 537-544.

Lumeng L, Li TK. The development of metabolic tolerance in the alcohol-preferring P rats: comparison of forced and free-choice drinking of ethanol. Pharmacol Biochem Behav. 1986; 25:1013–1020. [PubMed: 3786353]

Lynch WJ, Kushner MG, Rawleigh JM, Fiszdon J, Carroll ME. The effects of restraint stress on voluntary ethanol consumption in rats. Exp Clin Psychopharmacol. 1999; 7:318–323. [PubMed: 10609966]

Majewska MD, Schwartz RD. Pregnenolone-sulfate: An endogenous antagonist of the gamma-aminobutyric acid receptor complex in brain? Brain Res. 1987; 404:355–360. [PubMed: 3032339]

Martin-Garcia E, Darbra S, Pallares M. Intrahippocampal allopregnanolone decreases voluntary chronic alcohol consumption in non-selected rats. Prog Neuropsychopharmacol Biol Psychiatry. 2007; 31:823–831. [PubMed: 17329001]

Marx CE, Keefe RS, Buchanan RW, Hamer RM, Kilts JD, Bradford DW, Strauss JL, Naylor JC, Payne VM, Lieberman JA, Savitz AJ, Leimone LA, Dunn L, Porcu P, Morrow AL, Shampine LJ. Proof-of-concept trial with the neurosteroid pregnenolone targeting cognitive and negative symptoms in schizophrenia. Neuropsychopharmacology. 2009; 34:1885–1903. [PubMed: 19339966]

Matthews DB, Morrow AL, Tokunaga S, McDaniel JR. Acute ethanol administration and acute allopregnanolone administration impair spatial memory in the Morris water task. Alcohol Clin Exp Res. 2002; 26:1747–1751. [PubMed: 12436065]

McBride WJ, Murphy JM, Lumeng L, Li TK. Effects of Ro15–4513, fluoxetine and desipramine on intake of ethanol water and food in alcohol preferring and non-preferring lines of rats. Pharmacol Biochem Behav. 1988; 30:1045–1050. [PubMed: 3265788]

Meieran SE, Reus VI, Webster R, Shafton R, Wolkowotz OM. Chronic pregnenolone effects in normal humans: attenuation of benzodiazepine-induced sedation. Psychoneuroendocrinology. 2004; 29:486–500. [PubMed: 14749094]

Moran MH, Smith SS. Progesterone withdrawal I: Pro-convulsant effects. Brain Research. 1998; 807:84–90. [PubMed: 9757004]

Morrow AL, Janis GC, VanDoren MJ, Matthews DB, Samson HH, Janak PH, Grant KA. Neurosteroids mediate pharmacological effects of ethanol: A new mechanism of ethanol action? Alcohol Clin Exp Res. 1999; 23:1933–1940. [PubMed: 10630613]

Morrow AL, Pace JR, Purdy RH, Paul SM. Characterization of steroid interactions with -aminobutyric acid receptor-gated chloride ion channels: evidence for multiple steroid recognition sites. Mol Pharmacol. 1990; 37:263–270. [PubMed: 1689453]

Morrow AL, Suzdak PD, Paul SM. Steroid hormone metabolites potentiate GABA receptor-mediated chloride ion flux with nanomolar potency. Eur J Pharmacol. 1987; 142:483–485. [PubMed: 2828079]

Morrow AL, VanDoren MJ, Penland SN, Matthews DB. The role of GABAergic neuroactive steroids in ethanol action, tolerance and dependence. Brain Res Brain Res Rev. 2001; 37:98–109. [PubMed: 11744078]

Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, Lumeng L, Li TK. Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. Behav Genet. 2002; 32:363–388. [PubMed: 12405517]

Nie H, Janak PH. Comparison of reinstatement of ethanol- and sucrose-seeking by conditioned stimuli and priming injections of allopregnanolone after extinction in rats. Psychopharmacology. 2003; 168:222–228. [PubMed: 12719962]

Nohria V, Giller E. Ganaxolone. Neurotherapeutics. 2007; 4:102–105. [PubMed: 17199022]

O'Dell LE, Purdy RH, Covey DF, Richardson HN, Roberto M, Koob GF. Epipregnanolone and a novel synthetic neuroactive steroid reduce alcohol self-administration in rats. Pharmacol Biochem Behav. 2005; 81:543–550. [PubMed: 15950269]

Paul SM, Purdy RH. Neuroactive steroids. FASEB J. 1992; 6:2311–2322. [PubMed: 1347506]

Pierucci-Lagha A, Covault J, Feinn R, Nellissery M, Hernandez-Avila C, Oncken C, Morrow AL, Kranzler HR. GABRA2 alleles moderate the subjective effects of alcohol, which are attenuated by finasteride. Neuropsychopharmacology. 2005; 30:1193–1203. [PubMed: 15702134]

Pitel AL, Witkowski T, Vabret F, Guillery-Girard B, Desgranges B, Eustache F, Beaunieux H. Effect of episodic and working memory impairments on semantic and cognitive procedural learning at alcohol treatment entry. Alcohol Clin Exp Res. 2007; 31:238–248. [PubMed: 17250615]

Porcu P, O'Buckley TK, Alward SE, Marx CE, Shampine LJ, Girdler SS, Morrow AL. Simultaneous quantification of GABAergic 3alpha,5alpha/3alpha,5beta neuroactive steroids in human and rat serum. Steroids. 2009; 74:463–473. [PubMed: 19171160]

Purdy RH, Morrow AL, Blinn JR, Paul SM. Synthesis, metabolism, and pharmacological activity of 3alpha-hydroxy steroids which potentiate GABA-receptor-mediated chloride ion uptake in rat cerebral cortical synaptoneurosomes. J Med Chem. 1990; 33:1572–1581. [PubMed: 2160534]

Rassnick S, D'Amico E, Riley E, Koob GF. GABA antagonist and benzodiazepine partial inverse agonist reduce motivated responding for ethanol. Alcohol Clin Exp Res. 1993; 17:124–130. [PubMed: 8383923]

Roberts AJ, Cole M, Koob GF. Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. Alcohol Clin Exp Res. 1996; 20:1289–1298. [PubMed: 8904984]

Samson HH. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. Alcohol Clin Exp Res. 1986; 10:436–442. [PubMed: 3530023]

Sinnott RS, Mark GP, Finn DA. Reinforcing effects of the neurosteroid allopregnanolone in rats. Pharmacol Biochem Behav. 2002a; 72:923–929. [PubMed: 12062582]

Sinnott RS, Phillips TJ, Finn DA. Alteration of voluntary ethanol and saccharin consumption by the neurosteroid allopregnanolone in mice. Psychopharmacology. 2002b; 162:438–447. [PubMed: 12172699]

VanDoren MJ, Matthews DB, Janis GC, Grobin AC, Devaud LL, Morrow AL. Neuroactive steroid 3alpha-hydroxy-5alpha-pregnan-20-one modulates electrophysiological and behavioral actions of ethanol. J Neurosci. 2000; 20:1982–1989. [PubMed: 10684899]

Vengeliene V, Siegmund S, Singer MV, Sinclair JD, Li TK, Spanagel R. A comparative study on alcohol-preferring rat lines: effects of deprivation and stress phases on voluntary alcohol intake. Alcohol Clin Exp Res. 2003; 27:1048–1054. [PubMed: 12878910]

Volpicelli JR, Ulm RR, Hopson N. The bidirectional effects of shock on alcohol preference in rats. Alcohol Clin Exp Res. 1990; 14:913–916. [PubMed: 2088129]

Wiebe JP, De Gannes GC, Dallaire MJ. Synthesis of the allylic regulatory steroid, 3 alpha-hydroxy-4-pregnen-20-one, by rat granulosa cells and its regulation by gonadotropins. Biol Reprod. 1994; 50:956–964. [PubMed: 8199276]

Wieland S, Belluzzi JD, Stein L, Lan NC. Comparative behavioral characterization of the neuroactive steroids 3alpha-OH-5alpha-pregnan-20-one and 3alpha-OH,5beta-pregnan-20-one in rodents. Psychopharmacology. 1995; 118:65–71. [PubMed: 7597124]

Wu F-S, Gibbs TT, Farb DH. Pregnenolone sulfate: A positive allosteric modulator at the *N*-methyl-D-aspartate receptor. Molecular Pharmacology. 1991; 40:333–336. [PubMed: 1654510]

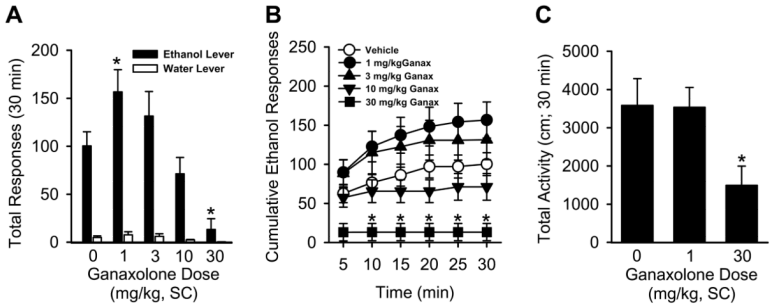

Figure 1.

Panel A. Mean (±SEM) total ethanol (15% v/v) and water responses 45 minutes after pregnenolone (0, 10, 20, 50, 75 mg/kg, IP; n=8) administration. Panel B. Mean (±SEM) cumulative ethanol responses over 30-minute self-administration testing session 45 minutes after pregnenolone pretreatment (n=8). Panel C. Mean (±SEM) total distance traveled (cm) 45 minutes after pregnenolone treatment (0, 50, 75 mg/kg, IP; n=8). *Notes a significant difference from vehicle (Student-Newman-Keuls' post hoc test, $p<0.05$).

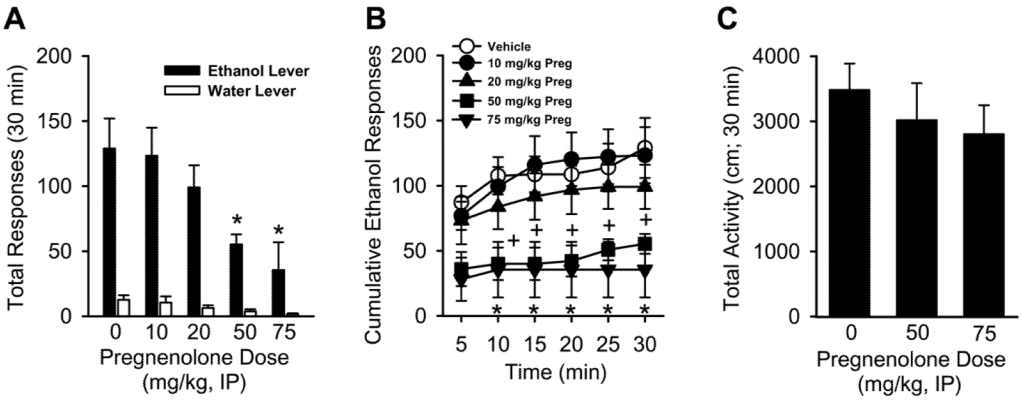

Figure 2.

Panel A. Mean (±SEM) total ethanol (15% v/v) and water responses 45 minutes after ganaxolone (0, 1, 3, 10, 30 mg/kg, SC; n=8) injection. Panel B. Mean (±SEM) cumulative ethanol responses over a 30-minute self-administration session following a 45 minute ganaxolone pretreatment (n=8). Panel C. Mean (±SEM) total distance traveled (cm) 45 minutes after ganaxolone pretreatment (0, 1, 3, 10, 30 mg/kg, SC; n=8). *Notes a significant difference from vehicle (Student-Newman-Keuls' post hoc test, $p<0.05$).

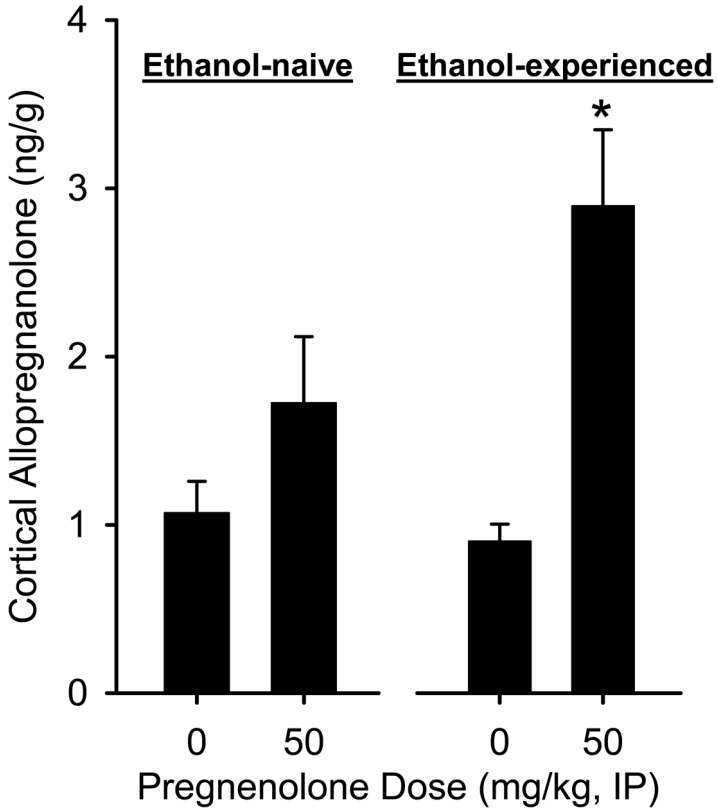

Figure 3.
Mean (±SEM) cortical allopregnanolone (ng/g) levels in ethanol-experienced (i.e., self-administration trained) and ethanol-naïve P rats determined after a 45-minute pregnenolone (0, 50 mg/kg, IP; n=5, n=6, respectively) pretreatment (t-test, $p<0.05$).

Table 1

Ethanol intake (g/kg) after ganaxolone and pregnenolone administration (mean ± SEM)

Ganaxolone dose (mg/kg, SC)				
0	**1**	**3**	**10**	**30**
0.55 ± 0.08	0.89 ± 0.11	0.69 ± 0.12	0.38 ± 0.10	**0.07 ± 0.06***
Pregnenolone dose (mg/kg, IP)				
0	10	20	50	75
0.72 ± 0.11	0.71 ± 0.12	0.57 ± 0.09	0.33 ± 0.04	**0.20 ± 0.12***

* $p<0.05$ relative to 0 (Student Newman Keuls' post hoc test)