Published in final edited form as:

Alcohol. 2011 June ; 45(4): 349–354. doi:10.1016/j.alcohol.2010.08.020.

Effects of 7-keto Dehydroepiandrosterone on Voluntary Ethanol Intake in Male Rats

Mary E. Worrel^{1,2}, Olga V. Gurkovskaya^{1,2}, Stuart T. Leonard¹, Peter B. Lewis¹, and Peter J. Winsauer^{1,2}

¹ Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Science Center, New Orleans, LA 70112, United States of America

² Alcohol and Drug Abuse Research Center, Louisiana State University Health Science Center, New Orleans, LA 70112, United States of America

Abstract

Administration of dehydroepiandrosterone (DHEA), a neurosteroid that can negatively modulate the GABA_A receptor, has been shown to decrease voluntary intake of ethanol in rats. In vivo, DHEA can be metabolized to a variety of metabolites, including 7-keto DHEA, a metabolite without the prohormonal effects of DHEA. This study compared the effectiveness of 7-keto DHEA to DHEA for reducing ethanol intake in the same group of rats. The subjects, previously trained to drink ethanol using a saccharin-fading procedure, had access to ethanol for thirty minutes daily, and the amount consumed was recorded. Subjects were administered 10 and 56 mg/kg of DHEA or 7-keto DHEA intraperitoneally 15 minutes prior to drinking sessions. Subjects received each particular dose daily until one of two criteria was met; that is, either ethanol intake did not differ by more than 20% of the mean for three consecutive days, or for a maximum of eight days. Both 10 and 56 mg/kg of 7-keto DHEA significantly reduced the dose of ethanol consumed. While 10 mg/kg of 7-keto DHEA produced decreases similar to those found with DHEA, the 56-mg/kg dose of 7-keto DHEA was significantly more effective at decreasing the dose of ethanol consumed than the same dose of DHEA. These results show that 7-keto DHEA is comparable to, or possibly more effective than, DHEA at decreasing ethanol consumption in rats, and that 7-keto DHEA is a compound deserving further investigation as a possible clinical treatment for alcohol abuse without the prohormonal effects of DHEA.

Keywords

DHEA; 7-ketoDHEA; neurosteroid; GABA_A receptor; ethanol intake; rats

Introduction

Gurkovskaya et al., (2009) found that the neuroactive steroid dehydroepiandrosterone (DHEA) can dose-dependently decrease voluntary intake of ethanol in male rats. In that

Please send all correspondence to: Ms. Mary E. Worrel, Dept. of Pharmacology, LSUHSC, 1901 Perdido St., New Orleans, LA 70112, 504-568-2071, mworre@lsuhsc.edu.

For reprint requests, please contact: Dr. Peter Winsauer, Dept. of Pharmacology, LSUHSC, 1901 Perdido St., New Orleans, LA 70112, 504-568-2063, pwinsa@lsuhsc.edu

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study, subjects trained to consume ethanol received doses of the neuroactive steroids pregnanolone and DHEA to compare their effects on ethanol consumption. That study showed that DHEA decreased ethanol intake more effectively than pregnanolone (Gurkovskaya et al., 2009).

DHEA produces a wide array of physiological effects, a characteristic that makes determining the specific mechanism by which it alters ethanol intake difficult. For instance, DHEA is a precursor for estradiol and testosterone in peripheral tissues (Lardy et al., 2005), and has intrinsic androgenic activity (Mo et al., 2006). DHEA is also a negative allosteric modulator of the GABA_A receptor complex (Majewska et al., 1990) that can increase the levels of other neuroactive steroids that modulate this receptor, such as allopregnanolone (Bernardi et al., 2005).

The fact that DHEA can serve as a precursor for estradiol and testosterone in peripheral tissues may be problematic for its use in a clinical setting (Lardy et al., 2005). Administration of exogenous DHEA has been found to cause acne, hirsutism, and altered menses in women due to its capacity for increasing testosterone levels (van Vollenhoven et al., 1999; Panjari and Davis 2007). Furthermore, alterations in the levels of sex steroids have been reported to affect ethanol consumption (Lakoza and Barkov 1980; Juarez et al., 2002).

The present study compared the effects of 3 β -acetoxyandrost-5-ene-7,17-dione (7-ketoDHEA) on ethanol intake to the effects of DHEA reported previously in the same group of rats (Gurkovskaya et al., 2009). 7-ketoDHEA is a metabolite of DHEA that is not converted to sex hormones (Lardy et al., 1998). 7-ketoDHEA is the acetyl ester of 7-oxoDHEA, a naturally occurring metabolite of DHEA (see Fig. 1). In vivo, widespread tissue esterases hydrolyze 7-ketoDHEA to free 7-oxoDHEA (Lund-Pero et al., 1994).

Materials and Methods

Animals

Twelve adult male Long-Evans rats, approximately 18 months of age at the time of this study, were trained to orally consume an 18% ethanol solution via a saccharin-fading procedure starting on postnatal day (PD) 75 (Gurkovskaya et al., 2009). All twelve subjects had a history of adolescent administration of either alcohol (n=6) or saline (n=6) between PD 35 and 63, but these groups were combined for the purpose of this experiment because no significant differences in ethanol intake were found after training (Gurkovskaya et al., 2009). Likewise, there was no difference between the alcohol and saline groups in the acute effects of DHEA and pregnanolone on ethanol intake when these drugs were administered during adulthood (PD 235–630). These subjects also represented only 12 of the 22 animals from the previously published study (Gurkovskaya et al., 2009) in order to maintain a within-subject comparison, and prevent the subjects that only received one drug (DHEA) from biasing the results. Data from this subset of 12 animals was statistically identical to the group as a whole; i.e., a two-way ANOVA (group $\times$ dose of DHEA) determined there was no effect of group (n=12 of the present study versus n=22 of the previously published study) ($F(1,62)=0.15$, $p=0.7$), no significant difference in the effect of DHEA dose between groups ($F(2,62)=2.432$, $p=0.096$), and no interaction between group and dose of DHEA ($F(2,62)=0.0573$, $p=0.944$).

Subjects were housed in polypropylene cages with hardwood chip bedding, and the colony room maintained at $21 \pm 2^\circ\text{C}$ with $50 \pm 10\%$ relative humidity on a 14L:10D light/dark cycle. Except during experimental sessions, water was provided *ad libitum*. Subjects were maintained at 95% of their free-feeding weight, which decreased the variability of ethanol absorption, allowed for minimal variation in day-to-day consumption, and increased the

comparability of doses across days. The mean weight for the group was 480 g. Daily ethanol training and experimental sessions occurred between 12:00 and 14:00 hours. Daily food rations were provided between 16:00 and 17:00 hours. All subjects were maintained in accordance with the Institutional Animal Care and Use Committee at Louisiana State University Health Sciences Center, and in compliance with the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animal Resources (National Research Council 1996).

Behavioral Procedure

The effects of 7-ketoDHEA on ethanol intake were determined by a two-bottle preference test (see Gurkovskaya et al., 2009). Briefly, during experimental sessions, two drinking tubes were available simultaneously for 30 minutes daily in the home cage. One tube contained an 18% (v/v) ethanol solution, the other tap water. Their positions were reversed daily to prevent positional bias. Following experimental sessions, water bottles were replaced and the volumes of water and ethanol consumed from the tubes were determined by comparing the mass of the tubes before and after each session.

Neuroactive Steroid Administration

DHEA was dissolved in vehicle consisting of 45% (w/v) (2-hydroxypropyl)- γ -cyclodextrin and saline. 7-ketoDHEA (kindly provided by Dr. Henry Lardy, Department of Biochemistry and Institute for Enzyme Research, University of Wisconsin-Madison) was dissolved in a vehicle consisting of 80% (v/v) propylene glycol, 8% polyethylene glycol, and 2% benzyl alcohol. Vehicles for both drugs have been found to be behaviorally inactive in previous studies (Quinton et al., 2005; Quinton et al., 2006; Gurkovskaya and Winsauer 2009). All drugs and control injections were administered intraperitoneally 15 minutes prior to the experimental sessions. Each dose (10 or 56 mg/kg) was administered daily until one of two criteria was met; that is, until either a stable level of intake was observed ($\pm$ 20% of the mean for 3 consecutive days) or a maximum of 8 days, in which case only the last 3 of those 8 days were averaged for comparability. The volume of both DHEA and 7-ketoDHEA injections was 0.1 ml/100g body weight (10 mg/kg) or 0.28 ml/100g body weight (56 mg/kg). Increasing the injection volume was necessary due to the limited solubility of the drugs. Following testing of each dose, subjects were returned to baseline (no injection) conditions of 18% ethanol intake until one of the two criteria described above was met to ensure drug treatments had not altered baseline levels of ethanol intake permanently and to minimize any "carry over" effects. The data for these redeterminations of baseline intake were pooled for analysis and presented as an overall mean in Figures 2, 3, and 4. The effects of the two doses of 7-ketoDHEA were determined after those of DHEA, with 56 mg/kg preceding 10 mg/kg.

Data Analyses

Mean data for each subject were grouped and analyzed for an effect of drug using a two-way repeated-measures ANOVA with dose and drug (Figures 2 and 3) or drug and day serving as factors (Figure 4). Following a significant interaction between factors, a one-way ANOVA and Holm-Sidak post-hoc tests were conducted to compare each dose with the respective baseline condition and to compare the drugs at each dose. Significance was accepted at α level ≤ 0.05 for all statistical tests.

Results

Baseline values represent multiple determinations of intake, averaged over three days, in the absence of drug treatment. Figure 2 depicts the average volume of ethanol and water intake following 10 and 56 mg/kg of DHEA or 7-ketoDHEA compared to baseline. With respect to the volume of intake, a two-way repeated-measures ANOVA revealed a significant main

effect of dose ($F(2,22)=38.82$, $p<0.001$) and a significant interaction between drug and dose ($F(2,22)=11.54$, $p<0.001$). Because of the significant interaction, separate one-way repeated-measures ANOVA tests were conducted, followed by Holm-Sidak post-hoc tests. For both DHEA ($F(2,22)=27.79$, $p<0.001$) and 7-ketoDHEA ($F(2,22)=30.55$, $p<0.001$), post-hoc analyses indicated that the 10- and 56-mg/kg doses significantly reduced ethanol intake compared to control. Post-hoc analyses following the one-way repeated-measures ANOVA tests for each dose also indicated there were no significant differences in intake under baseline conditions or after 10 mg/kg ($p>0.05$) of each drug, whereas 7-ketoDHEA produced a significantly greater decrease in intake than DHEA after 56 mg/kg ($F(1,11)=12.27$, $p=0.005$).

The bottom panel of Figure 2 shows the effects of DHEA and 7-ketoDHEA on water intake. A two-way repeated-measures ANOVA indicated a significant difference between the effects of DHEA and 7-ketoDHEA on water consumption ($F(1,11)=11.46$, $p=0.006$). However, this difference can be attributed largely to the fact that subjects consistently consumed more water under baseline conditions during determinations of 7-ketoDHEA than during determinations of DHEA. There was no significant main effect of dose on water intake ($F(2,22)=0.015$, $p=0.985$) and no significant interaction between drug and dose ($F(2,22)=1.01$, $p=0.382$).

Figure 3 demonstrates the dose of ethanol (g/kg) consumed following 10 and 56 mg/kg of DHEA or 7-keto DHEA. A significant main effect of dose ($F(2,22)=43.03$, $p<0.001$) and a significant interaction between drug and dose ($F(2,22)=12.29$, $p<0.001$) also occurred for the dosage of ethanol consumed. Separate one-way repeated-measures ANOVA tests revealed the dosages of ethanol consumed after 10 and 56 mg/kg of DHEA ($F(2,22)=28.15$, $p<0.001$) and 7-ketoDHEA ($F(2,22)=34.81$, $p<0.001$) were significantly different than control. Similar to effects of each drug on the volume of ethanol consumed, there was no significant difference in dosage of ethanol consumed between 10 mg/kg of DHEA and 7-ketoDHEA ($p>0.05$), whereas 7-ketoDHEA produced a significantly greater decrease than DHEA at 56 mg/kg ($F(1,11)=12.07$, $p=0.005$).

Figure 4 shows ethanol intake on the first day of treatment compared to the average intake over the final three days of treatment, with the data for 10 mg/kg of each drug shown in the top panel and the data for 56 mg/kg shown in the bottom panel. A two-way repeated-measures ANOVA indicated a significant main effect of day for the 10 mg/kg dose ($F(2,22)=9.124$, $p=0.001$), but no effect of drug and no interaction between drug and day. A post-hoc analysis of the effect of day (collapsed across the two drug treatments) indicated that the intake on the criterion day was significantly different from both baseline intake and intake after the first injection. For the 56-mg/kg dose, there was a significant main effect of drug ($F(1,22)=44.441$, $p<0.001$), a main effect of day ($F(2,22)=40.779$, $p<0.001$), as well as a significant interaction between drug and day ($F(2,22)=21.609$, $p<0.001$). Separate one-way repeated-measures ANOVA tests followed by Holm-Sidak post-hoc tests showed that average intake over the final three days of DHEA administration was significantly different from baseline ($F(1,11)=16.680$, $p<0.002$) and from first-day intake ($F(1,11)=30.596$, $p<0.001$). In contrast, average intake after administration of 56 mg/kg of 7-ketoDHEA significantly differed from baseline after the first injection ($F(1,11)=44.317$, $p<0.001$) and after the 3-day criterion was met ($F(1,11)=66.251$, $p<0.001$). Therefore, unlike DHEA, 56 mg/kg of 7-ketoDHEA had a significant effect on ethanol intake on the first day of administration.

Discussion

DHEA was shown previously to decrease voluntary intake of ethanol in male Long-Evans rats (Gurkovskaya et al., 2009). The present study determined that 7-ketoDHEA, a metabolite without the prohormonal effects of DHEA, was also effective at decreasing both the volume and dose of ethanol intake. While 10 mg/kg of both neurosteroids reduced alcohol consumption similarly, 56 mg/kg of 7-ketoDHEA decreased intake significantly more than DHEA, suggesting that 7-ketoDHEA may be more effective than DHEA. Furthermore, the difference between 7-ketoDHEA and DHEA did not appear to be a difference in potency, because an increased potency alone would have resulted in greater effects at both doses tested. Another difference between DHEA and 7-ketoDHEA was the onset of their effect on ethanol intake. 7-ketoDHEA significantly decreased ethanol intake on the first day of treatment with 56 mg/kg. This is in contrast to DHEA, which was only effective at decreasing ethanol intake after multiple injections with each dose. Notably, neither DHEA nor 7-ketoDHEA significantly reduced water intake.

DHEAS, the sulfated form of DHEA, is the most abundant hormone in human circulation (Lardy et al., 2005). DHEAS levels in rat circulation are much lower than those found in humans (Lardy et al., 1995). However, rat brain contains up to 20 times more DHEAS than rat plasma (Corpechot et al., 1981). In both species, the adrenal gland is the main site of DHEA production (Lardy et al., 2002; Lardy et al., 2005). Formation also occurs in human and rat ovaries, testes, and brain. In rats, DHEA production in the brain has been shown to be independent of adrenal or gonadal production (Lardy et al., 2005).

DHEA is a precursor for estrogens and androgens (Lardy et al., 2005), and is estimated to be responsible for 50% of total androgens in adult male prostate and 75% of estrogen found in peripheral tissue of premenopausal women (Labrie et al., 2001). DHEA may affect ethanol intake through its actions as a steroid, steroid precursor, or an abundance of nongenomic effects (Losel et al., 2003). One prominent nongenomic effect of DHEA is at the GABA_A receptor complex, where it has been shown to negatively modulate chloride flux (Imamura and Prasad 1998) and GABA-induced membrane currents (Park-Chung et al., 1999). Decreasing ethanol intake through the GABA_A receptor complex would liken DHEA to the GABA_A antagonist picrotoxin (Boyle et al., 1993) and the negative GABA_A modulator RO15-4513, which have also been shown to decrease ethanol intake (Suzdak et al., 1986; Samson et al., 1987; Rassnick et al., 1993). While the direct effects of 7-ketoDHEA on the GABA_A receptor have not yet been investigated, there is evidence that 7-ketoDHEA, like DHEA, reverses scopolamine-induced memory deficits in young mice (Shi et al., 1999). The proposed mechanism for this effect of DHEA, and presumably 7-ketoDHEA, is via the influence of GABAergic neurons on the cholinergic system (i.e., the reduction of GABAergic inhibition increases acetylcholine release and antagonizes the effect of scopolamine).

Many neurosteroids have been investigated for their action at the GABA_A receptor (Harrison et al., 1987; Lambert et al., 1995; Imamura and Prasad 1998; Park-Chung et al., 1999; Losel et al., 2003), especially in light of the complex interactions of the neurosteroids with ethanol – a positive GABA_A modulator (Grobin et al., 1998; Koob 2004) that can influence the expression and trafficking of specific GABA_A receptor subunits (Morrow et al., 1990; Charlton et al., 1997; Devaud et al., 1997; Kumar et al., 2004; Hemby et al., 2006). For example, ethanol intake alters the levels of several neurosteroids (Barbaccia et al., 1999; Breese et al., 2006), and some neurosteroids have been implicated in mediating the effects of ethanol (Morrow et al., 1999). These complex interactions suggest that the effects of DHEA on ethanol intake may be due to one or more of its many effects, although the delayed effect of DHEA reported by Gurkovskaya, et al (2009) could be viewed as

inconsistent with an ion-channel mediated mechanism of action. There is also the possibility that DHEA could alter the metabolism of ethanol, although neither DHEA nor DHEAS was shown to alter blood ethanol concentration over time compared to control in a previous study (Melchior and Ritzmann 1992). Also, If DHEA was affecting ethanol intake through its capacity to negatively modulate the GABA_A receptor complex, it could increase the liability for anxiogenic and pro-convulsant side effects.

Unlike DHEA, 7-ketoDHEA does not act as a precursor for the sex hormones (Lardy et al., 1998). The fact that 7-ketoDHEA is comparable to DHEA in reducing voluntary ethanol intake has two important implications: 1) 7-ketoDHEA (or related compounds) may have a therapeutic advantage over DHEA for clinical treatment of alcohol abuse and dependence, because it is free of the adverse effects associated with increased production of testosterone and estradiol; and 2) DHEA may exert effects on ethanol intake independently of its role as a hormone precursor. Another potential advantage of 7-ketoDHEA was its comparatively rapid effects, which suggest its effects may not be due to changes in protein expression or metabolism. While the mechanism by which DHEA decreases ethanol intake is unknown, establishing the effectiveness of 7-ketoDHEA is the first step in helping to determine the potential mechanisms responsible. Additional studies are certainly warranted and will be needed as the subjects used in this study had a considerable history of both behavioral and pharmacological manipulations. Determining the indirect effects of 7-ketoDHEA on the metabolism of ethanol, and investigating the direct interaction of 7-ketoDHEA with the GABA_A receptor, are just a few examples of studies that will be needed. Nevertheless, the present data suggest that 7-ketoDHEA shows greater potential as a potential therapeutic for alcohol abuse and dependence than DHEA.

Acknowledgments

This work was supported by USPHS AA09803 (P.J.W.) and T32AA07577 (O.V.G.) from the National Institute on Alcohol Abuse and Alcoholism. 7-ketoDHEA was kindly provided by Dr. Henry Lardy, Department of Biochemistry and Institute for Enzyme Research, University of Wisconsin-Madison. Special thanks to Mr. Russell Amato and Ms. Jessie Sutton for their expert assistance.

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Figure 1.
Structures of (A) dehydroepiandrosterone, (B) 7-keto dehydroepiandrosterone (3β-acetoxyandrost-5-ene-7,17-dione), and (C) 7-oxo dehydroepiandrosterone (3β-hydroxyandrost-5-ene-7,17-dione).

Figure 2.

Effects of DHEA and 7-keto DHEA on the volume of ethanol consumed during 30-minute two-bottle preference sessions in rats (top panel). Baseline values represent multiple determinations of intake, averaged over three days, in the absence of drug treatment. The concentration of ethanol available to the subjects was 18% (v/v). The effects of each drug on water intake are shown in the bottom panel. Each dose was administered until 1 of 2 criteria was met; that is, until either intake did not vary by more than $\pm 20\%$ for 3 days or a total of 8 days, in which case the last 3 of those 8 days were averaged for comparison. Asterisks indicate significant differences for both drugs from the volume of ethanol consumed during baseline sessions. The pound sign indicates a significant difference between DHEA and 7-keto DHEA as determined by a one-way ANOVA and Holm-Sidak post-hoc tests after a significant interaction was revealed by the two-way repeated-measures ANOVA.

Figure 3. Effects of DHEA and 7-keto DHEA on the dose of ethanol consumed during 30-minute two-bottle preference sessions in rats. Baseline values represent multiple determinations of average intake in the absence of drug treatment. For additional details, see legend for Fig 2.

Figure 4.

Effects of DHEA and 7-ketoDHEA on volume of ethanol intake on the first day of injection compared to the intake after meeting the three-day criteria. Asterisks indicate significant differences from baseline, and pound signs indicate significant differences from intake after the first injection.