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Laura O'Dell, *University of Texas at El Paso*

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Epipregnanolone and a novel synthetic neuroactive steroid reduce alcohol self-administration in rats

L.E. O'Dell[a,b], R.H. Purdy[a,c,d], D.F. Covey[e], H.N. Richardson[a], M. Roberto[a], G.F. Koob[a,*]

[a]*Department of Neuropharmacology, The Scripps Research Institute, CVN-7, 10550 North Torrey Pines Rd., La Jolla, CA, 92037, USA*
[b]*Department of Psychology, The University of Texas at El Paso, El Paso, TX, USA*
[c]*Department of Psychiatry, University of California San Diego, La Jolla, CA, USA*
[d]*Department of Veterans Affairs Medical Center and Veterans Medical Research Foundation, San Diego, CA, USA*
[e]*Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St Louis, MO, USA*

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Abstract

This study was designed to compare the effects of several neuroactive steroids with varying patterns of modulation of γ-aminobutyric acid $(GABA)_A$ and NMDA receptors on operant self-administration of ethanol or water. Once stable responding for 10% (w/v) ethanol was achieved, separate test sessions were conducted in which male Wistar rats were allowed to self-administer ethanol or water following pre-treatment with vehicle or one of the following neuroactive steroids: (3β,5β)-3-hydroxypregnan-20-one (epipregnanolone; 5, 10, 20 mg/kg; $n=12$), (3α,5β)-20-oxo-pregnane-3-carboxylic acid (PCA; 10, 20, 30 mg/kg; $n=10$), (3α,5β)-3-hydroxypregnan-20-one hemisuccinate (pregnanolone hemisuccinate; 5, 10, 20 mg/kg; $n=12$) and (3α,5α)-3-hydroxyandrostan-17-one hemisuccinate (androsterone hemisuccinate; 5, 10, 20 mg/kg; $n=11$). The effect of the 3β-epimer of PCA, (3β,5β)-20-oxo-pregnane-3-carboxylic acid (10, 20, 30 mg/kg; $n=9$), on ethanol self-administration was also examined. The compounds were administered using a Latin-square design 45 min prior to the weekly test sessions. The effects of the 30 mg/kg dose of the steroidal hemisuccinates on ethanol intake were also examined 5 min after administration of these drugs. Both epipregnanolone and PCA attenuated ethanol self-administration. However, neither of the hemisuccinate compounds significantly altered this behavior. The steroidal hemisuccinates (30 mg/kg; $n=7$) were also tested 5 min before behavior testing and had no effect on ethanol intake 5 min after administration. The 3β-epimer of PCA also failed to alter ethanol intake. None of the test compounds altered water intake. In electrophysiological studies, the effects of PCA and androsterone hemisuccinate on evoked $GABA_A$ receptor-mediated inhibitory postsynaptic currents ($GABA_A$-IPSCs) was examined in brain slices of the amygdala. PCA had a stimulatory effect at concentrations of 5 and 25 μM. Androsterone hemisuccinate had no agonist activity. The ability of epipregnanolone and PCA to alter ethanol intake appears to be related to different inhibitory actions of these compounds on either $GABA_A$ or NMDA receptors, respectively. Thus, dual modulation of these systems by selected neuroactive steroids may offer potential for modifying the reinforcing effects of alcohol.

Keywords: Alcohol; Self-administration; Epipregnanolone; Neuroactive steroid; Ethanol; Rat

1. Introduction

γ-Aminobutyric acid (GABA)-ergic neuroactive steroids have generated significant interest in the field of alcohol addiction because of their similar behavioral and inherent pharmacological activities with ethanol. For example, some neuroactive steroids like allopregnanolone, which is a potent positive modulator of $GABA_A$ receptors (Covey et al., 2001; Park-Chung et al., 1999), share behavioral properties with ethanol that include anxiolytic, anesthetic, and reinforcing effects (Kumar et al., 2004). Allopregnanolone also has been demonstrated to decrease the behavioral effects produced by withdrawal from ethanol

* Corresponding author. Tel.: +1 858 784 7062; fax: +1 858 784 7405.
E-mail address: gkoob@scripps.edu (G.F. Koob).

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(Finn et al., 2004). It is well established that the rewarding effects of ethanol are attenuated by administration of compounds that block $GABA_A$ receptors (Rassnick et al., 1993). Therefore, it is hypothesized that neuroactive steroids that serve to inhibit GABA transmission might decrease ethanol self-administration.

Glutamatergic systems are also targets for the actions of ethanol via its antagonism of the *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor. The major pharmacological sites of action for ethanol and for anionic neuroactive steroids, such as pregnanolone sulfate, include effects at GABA- and NMDA-receptor complexes (Park-Chung et al., 1997, 1999). Therefore, anionic neuroactive steroids might also alter the behavioral effects of ethanol by altering glutamate neuronal transmission.

The present study examined the effects of five different neuroactive steroids on ethanol self-administration in rats. These compounds differ in their structure as well as their effects on GABA and NMDA receptors (see Fig. 1 and Table 1). Epipregnanolone (Fig. 1A) is a 3β-hydroxysteroid that has been shown to serve as an antagonist of endogenous 3α-hydroxysteroids that are positive modulators of $GABA_A$ receptors (Prince and Simmonds, 1993). Epipregnanolone has also been shown in electrophysiological studies to antagonize pregnanolone-induced enhancement of GABA currents (Garrett and Gan, 1998; Maitra and Reynolds, 1998) and population spikes in rat hippocampal neurons (Wang et al., 2002). Further electrophysiological studies have demonstrated that epipregnanolone is not a direct antagonist of potentiating GABAergic steroids, but rather is a non-competitive blocker of $GABA_A$ receptors (Wang et al., 2002).

Two different hemisuccinate compounds were also tested for their influence on ethanol self-administration. These anionic compounds are of interest because of their effects on glutamate transmission. Pregnanolone hemisuccinate (Fig. 1B) acts as an NMDA receptor antagonist, and has been shown to be neuroprotective in rats (Weaver et al., 2000; Lapchak, 2004). The effects of the structurally related androsterone hemisuccinate, with a 17-keto group instead of the acetyl side chain, were also examined. The use of these two hemisuccinates provided an opportunity to examine the behavioral effects of anionic steroids that cross the blood–brain barrier on ethanol self-administration.

Table 1
Neuroactive steroid actions at GABA and NMDA receptors

Compound	Electrophysiological effects	
	$GABA_A$	NMDA
Epipregnanolone (Fig. 1A)	Inhibitory[a]	[Not active]
Pregnanolone hemisuccinate (Fig. 1B)	Inhibitory[b]	Inhibitory[c]
Androsterone hemisuccinate	[Not active][d]	[Not active][d]
PCA (non-ionized form shown in Fig. 1C)	Facilitory[d]	Inhibitory[d,e]
3β-Epimer of PCA (Fig. 1D)	Inhibitory[e]	[Not active][f]

[a] Wang et al. (2002).
[b] Park-Chung et al. (1999).
[c] Park-Chung et al. (1997).
[d] This paper.
[e] Mennerick et al. (2001).
[f] Zeng et al. (1999).

Finally, the discovery by Mennerick et al. (2001) of (3α,5β)-20-oxo-pregnane-3-carboxylic acid (PCA; Fig. 1C), a synthetic non-hydrolizable and CNS-available carboxylic acid analog of pregnanolone hemisuccinate, provides a unique neuroactive steroid that has been shown to have a negative modulatory action on NMDA receptors and to potentiate $GABA_A$ receptors in its non-ionized form. The 3β-epimer of PCA also inhibited GABA currents in cell cultures (Mennerick et al., 2001), but did not significantly affect NMDA currents (Zeng et al., 1999).

The goal of the present study was to determine if any of the above neuroactive steroids was capable of reducing ethanol self-administration in rats. Briefly, rats were trained in an operant procedure to self-administer ethanol. Once responding was stabilized, separate groups of rats received pre-treatment with one of the five neuroactive steroids described above, and 45 min later were given access to self-

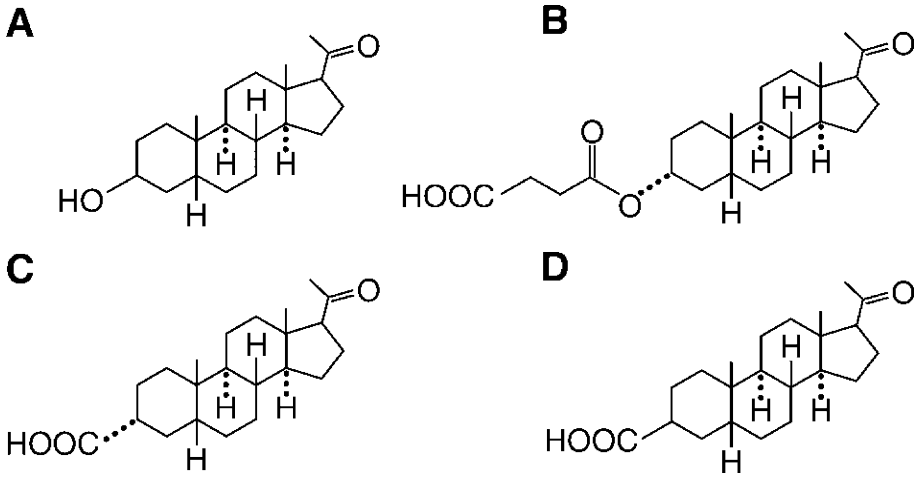

Fig. 1. Structures of neuroactive steroids: (A) (3β,5β)-3-hydroxypregnan-20-one (epipregnanolone); (B) (3α,5β)-3-hydroxypregnan-20-one hemisuccinate (pregnanolone hemisuccinate); (C) (3α,5β)-20-oxo-pregnane-3-carboxylic acid (PCA); (D) (3β,5β)-20-oxo-pregnane-3-carboxylic acid (3β-epimer of PCA).

administer ethanol. Various doses of the drugs were examined in a Latin-square design, and animals were allowed to respond for ethanol between the test days. In addition, experiments were conducted to identify the cellular actions of androsterone hemisuccinate which were not available from the literature. The previously demonstrated GABA agonist actions of PCA by Mennerick et al. (2001) were also extended. It was our working hypothesis that one or more of these neuroactive steroids would significantly reduce ethanol self-administration in a dose-dependent manner.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 150–200 g at the start of the experiment were obtained from Charles River Laboratory (Kingston, NY). Rats were housed 2–3 per cage with food and water available ad libitum, except for 3 days of water restriction during the initiation of operant training. Separate groups of rats were used to examine the effects of the various neuroactive steroids. Lights were on a 12-h light/dark cycle, with lights on at 6:00 AM. All procedures met the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

PCA and its 3β-epimer were synthesized as described by Zeng et al. (1999). The hemisuccinates of epipregnanolone and androsterone were prepared as described elsewhere (Park-Chung et al., 1997). Epipregnanolone was prepared by hydrolysis of 3β-acetoxy-5β-pregnan-20-one (Steraloids, Inc., Newport, RI) and purification by column chromatography. The test compounds were delivered in a 3 ml/kg injection volume using 40% (w/v) β-hydroxypropyl-β-cyclodextrin (Aldrich, Milwaukee, WI) as the vehicle.

2.3. Operant ethanol self-administration

Ethanol (10% w/v) was prepared with 95% ethyl alcohol and water. Saccharin (Sigma Chemical Co., St. Louis, MO) was added to water or the ethanol solutions to achieve a concentration of 0.2% w/v. Ethanol self-administration was established in standard operant chambers (Coulbourn Instruments, Allentown, PA) that were housed in sound-attenuated ventilated cubicles. A continuous reinforcement (FR1) schedule was used, such that each response resulted in delivery of 0.1 ml of fluid delivered into either of two stainless steel drinking cups mounted 4 cm above the grid floor in the middle of one side panel. Two retractable levers were located 4.5 cm to either side of the drinking cups. Fluid delivery and recording of operant responding were controlled by microcomputer. During the 0.5 s in which the pumps were activated, lever presses were not recorded.

Rats were trained to self-administer ethanol using a sweet saccharin solution fading procedure. This paradigm has been shown to produce pharmacologically relevant BALs with limited access to ethanol over approximately a 6-week period (see Roberts et al., 1999). Briefly, rats had restricted water access (available only for 3 h each day) for the first 3 days of training. During training, animals were allowed access to the operant boxes where responding on the one extended lever resulted in the delivery of a saccharin solution. Thereafter, water restriction was discontinued. Ethanol concentrations increased from 5% to 8% to a final concentration of 10% over the following 20 days, with each new increased concentration of ethanol first being mixed with saccharin. The ethanol was then administered alone prior to the next higher dose of ethanol that was initially presented with saccharin. During this saccharin fading procedure, both levers were extended, with one lever producing ethanol/saccharin and the other lever producing water. Initially, the levers associated with each solution were alternated between left and right on consecutive days, and then ultimately one of the levers produced ethanol and the other lever produced water. All operant sessions were 30 min long and were conducted 5 days/week between 9:00 AM and 12:00 PM.

2.4. Behavioral testing procedures

The goal of this study was to compare the effects of various neuroactive steroid compounds on operant self-administration of ethanol in male Wistar rats. Prior to testing, rats were allowed to respond for 10% (w/v) ethanol versus water for 4–6 weeks until responding across 3 consecutive days varied less than 25% and preference for ethanol over water was at least 60%. Once operant responding was stable, a pre-test was conducted using intraperitoneal administration of saline (1 ml) in order to acclimate the rats to the injection procedure prior to testing. On the test days, the rats received intraperitoneal administration of vehicle (40% β-cyclodextrin) or various doses of the following neuroactive steroids in a Latin-square design in separate groups of rats: epipregnanolone (5, 10, and 20 mg/kg; $n=12$), PCA (10, 20, and 30 mg/kg; $n=10$), pregnanolone hemisuccinate (5, 10, and 20 mg/kg; $n=12$) and androsterone hemisuccinate (5, 10, and 20 mg/kg; $n=11$). The effect of the 3β-epimer of PCA (Fig. 1D), (3β,5β)-20-oxo-pregnane-3-carboxylic acid (10, 20, 30 mg/kg; $n=9$), on ethanol self-administration was also examined. The doses of each drug were administered in a balanced manner such that each dose was equally represented on every test day. The compounds were administered in separate experiments to different cohorts of rats. The rationale for the choice of neuroactive steroid doses was based on two criteria. First, we conducted preliminary studies examining potential sedative effects of

the compounds. Since any compound with sedative effects might attenuate ethanol self-administration, we did not administer any of the compounds at dose ranges that produced sedation. Second, the goal of this report is to characterize the effects of various neuroactive steroids on ethanol self-administration. Therefore, in order to present a full dose–response characterization of these effects, we report our behavioral measures across doses that are both effective and ineffective at reducing self-administration behavior. The effects of the 3β-epimer of PCA (10, 20, 30 mg/kg; $n=5$) on ethanol self-administration were also examined. The test compounds were administered 45 min prior to the test sessions in order to minimize the effect of stress produced by IP injections and to optimize distribution and uptake of the steroids into the CNS. An additional experiment was conducted where animals received 30 mg/kg ($n=7$) of each of the hemisuccinates, and were tested for ethanol self-administration 5 min after the injection to reduce the period of possible brain metabolism of these hemisuccinates. The tests were run once per week, with 2–3 intervening days where the animals received ethanol self-administration sessions in the absence of neuroactive steroid pretreatment. The ethanol self-administration training sessions were run 5 days/week (Monday–Friday), and since responding is most stable in the middle of the week, all of the test sessions were conducted in the middle of the week.

2.5. Electrophysiology

Slices of amygdala were prepared from male rats (200–250 g) as described in Roberto et al. (2003). Androsterone hemisuccinate (10 μM) was dissolved in artificial cerebrospinal fluid (aCSF) and PCA was dissolved in DMSO (5:1000), and then added to aCSF from stock solutions at known concentrations. Both compounds were superfused on the slices for 20–30 min. Recordings were made from central amygdala (CeA) neurons (medial division of CeA) with sharp micropipettes (3M KCl) using discontinuous voltage- or current-clamp mode. A switching frequency of 3 to 5 kHz was used with continuously monitored electrode settling time and capacitance neutralization at the headstage. Data were acquired with an Axoclamp-2A preamplifier and stored for later analysis using pClamp software (Axon Instruments, Foster City, CA). Pharmacologically isolated $GABA_A$ receptor-mediated inhibitory postsynaptic currents ($GABA_A$-IPSCs) were evoked by stimulating locally within the CeA through a bipolar stimulating electrode while superfusing the glutamate receptor blockers 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM) and DL-2-amino-5-phosphonovalerate (APV; 30 μM) together with 1 μM CGP 55845A (a $GABA_B$ receptor antagonist. The NMDA excitatory postsynaptic currents (NMDA-EPSCs) were evoked in low Mg+aCSF (0.75 mM instead of 1.5 mM) using 30 μM bicuculline, 1 μM CGP 55845A, and 10 μM CNQX (Roberto et al., 2004). To determine the response parameters for each cell, an input/output (I/O) protocol was performed. A range of currents (typically between 50 and 200 μA) was used to stimulate $GABA_A$-IPSCs or NMDA-EPSCs. The stimulus strength was increased in 3 steps of 30 μA (rate of 1 pulse per 8 s) until the voltage required to elicit the maximum amplitude was reached. Most neurons were held near their resting membrane potential (RMP=−77 mV). Hyperpolarizing and depolarizing current steps (200 pA increments, 750 ms duration) were applied to generate voltage–current (V/I) curves. The evoked $GABA_A$-IPSCs and NMDA-EPSC amplitudes and V/I responses were quantified by Clampfit software (Axon Instruments, Foster City, CA).

2.6. Statistical analyses

The number of ethanol or water deliveries was first analyzed using separate one-way analysis of variance (ANOVA) across various doses of the neuroactive steroids. Significant overall effects were further analyzed using the Fisher's PLSD test ($p<0.05$) to compare vehicle-treated rats to animals that received various doses of the neuroactive steroids. Electrophysiological data were subjected to a within-subject ANOVA with repeated measures with $p<0.05$ considered statistically significant.

3. Results

The average baseline intake for each study was about 40 lever presses for the 10% ethanol solution. The differences in baseline ethanol self-administration that were observed in this study are commonly observed between animal shipments, and might be expected from this outbred strain of rats. Although the values for the PCA study are admittedly higher, the overall level of variability is within the range of different studies in our laboratory using this model (i.e., 25–30 responses in Valdez et al., 2002, 33–36 responses in Roberts et al., 1999, and 40 responses in Schulteis et al., 1996). Higher baseline values in the PCA experiment do not confound the major conclusions of this report, since the attenuation observed at the high dose of PCA (22 responses) would be significantly lower than the average baseline intake across studies (40 responses). In each experiment, animals had stable baseline intake prior to the beginning of the Latin-square testing, and the conclusions were drawn from independent experiments.

Based on an average weight of 500 g and 40 responses for 10% (w/v) ethanol, the dose of ethanol consumed was approximately 0.8 g/kg. BALs were not directly measured in this study. However, previous research in our laboratory has demonstrated that oral intake of 0.5 g/kg produces a BAL of approximately 30 mg% (see Roberts et al., 1999). Therefore, it is expected that the BAL of rats in this study was approximately 30–40 mg% or higher after each 30 min session.

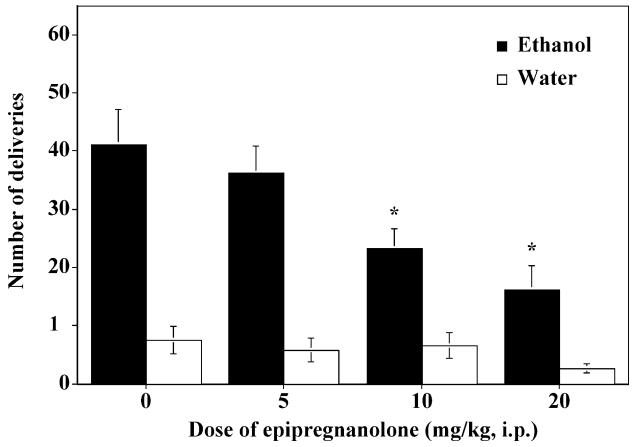

Fig. 2. Mean deliveries (+ S.E.M.) of ethanol (black bars) or water (white bars) following administration of vehicle or various doses of epipregnanolone (5, 10, and 20 mg/kg, i.p.; $n=12$). Once responding for 10% (w/v) ethanol was stable, rats received each dose of epipregnanolone in a Latin-square design 45 min prior to a weekly test session where they were allowed to self-administer ethanol or water for 30 min. Asterisks (*) reflect a significant decrease in operant self-administration of ethanol relative to the vehicle test day ($p<0.05$).

The effects of epipregnanolone on ethanol and water self-administration are shown in Fig. 2. Epipregnanolone (see Fig. 1A) dose-dependently attenuated ethanol self-administration [$F(3,44)=5.9$, $p<0.001$], with significant effects observed at the 10 and 20 mg/kg dose relative to vehicle treatment (Fisher's PLSD test, $p<0.05$). This compound did not alter water self-administration [$F(3,44)=1.11$, $p=0.35$].

The effects of the hemisuccinates of pregnanolone (see Fig. 1B) and androsterone (structure not shown) on ethanol and water self-administration are displayed in Figs. 3 and 4, respectively. Neither of these compounds significantly altered ethanol or water self-administration [$F(3,44)<1.0$, $p=0.43$ for pregnanolone hemisuccinate effects on ethanol intake, and $F(3,40)=0.92$, $p=0.43$ for androsterone hemisuccinate effects on ethanol intake]. These compounds were also tested at an earlier time point to determine if they were ineffective because of rapid metabolism. However, when animals ($n=7$) received 30 mg/kg of these hemisuccinates and were tested 5 min later, no significant differences on ethanol or water self-administration were observed (data not shown).

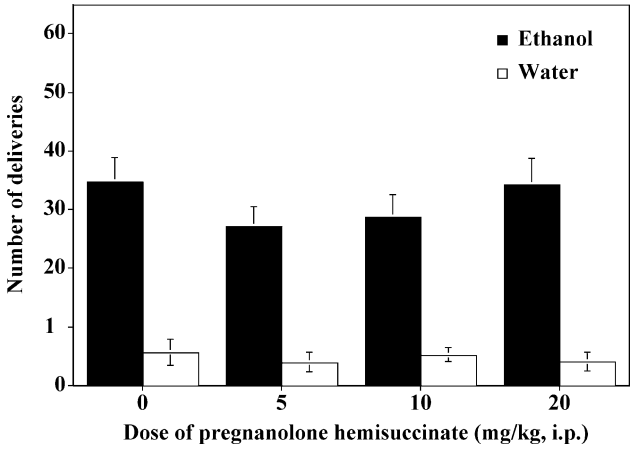

Fig. 3. Mean deliveries (+ S.E.M.) of ethanol (black bars) or water (white bars) following administration of vehicle or various doses of pregnanolone hemisuccinate (5, 10, and 20 mg/kg, i.p.; $n=12$). Once responding for 10% ethanol was stable, rats received each dose of pregnanolone hemisuccinate in a Latin-square design 45 min prior to a weekly test session where they were allowed to self-administer ethanol or water for 30 min.

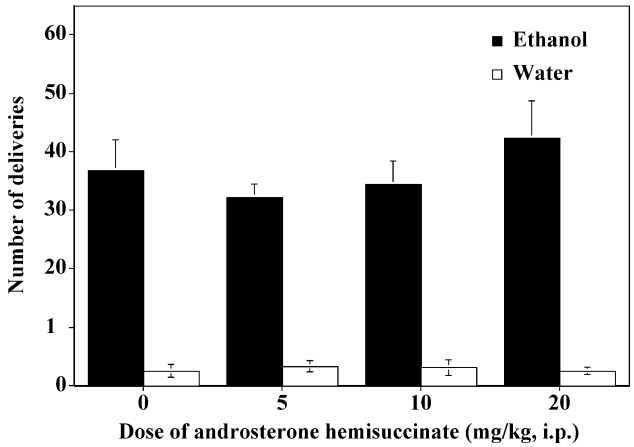

Fig. 4. Mean deliveries (+ S.E.M.) of ethanol (black bars) or water (white bars) following administration of vehicle or various doses of androsterone hemisuccinate (5, 10, and 20 mg/kg, i.p.; $n=11$). Once responding for 10% ethanol was stable, rats received each dose of androsterone hemisuccinate in a Latin-square design 45 min prior to a weekly test session where they were allowed to self-administer ethanol or water for 30 min.

The effects of PCA (see Fig. 1C) on ethanol and water self-administration are shown in Fig. 5. PCA dose-dependently attenuated ethanol self-administration [$F(3,36)=5.9$, $p<0.002$], with significant effects observed at the 20 and 30 mg/kg dose relative to the vehicle treatment (Fisher's PLSD test, $p<0.05$). PCA did not alter water self-administration [$F(3,36)=0.85$, $p=0.47$]. There was no effect of the 3β-

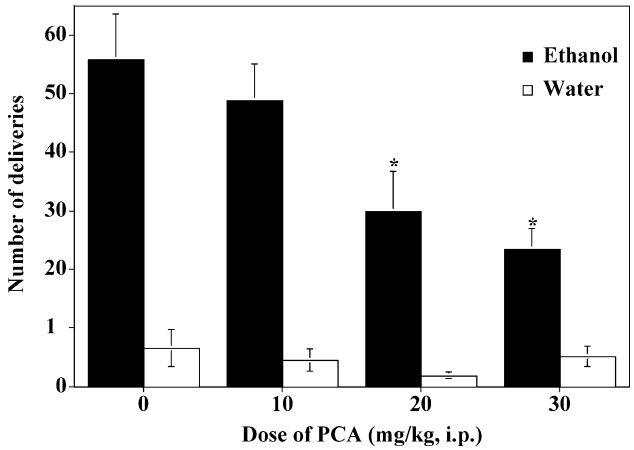

Fig. 5. Mean deliveries (+ S.E.M.) of ethanol (black bars) or water (white bars) following administration of vehicle or various doses of PCA (10, 20, and 30 mg/kg, i.p.; $n=10$). Once responding for 10% ethanol was stable, rats received each dose of PCA in a Latin-square design 45 min prior to a weekly test session where they were allowed to self-administer ethanol or water for 30 min. Asterisks (*) reflect a significant decrease in operant self-administration of ethanol relative to the vehicle test day ($p<0.05$).

epimer of PCA (Fig. 1D) on alcohol self-administration at any dose that was examined ($F(3,64)<0.8$, $p=0.5$; data not shown).

In the electrophysiological study, 36 CeA neurons were recorded intracellularly with a mean RMP of − 77±2 mV and a mean input resistance of 108±4 MW. $GABA_A$-IPSCs and NMDA-EPSPCs evoked by local electrical stimulation within the CeA were studied. Androsterone hemisuccinate (10 μM) superfused for 20–25 min had no significant ($p>0.1$) effect on the amplitude of the $GABA_A$-IPSCs (103±6% of baseline; $n=6$) measured across the stimulus strengths used. Androsterone hemisuccinate did not alter basic membrane properties (such as membrane potential, input resistance, or spike amplitude). We evoked the D-AP5 sensitive, voltage-dependent NMDA-EPSCs by using a low Mg^+ aCSF. Androsterone hemisuccinate (10 μM, 25 min application) had no significant ($p>0.1$), inhibitory effect (to 89±9%; $n=7$) over the stimulus strengths used. PCA (0 to 25 μM) was also tested on evoked $GABA_A$-IPSCs in CeA neurons (Fig. 6). PCA at concentrations of 5 and 25 μM significantly increased the amplitude of $GABA_A$-IPSCs ($p<0.05$, 132.7±10%; $n=5$; $p<0.05$, 150.3±13%; $n=8$, respectively), whereas the lower concentration tested (1 μM) was ineffective ($p>0.05$, 99±7%; $n=6$). PCA at concentration of 25 μM significantly decreased the amplitude of NMDA-EPSCs.

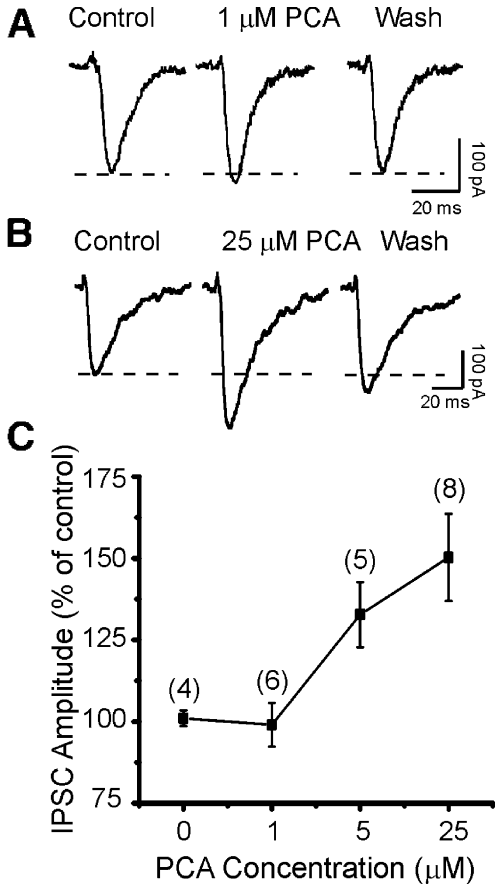

Fig. 6. Electrophysiological effect of PCA on $GABA_A$-IPSCs in CeA neurons. Representative recordings of $GABA_A$-IPSCs in a CeA neuron recorded before, during and after superfusion of 1 (A) and 25 (B) μM PCA, respectively. Note that superfusion of PCA significantly increased the mean $GABA_A$-IPSC amplitude with recovery on PCA washout (20–30 min). (C) Concentration–response relationship for PCA enhancement of mean $GABA_A$-IPSC amplitudes in CeA neurons, expressed as percent of control (PCA superfused for 20–30 min, number of cells in parentheses). Asterisks (*) reflect a significant increase in the IPSC amplitude relative to control ($p<0.05$).

4. Discussion

Neuroactive steroids have specific interactions with ethanol, many of which are mediated by $GABA_A$ or NMDA receptors. Drug discrimination studies have demonstrated that the discriminative effects of ethanol are potentiated by neuroactive steroids that facilitate $GABA_A$ receptors and block NMDA receptors (Hodge et al., 2001). Neuroactive steroids that modulate $GABA_A$ or NMDA receptors in a manner opposite to that produced by ethanol have been hypothesized to attenuate the effects of ethanol self-administration (Bowen et al., 1999a,b; Engel and Grant, 2001). In fact, the neurosteroid allopregnanolone, a positive modulator of $GABA_A$ receptors, increases ethanol intake in self-administration studies in rats (Janak and Gill, 2003) and mice (Sinnott et al., 2002). Since both of these receptor systems have been implicated as modulatory substrates for ethanol self-administration, the present study was designed to explore the effects of a group of neuroactive steroids on ethanol self-administration based on electrophysiological evidence of their interaction with $GABA_A$ and/or NMDA receptors.

The present study demonstrates that epipregnanolone and PCA attenuate ethanol self-administration, whereas the hemisuccinates do not alter this behavioral measure. The ability of epipregnanolone to reduce ethanol self-administration was not surprising, considering the noncompetitive nature of its interaction in electrophysiological studies between this compound and GABAergic 3α-hydroxy neuroactive steroids of similar structure (Garrett and Gan, 1998; Maitra and Reynolds, 1998; Wang et al., 2002). The lack of structural specificity of 3β-hydroxysteroids like epipregnanolone in blocking different neuroactive steroids that potentiate GABA currents suggests that there is not a well-defined binding site for this class of steroids on these receptors. Instead, 3β-hydroxysteroid antagonism appears to be explained by a noncompetitive mechanism dependent on receptor activation (Wang et al., 2002). We also predicted that compounds that inhibit GABA currents, such as epipregnanolone, might serve as functional antagonists of postsynaptic GABAergic function. Such GABA-antagonistic action may underlie the ability of epipregnanolone to reduce ethanol self-administration in the present study.

Electrophysiological studies have shown that both the geometry and nature of the charged group at C3 of the

steroid determine the pharmacological interaction of neuroactive steroid sulfates and dicarboxylic acid ester analogs of pregnenolone sulfate and pregnanolone sulfate with NMDA receptors (Weaver et al., 2000). In drug discrimination studies using systemic administration of structurally related pregnane sulfates, none of the sulfates were effective in substituting for ethanol as compared to pregnanolone (Bowen et al., 1999a,b), presumably because the sulfated compounds lacked the ability to significantly penetrate the CNS. Therefore, we selected the hemisuccinate of pregnanolone with glutamatergic activity, which penetrates the blood–brain barrier, to test the hypothesis that it might alter ethanol self-administration via inhibition of NMDA receptor function (Weaver et al., 2000). Androsterone hemisuccinate, which lacks glutamatergic activity, was used as a control. Both these hemisuccinates were ineffective in blocking ethanol self-administration at systemically administered doses up to 20 mg/kg. Pregnanolone hemisuccinate administered systemically (10 mg/kg) inhibits NMDA-mediated motor activity and dopamine release in the rat striatum, and it is rapidly metabolized (Sadri-Vakili et al., 2003). However, in the present study neither of the hemisuccinates altered ethanol self-administration 5 min after administration, suggesting that the lack of effect with the pregnanolone and androsterone hemisuccinates on ethanol self-administration was not due to rapid metabolism.

There is limited information on other behavioral effects of the neuroactive steroids used in this study. Epipregnanolone is metabolically oxidized to the 3-ketone (5β-pregnane-3,20-dione), which is not active at GABA or NMDA receptors. This ketone is subsequently reduced to the behaviorally active 3α-hydroxysteroid, pregnanolone, that has anxiolytic, sedative-hypnotic, and anesthetic effects at higher concentrations (Mellon, 2004). However, we did not observe sedative effects at the 20 mg/kg dose of epipregnanolone. It has recently been reported by Janak et al. (2004) that epipregnanolone attenuated the ability of allopregnanolone to reinstate ethanol-seeking behavior in rats. This is consistent with our finding that epipregnanolone reduces ethanol self-administration in non-dependent animals.

The novel discovery by Mennerick et al. (2001) of inhibition of NMDA receptor and complex modulatory action of GABA receptor function by PCA provided a neuroactive steroid with a unique pharmacological profile in vitro for evaluation in our animal model system (Fig. 5). PCA was originally synthesized as an analog of pregnanolone hemisuccinate that could not be hydrolyzed to pregnanolone in vivo. In vitro electrophysiological evidence from cell cultures has shown complex modulatory actions of PCA at $GABA_A$ receptors that depend on both the concentration and ionization state of this neuroactive steroid (pK of about 6.4). This is likely explained by the non-ionized and ionized form of PCA binding to different sites on $GABA_A$ receptors (Park-Chung et al., 1999). Therefore, we measured the effect of PCA on evoked $GABA_A$ receptor-mediated inhibitory postsynaptic currents ($GABA_A$-IPSCs) in brain slices of the amygdala, and found that this drug has stimulatory effects at concentrations of 5 and 25 μM (Fig. 6). Thus, since at similar concentrations epipregnanolone and PCA have opposite actions at $GABA_A$ receptors, but similar ability to attenuate alcohol self-administration, it is concluded that the actions of PCA at $GABA_A$ receptors are not the sole or even the major determinant of the behavioral actions of PCA in this study. Moreover, because the 3β-epimer of PCA (Fig. 1D), a compound that only blocks GABA-mediated currents in vitro (Mennerick et al., 2001) and lacks the ability to block NMDA currents at a concentration as high as 30 μM in vitro (Zeng et al., 1999), failed to attenuate alcohol self-administration in vivo, we hypothesize that the blocking action of PCA at NMDA receptors contributes to this compound's ability to attenuate alcohol self-administration. In summary, the present results show that epipregnanolone decreased ethanol self-administration in non-dependent rats through a hypothesized indirect blockade of GABA function, and PCA decreased ethanol self-administration in non-dependent rats through a hypothesized blockade of NMDA receptors. These results suggest novel targets for modifying the reinforcing actions of alcohol.

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