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GABA_A receptor modulation during adolescence alters adult ethanol intake and preference in rats

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Abstract

Background—To address the hypothesis that GABA_A receptor modulation during adolescence may alter the abuse liability of ethanol during adulthood, the effects of adolescent administration of both a positive and negative GABA_A receptor modulator on adult alcohol intake and preference were assessed.

Methods—Three groups of adolescent male rats received 12 injections of lorazepam (3.2 mg/kg), dehydroepiandrosterone (DHEA, 56 mg/kg), or vehicle on alternate days starting on postnatal day (PD) 35. After this time, the doses were increased to 5.6 and 100 mg/kg, respectively, for three more injections on alternate days. Subjects had access to 25–30 g of food daily during the period of the first six injections, and 18–20 g thereafter. Food intake of each group was measured 60-min after food presentation, which occurred immediately after drug administration on injection days or at the same time of day on non-injection days. When subjects reached adulthood (PD 88), ethanol preference was determined on two separate occasions, an initial 3-day period and a 12-day period in which increasing concentrations of ethanol were presented. During each preference test, intake of water, saccharin, and an ethanol/saccharin solution was measured after each 23-hour access period.

Results—During adolescence, lorazepam increased 60-minute food intake, and this effect was enhanced under the more restrictive feeding schedule. DHEA had the opposite effect on injection days, decreasing food intake compared to non-injection days. In adulthood, the lorazepam-treated group preferred the two lowest concentrations of ethanol/saccharin more than saccharin alone compared to vehicle-treated subjects, which showed no preference for any concentration of ethanol/saccharin over saccharin. DHEA-treated subjects showed no preference among the three solutions.

Conclusions—These data demonstrate that GABA_A receptor modulation during adolescence can alter intake and preference for ethanol in adulthood, and highlights the importance of drug history as an important variable in the liability for alcohol abuse.

Keywords

adolescence; alcohol preference; DHEA; lorazepam; GABA_A receptors

Alcohol use during adolescence has been associated with increased alcohol abuse and dependence during adulthood (Grant & Dawson, 1997; Hawkins et al., 1997). This association is particularly worrisome because, in 2009, the average age of initiating alcohol use was 16.9 years and adolescents drink, on average, twice as much as adults drink per episode (Spear & Varlinskaya, 2010; Substance Abuse and Mental Health Services Administration 2006; Substance Abuse and Mental Health Services Administration 2010). Moreover, adolescents may be particularly vulnerable to the deleterious effects of drug use due to the increased neuronal plasticity that occurs during development (Tucker, 1985). For instance, there is a developmental shift in the composition of GABA_A receptor subunits that is sensitive to GABA_A receptor modulation. Under normal conditions, this shift is putatively regulated by age-dependent levels of neurosteroids (Smith et al., 2007), endogenous substances that can modulate GABA_A receptor activity (Imamura & Prasad, 1998; Kellogg et al., 1998; Majewska et al., 1990); however, both endogenous and exogenous GABA_A receptor modulators may have the capacity to alter GABA_A receptor activity. For example, prenatal diazepam administration altered the *in vitro* response of GABA_A receptors from adults by reducing diazepam's capacity to facilitate chloride uptake compared to prenatal vehicle administration (Kellogg et al., 1991). Such alterations in the response of GABA_A receptors exemplify how GABA_A receptor modulation by exogenous modulators could affect ontogenetic development. Because of the direct action of ethanol on GABA_A receptors (Breese et al., 2006) and its capacity to affect the levels of several neurosteroids in the brain (Grobin et al., 1998; Morrow et al., 1999; Morrow et al., 2001), the GABAergic system is also susceptible to the effects of alcohol use in adolescence.

To investigate the specific interaction between exogenous GABA_A receptor modulation and subsequent adult ethanol intake and preference, both a positive modulator (lorazepam) and a neurosteroid (dehydroepiandrosterone, DHEA) were administered to adolescent rats. Ethanol preference was assessed 24 days after the final injection of each drug when the rats had reached adulthood. Both rat and mouse models have been used to investigate the relationship between adolescent and adult alcohol intake. While several studies with these species of rodent have found that early experience with alcohol can affect intake later in life (Hayashi & Tadokoro, 1985; Ho et al., 1989), other studies have found little or no alteration of alcohol intake or its reinforcing effects (Gurkovskaya et al., 2009; Siegmund et al., 2005; Slawecki & Betancourt, 2002; Tolliver & Samson, 1991). However, these different findings could be attributable to differences in a variety of experimental variables, including the route and dosage of ethanol administered during adolescence. Other reasons for these disparate findings could be that receptors other than the GABA_A receptor are largely responsible for mediating ethanol's effects at the doses tested in adolescents, or that the procedures used to train the subjects to consume ethanol overshadowed any pre-existing preferences that may have developed during adolescence. For example, in a study by Gurkovskaya et al. (2009), adult intake and preference for ethanol did not significantly differ between subjects that received 2 g/kg of ethanol or saline every other day from postnatal day (PD) 35 to 63. Adult ethanol intake and preference in this study was also assessed after a well-established sucrose-fading procedure.

The purpose of using both a positive GABA_A receptor modulator and a neurosteroid that negatively modulates the GABA_A receptor was two-fold. First, using both types of modulator provided a comparison of the differential modulation of adolescent GABAergic activity. Second, although DHEA has a variety of actions other than negative modulation of GABA_A receptors, it has been shown specifically to decrease ethanol intake (Gurkovskaya et al., 2009). In addition to the actions of DHEA at the GABA_A receptor, where it decreases GABA-mediated chloride flux (Imamura & Prasad, 1998; Majewska et al., 1990; Park-Chung et al., 1999), it also acts as a positive allosteric modulator of the NMDA glutamate receptor and the σ -1 receptor (Baulieu, 1998; Bergeron et al., 1996; Compagnone & Mellon,

1998; Compagnone & Mellon, 2000; Maninger et al., 2009; Yadid et al., 2010). The capacity of DHEA to positively modulate the NMDA glutamate receptor is an additional mechanism by which DHEA might potentially antagonize the effects of ethanol, which negatively modulates the NMDA glutamate receptor (Kostowski & Bienkowski, 1999). Finally, DHEA also has well-documented anti-glucocorticoid effects (Hu et al., 2000; Maninger et al., 2009). Each of these systems interact with the mesolimbic dopamine pathway involved in drug abuse and addiction (Yadid et al., 2010).

The benzodiazepine lorazepam was selected for exogenous administration instead of ethanol because it positively modulates the GABA_A receptor similarly to ethanol, but the two drugs have different mechanisms of action. Ethanol has been shown *in vitro* to enhance GABA-mediated currents at GABA_A receptors containing a delta subunit (which, *in vivo*, are found almost exclusively extrasynaptically) at doses of ethanol consistent with those achieved during typical episodes of social drinking in humans (Breese et al., 2006a; Santhakumar et al., 2007; Wallner et al., 2003). In addition to direct effects on the GABA_A receptor complex, ethanol has also been shown to increase presynaptic GABA release (Carta et al., 2004; Roberto et al., 2003; Ziskind-Conhaim et al., 2003). Benzodiazepines, by contrast, bind to specific sites on the GABA_A receptor, which facilitates GABA binding and enhances GABA-mediated chloride influx (for review, see Polc, 1988 and Sieghart, 1994). Therefore, using lorazepam should produce a more specific effect on GABA_A receptors, and, presumably, a more significant behavioral effect on adult ethanol preference than ethanol if this receptor complex is a key mediator. Furthermore, from a clinical perspective, investigating the effect of adolescent benzodiazepine use on alcohol preference in adulthood was important considering the co-abuse of alcohol and prescription drugs. A 2007 survey by McCabe et al. determined that illicit use of prescription drugs was the second most common form of illicit drug use (after marijuana) in a population of undergraduate students. In addition, 4 of 5 participants who reported illicit use of prescription drugs also reported heavy episodic drinking within the past two weeks (McCabe et al., 2006). These data also reflect a more general increase in visits to the emergency department for the nonmedical use of prescription drugs that occurred between 2004 and 2008. Of these, 89% were associated with benzodiazepine use, 25% of which were associated with a combination of benzodiazepine and alcohol misuse (CDC, 2010).

A final difference between this study and previous studies from this laboratory was the procedure for determining ethanol preference. Instead of training the subjects to consume ethanol using a saccharin-fading procedure, ethanol preference was determined without prior training over two discrete test periods, a 3-day initiation period and a 12-day period. During each day of these periods, subjects had 23-hour access to three solutions: saccharin, water, and an ethanol/saccharin solution. The purpose of the initial 3-day preference test was to allow the subjects to become familiar with each solution and the three-bottle choice procedure, whereas the 12-day preference test provided the opportunity to present increasing concentrations of ethanol. The availability of concurrent reinforcers such as ethanol and saccharin was assumed to provide a more comprehensive measure of ethanol preference.

MATERIALS AND METHODS

Animals

Twenty-one male Long-Evans hooded rats purchased at postnatal day (PD) 25 served as subjects. Subjects were housed individually and provided a diet of 25–30 g of standard rodent chow (Rodent Diet 5001; PMI Inc., St. Louis, MO) daily until PD 46, at which point subjects received 18–20 g of chow daily. This amount of chow was sufficient for growth and weight gain while eliminating continuous access to food between daily sessions. Water was provided *ad libitum* in the home cage. Subjects were housed in polypropylene cages with

hardwood chip bedding and nestlets (Ancare, Bellmore, NY). The colony room was maintained at $21 \pm 2^\circ\text{C}$ with $50 \pm 10\%$ relative humidity on a 14:10 light/dark cycle.

All subjects were maintained in accordance with the Institutional Animal Care and Use Committee, 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).

Adolescent Drug Administration and Food Intake Measurements

Prior to drug administration, subjects were randomly divided into three groups with a similar mean body weight. From PD 35 to 58, one group received 3.2 mg/kg of lorazepam ($n=7$), another received 56 mg/kg of DHEA ($n=7$), and the third received comparable volumes of vehicle (lorazepam vehicle, $n=4$; DHEA vehicle, $n=3$). From PD 59 to 64, the doses of lorazepam and DHEA were increased to 5.6 and 100 mg/kg, respectively (see Figure 1 for treatment timeline). The injection volume for lorazepam and lorazepam vehicle was 0.1 ml/100 g body weight, whereas the injection volume for DHEA and DHEA vehicle varied with the dose (i.e., 0.175 ml/100 g for 56 mg/kg and 0.313 ml/100 g for 100 mg/kg). All injections were administered intraperitoneally every other day for a total of 15 injections. Each injection immediately preceded the presentation of the daily ration of chow. On both injection and non-injection days, this occurred between 1100 and 1200 hours. Sixty minutes following food presentation, any remaining food was collected and weighed. This amount, which reflected the uneaten balance of each subjects' total daily ration, was always returned to the home cage after weighing. This method of assessing food intake was shown by Winsauer et al. (1994) to produce reliable consumption over the first 60 minute period after food presentation, and to produce stable consumption and body weight gain from day to day.

Determination of Adult Ethanol Preference

Four weeks after completion of lorazepam, DHEA, or vehicle administration (at PD 88), ethanol preference was determined by presenting each group with three solutions for 23 hours per day. The solutions were: water, 0.05% saccharin, and a solution containing a mixture of ethanol and 0.05% saccharin. Solutions were provided in 50-ml centrifuge tubes fitted with rubber stoppers and sipper tubes, and the position of each tube was rotated from day to day to prevent positional bias. The amount consumed was determined by weighing the tubes before and after each session. Ethanol preference was determined on two separate occasions, an initial 3-day period in which a single concentration of ethanol was tested and a 12-day period in which increasing concentrations of ethanol were tested. These periods of testing were separated by 21 days of abstinence. During the initial 3-day test, the concentration of ethanol in the ethanol/saccharin solution remained at 5%, whereas during the 12-day test, the concentration of ethanol in the ethanol/saccharin solution was increased every three days. The concentrations of ethanol were 5%, 10%, and 18%. After 18% ethanol/0.05% saccharin was presented for three days, an 18% ethanol solution without saccharin was presented. Ethanol preference was calculated as the percent ratio between ethanol consumption and total fluid intake (i.e., ethanol intake/total fluid intake $\times 100$).

Data Analysis

Data for the amount of food consumed on injection days was compared directly with the amount of food consumed on non-injection days using a two-way repeated-measures ANOVA (treatment $\times$ day) followed by one-way ANOVA tests and Holm Sidak post-hoc tests when a significant interaction was detected (SigmaStat Statistical Software, SYSTAT Software, Inc. Point Richmond, CA).

Preference data from the initial 3-day test period were compared to data from the initial three days of the 12-day exposure (i.e., 5%/0.05%) using a two-way repeated-measures ANOVA (solution $\times$ determination) followed by one-way ANOVA tests and Holm Sidak post-hoc tests when a significant interaction was detected. To compare the preferences of each treatment group across ethanol concentrations during the 12-day exposure, a three-way ANOVA (treatment $\times$ solution $\times$ ethanol concentration) was conducted. When significant interactions were found, a two-way repeated-measures ANOVA was performed. One-way repeated-measures ANOVA tests then followed if significant interactions were found, as well as Holm Sidak post-hoc tests to compare intake of the different solutions or ethanol concentrations. Significance was accepted at α level ≤ 0.05 for all statistical tests.

RESULTS

Effect of Adolescent Treatment on Food Intake

Figure 2 shows the effects of adolescent administration of lorazepam (3.2 and 5.6 mg/kg), DHEA (56 and 100 mg/kg), or vehicle on 60-minute food intake under the different levels of food restriction. The top panel illustrates the effects produced by 3.2 mg/kg of lorazepam and 56 mg/kg of DHEA on food intake when 25–30 grams of food were available. As shown, a two-way repeated-measures ANOVA indicated there was a significant interaction between drug treatment and day (no injection or injection) ($F(2,18)=11.424$, $p<0.001$), but no main effects for treatment ($F(2,18)=1.352$, $p=0.284$) or day ($F(1,18)=0.122$, $p=0.731$). One-way repeated-measures ANOVA tests on food intake within each treatment group indicated no significant effect between days on food intake in the group treated with vehicle ($F(1,6)=1.058$, $p=0.343$), but a significant effect was obtained for the groups treated with lorazepam ($F(1,6)=10.549$, $p=0.018$) and DHEA ($F(1,6)=13.603$, $p=0.010$). Across treatments, one-way repeated-measures ANOVA tests indicated that there was a significant effect of treatment on non-injection days ($F(2,18)=12.264$, $p<0.001$), but no effect of treatment on injection days ($F(2,18)=0.742$, $p=0.49$). In particular, Holm-Sidak post-hoc tests indicated that food intake on non-injection days was significantly different after lorazepam, but not after vehicle or DHEA. Taken together, these analyses indicate that 56 mg/kg of DHEA significantly decreased food intake following injection, and 3.2 mg/kg of lorazepam significantly increased food intake following injection. Furthermore, treatment with 3.2 mg/kg of lorazepam produced a significant decrease in food intake on days when the drug was not administered.

The middle panel of Figure 2 shows the effects of the same doses of lorazepam and DHEA (3.2 and 56 mg/kg, respectively) on food intake when food availability was restricted to 18–20 grams. A two-way repeated-measures ANOVA indicated there was a significant main effect of day ($F(1,18)=9.211$, $p=0.007$) as well as a significant interaction between treatment and day ($F(2,18)=29.149$, $p<0.001$); no main effect of treatment was found ($F(2,18)=1.115$, $p=0.349$). Although overall intake in grams was larger during the period of restriction than non-restriction (compare top and middle panels), one-way ANOVA tests indicated that intake was not significantly different between injection and non-injection days in the vehicle-treated group ($F(1,6)=3.020$, $p=0.133$). In contrast, there were significant differences between injection and non-injection days in both the lorazepam-treated ($F(1,6)=27.176$, $p=0.002$) and DHEA-treated groups ($F(1,6)=7.556$, $p=0.033$), verifying that 3.2 mg/kg of lorazepam acutely increased food intake, and 56 mg/kg of DHEA acutely decreased food intake. An analysis of food intake across treatments indicated that there were no significant effects on non-injection days ($F(2,18)=0.436$, $p=0.654$), but a significant main effect on injection days ($F(2,18)=4.931$, $p=0.020$), which was largely due to the significant increase in food intake produced by 3.2 mg/kg of lorazepam.

The bottom panel of Figure 2 shows the effects produced by 5.6 mg/kg of lorazepam and 100 mg/kg of DHEA on food intake in adolescent rats fed 18–20 g of chow. The effects on food intake obtained with these larger doses of lorazepam and DHEA were similar to those obtained with the lower doses. That is, a significant main effect of day ($F(1,18)=16.493$, $p<0.001$) occurred as well as a significant interaction between treatment and injection day ($F(2,18)=58.791$, $p<0.001$), with no significant effect of treatment ($F(2,18)=3.204$, $p=0.065$). One-way ANOVA tests also indicated no significant difference between treatment groups on non-injection days ($F(2,18)=1.670$, $p=0.216$), and a significant effect on injection days ($F(2,18)=12.867$, $p<0.001$) for the lorazepam-treated group compared to the vehicle- and DHEA-treated groups.

Effect of Adolescent Treatment on Initial Ethanol Preference

When data from the initial 3-day preference test were compared to the data from the first three days of the 12-day preference test (when the same 5% ethanol/saccharin solution was available) using a two-way repeated-measures ANOVA, a significant interaction between solution and determination was found for the vehicle-treated group ($F(2,12)=3.980$, $p=0.047$). Subsequent one-way ANOVA tests indicated that there was no main effect of solution during the first determination ($F(2,18)=1.286$, $p=0.301$), but there was a significant effect of solution during the second determination ($F(2,18)=5.846$, $p=0.011$). Holm-Sidak post-hoc tests showed a significant difference between ethanol and water as well as saccharin and water ($p<0.05$), verifying that more ethanol and saccharin were consumed than water during the second determination of solution preference. With respect to the differences between the first and second determinations for each solution in the vehicle-treated group, a one-way repeated measures ANOVA determined there was a difference in water intake ($F(1,6)=7.289$, $p=0.036$), no difference in saccharin intake ($F(1,6)=0.375$, $p=0.563$), and only a trend for ethanol intake ($F(1,6)=5.152$, $p=0.064$). Thus, the only difference between the first and second determinations in vehicle-treated subjects was a decrease in water intake.

In the lorazepam-treated group, a two-way repeated-measures ANOVA indicated there was a main effect of solution ($F(2,12)=24.300$, $p<0.001$), determination ($F(1,12)=33.302$, $p=0.001$), and a significant interaction between solution and determination ($F(2,12)=6.822$, $p=0.011$). This interaction was further confirmed by one-way ANOVA tests, which indicated that there were significant main effects of solution within the first determination ($F(2,18)=3.844$, $p=0.041$) and within the second determination ($F(2,18)=83.436$, $p<0.001$). For example, during the first determination, lorazepam-treated subjects consumed significantly more ethanol than water, while during the second determination, these subjects consumed more ethanol and saccharin than water, and more ethanol than saccharin. In addition, one-way repeated-measures ANOVA tests found no difference in saccharin intake between the first and second determinations ($F(1,6)=0.0474$, $p=0.835$), but there was a significant difference in ethanol ($F(1,6)=18.224$, $p=0.005$) and water ($F(1,6)=8.221$, $p=0.029$) intake between determinations.

In contrast to the findings for both the vehicle- and lorazepam-treated groups, a two-way repeated-measures ANOVA determined there were no main effects for solution ($F(2,12)=2.487$, $p=0.125$) or determination ($F(1,12)=1.267$, $p=0.303$), and no significant interaction ($F(2,12)=2.513$, $p=0.123$) of these two factors in the DHEA-treated group.

Effects of Adolescent Treatment on Ethanol Preference for Increasing Concentrations

Initially, a three-way ANOVA was conducted to determine the overall effects of each factor (adolescent treatment $\times$ solution $\times$ ethanol concentration). This analysis indicated there was a significant main effect of the type of solution ($F(2, 216)=39.317$, $p<0.001$) and of the

concentration of ethanol ($F(3, 216)=5.287$, $p=0.002$) on intake, but no effect of adolescent treatment ($F(2, 216)=2.126$, $p=0.122$). However, there was a significant interaction between the adolescent treatment and the type of solution consumed ($F(4, 216)=4.633$, $p=0.001$) as well as the type of solution and the ethanol concentration ($F(6, 216)=14.751$, $p<0.001$); the interaction between the adolescent treatment and the ethanol concentration was not significant ($F(6,216)=0.112$, $p=0.995$).

To isolate where the interaction occurred between adolescent treatment and the type of solution, the amount of solution consumed by each group was plotted for each ethanol concentration and analyzed using a two-way ANOVA. As shown in Figure 4, within the vehicle-treated group (top panel), there were main effects of solution ($F(2,36)=6.578$, $p=0.012$), ethanol concentration ($F(3,36)=12.182$, $p<0.001$), and a significant interaction ($F(6,36)=6.779$, $p<0.001$). To further isolate these effects, one-way repeated-measures ANOVA tests were then conducted to determine the effects of ethanol concentration on the intake of each of the solutions ($F(3,18)=9.431$, $p<0.001$) and on the type of solution consumed at each ethanol concentration ($F(3,18)=5.225$, $p=0.009$). For example, Holm-Sidak post-hoc tests indicated that the intake for the 5% ethanol/saccharin solution was significantly different from the intake for 18% ethanol, either with saccharin or alone (i.e., for the filled circles in the top panel, “a” is different from “b”). For saccharin intake in the vehicle-treated group, the only significant differences in intake occurred between the 10 % ethanol/saccharin solution and 18% ethanol alone.

Multiple one-way repeated-measures ANOVA tests also indicated that the amount of each solution consumed varied with the concentration of ethanol in the vehicle-treated group. For example, there was a significant difference between the intake of the 5% ethanol/saccharin solution and water ($F(2,12)=5.143$, $p=0.024$), and between the intake of saccharin and water when both 18% ethanol solutions were available ($F(2,12)=5.444$, $p=0.021$ and $F(2,12)=19.759$, $p<0.001$). Subjects in the vehicle-treated group also consumed significantly less ethanol than saccharin when 18% ethanol was presented without saccharin ($F(2,12)=19.759$, $p<0.001$). In summary, the vehicle-treated group consumed more ethanol than water when the lowest concentration of ethanol was available (5%), while smaller amounts of ethanol were consumed as the concentration of ethanol increased. In contrast, the amount of saccharin consumed was relatively stable when low ethanol concentrations were available, but significantly greater than water and ethanol when 18% ethanol alone was available.

Mean intake of the same three solutions in lorazepam-treated subjects is shown in the middle panel of Figure 4. Following a two-way repeated-measures ANOVA of the intake data for this group, which indicated there were main effects of solution ($F(2,36)=11.724$, $p=0.002$), ethanol concentration ($F(3,36)=35.253$, $p<0.001$), and a significant interaction ($F(6,36)=42.023$, $p<0.001$), one-way repeated-measures ANOVA tests were conducted to isolate the effects of solution and ethanol concentration on intake. For ethanol, mean intake was significantly different at every concentration of ethanol presented ($F(3,18)=9.431$, $p<0.001$). For saccharin, intake was relatively stable when each of the different concentrations of ethanol was presented. The only exception was the increase in saccharin intake when 18% ethanol was presented without saccharin ($F(3,18)=7.292$, $p=0.002$).

Multiple one-way repeated-measures ANOVA tests examining differences in intake among the three solutions at each concentration of ethanol indicated that lorazepam-treated subjects consumed significantly more ethanol than water when the 5% and 10% ethanol/saccharin solutions were available and more saccharin than water when 5% ethanol with saccharin and 18% ethanol alone were available ($F(2,12)=101.135$, $p<0.001$). Lastly, lorazepam-treated subjects consumed significantly more ethanol than saccharin when the 5% and 10% ethanol

solutions were available, and significantly less ethanol than saccharin when 18% ethanol alone was presented. In summary, the lorazepam-treated group consumed more ethanol than both water and saccharin when the 5 and 10% ethanol/saccharin solutions were present, and only consumed significantly more saccharin than ethanol when 18% ethanol was presented.

For the DHEA-treated subjects, which are represented by the data in the bottom panel of Figure 4, a two-way repeated-measures ANOVA indicated there was a significant main effect of ethanol concentration ($F(3,36)=5.966$, $p=0.005$) as well as an interaction between ethanol concentration and the type of solution ($F(6,36)=8.061$, $p<0.001$). There was no main effect for the type of solution ($F(2,36)=0.915$, $p=0.427$). To analyze the interaction, one-way repeated-measures ANOVA tests were conducted to determine the effect of ethanol concentration on the intake of each solution, and these tests confirmed that ethanol concentration significantly affected both ethanol ($F(3,18)=13.010$, $p<0.001$) and saccharin ($F(3,18)=4.237$, $p=0.020$) intake. Furthermore, Holm-Sidak post-hoc tests revealed that intake of the 5% ethanol/saccharin concentration differed significantly from ethanol intake when the higher ethanol concentrations were available. In contrast, only 18% ethanol alone significantly altered the intake of saccharin.

The concentration of ethanol presented did not significantly affect the intake of water in the vehicle-, lorazepam-, or DHEA-treated groups ($F(3,18)=0.333$, $p=0.801$; $F(3,18)=2.821$, $p=0.068$; and $F(3,18)=0.255$, $p=0.857$; respectively).

Table 1 shows ethanol preference and the dose of ethanol consumed for each treatment group when each concentration of ethanol was available. In general, ethanol preference decreased in each treatment group as the concentration of ethanol increased. There were notable differences in the initial preference ratios, however, as the preference ratio for the lorazepam-treated group was approximately 25% higher than that for the vehicle-treated group. The preference ratio for the lorazepam-treated group also showed a slower decline as the ethanol concentration increased. In contrast to the preference ratios, the mean dose of ethanol consumed increased at the 10 and 18% ethanol/saccharin concentrations prior to decreasing when 18% ethanol alone was available. Also of importance was the fact that the DHEA-treated group consumed the smallest mean dose of ethanol at all of the ethanol/saccharin concentrations.

DISCUSSION

The present study examined the effects of adolescent administration of lorazepam and DHEA on ethanol preference during adulthood, while using the effect of each drug on food intake as an indicator of their effectiveness during adolescence. Adolescent rats that received lorazepam consumed more food during the 60-minute session on injection days than on non-injection days and when food access was both unrestricted and restricted. Lorazepam-treated subjects also consumed more food than vehicle-treated subjects when the dosage was increased. In contrast, DHEA injection decreased the amount of food consumed compared to non-injection days under both food-access conditions, but did not significantly decrease levels of food intake below those for the vehicle-treated subjects. Using two doses of each drug also showed that the acute effects of lorazepam and DHEA were dose-dependent. Furthermore, using two different food-access conditions demonstrated the hyperphagic effects of lorazepam and the hypophagic effects of DHEA in both food-restricted and non-restricted subjects, which have only been demonstrated in adult subjects (Cooper, 1983; Porter & Svec, 1995).

Adolescent administration of lorazepam and DHEA also affected ethanol preference in adulthood compared to vehicle treatment. The effects of increasing the concentration of

ethanol produced similar patterns of drinking among the three treatment groups (i.e., as the concentration of ethanol increased, ethanol intake decreased and saccharin intake increased). However, each group differed significantly in their preference for the three solutions, and these differences were evident in the ethanol preference values between treatment groups. More specifically, lorazepam-treated subjects preferred low concentrations of ethanol with saccharin over saccharin alone or water, and these subjects did not significantly increase their preference for saccharin until 18% ethanol alone was presented. In contrast, DHEA-treated subjects showed the least preference for ethanol among the three groups as no concentration of ethanol was consumed significantly more than water. Notably, differences between treatment groups were enhanced with additional determinations (and deprivation) as the aforementioned significant differences were more apparent during the 12-day test that occurred three weeks after the initial exposure to ethanol and the three-bottle choice procedure. Thus, one could conclude that the effects of adolescent treatment with lorazepam or DHEA on ethanol preference are contingent upon repeated ethanol exposure.

The hyperphagic effect of positive GABA_A receptor modulators is a well-documented effect (Cooper, 1980; Cooper, 1987; Cooper & Francis, 1980; Reddy & Kulkarni, 1999; Wegelius et al., 1994). A study by Cooper et al. (1980) demonstrated that the benzodiazepines diazepam and chlordiazepoxide reduced the latency to feed and increased the number of feeding “bouts” in rats that had been fasted overnight. Allopregnanolone, a neurosteroid that is also a positive allosteric modulator of the GABA_A receptor, produced hyperphagic effects similar to the benzodiazepine triazolam in male and female rats upon food presentation after a 24-hour fast (Reddy & Kulkarni, 1999).

Unlike the positive modulators, several negative modulators of the GABA_A receptor have been shown to reduce food intake. Specifically, the inverse agonist RO 15-4513 and certain β -carboline have been shown to decrease food intake (Cooper, 1985; Reddy & Kulkarni, 1999; Svec & Porter, 1996; Wegelius et al., 1994). DMCM and FG 7142, two beta carboline that act as inverse agonists at the benzodiazepine binding site on the GABA_A receptor complex, produced anorectic effects in free-fed animals presented with highly palatable food, and these effects were not reversed by food deprivation prior to the test. This response also directly opposed the hyperphagic effects of various benzodiazepines in the same procedure (Cooper, 1985). The neurosteroid and negative GABA_A receptor modulator DHEA has been shown to decrease food intake (Reddy & Kulkarni, 1999; Svec & Porter, 1996). The administration of the sulfated form, DHEA-S, produced a hypophagic effect in both male and female rats upon food presentation following a 24-hour fast (Reddy & Kulkarni, 1999). Thus, the present data extend the bidirectional effects of GABA_A receptor modulation on food intake from adult to adolescent subjects.

Although positive allosteric modulators of the GABA_A receptor have been shown to alter ethanol intake, these effects have not been as consistent as the effects on food intake. For example, administration of the benzodiazepines flunitrazepam and diazepam had no effect on alcohol intake in either a home-cage drinking procedure (Leonard et al., 2006; Rimondini et al., 2002) or an operant procedure (Rimondini et al., 2002) where rats had relatively high baseline levels of intake (i.e., approximately 1.5 and 1 g/kg, respectively) during a 30-minute session. In contrast, treatment with 5 mg/kg of diazepam or 5–10 mg/kg of midazolam in free-fed male rats increased home-cage intake of ethanol when baseline intake levels were low (approximately 0.7 g/kg/2 hours) (Soderpalm & Hansen, 1998). Studies have also reported that up to 20 mg/kg of diazepam can decrease ethanol intake in subjects with high baseline intake (3.2 g/kg) (Hedlund & Wahlstrom, 1998). With respect to positive modulators other than the benzodiazepines, the GABA_A receptor agonist THIP (gaboxadol) has been shown not only to increase home-cage ethanol consumption, but also to aid in the acquisition of drinking behavior (Boyle et al., 1993; Smith et al., 1992). Additionally, the

neurosteroid and positive GABA_A receptor modulator allopregnanolone increased operant responding for ethanol in rats (Janak et al., 1998). These differences in the effects of GABA_A receptor modulation on ethanol intake indicate that study design, including baseline ethanol intake, doses of drug administered, and even the availability of concurrent reinforcers (Samson & Grant, 1985), are critical variables to consider when interpreting the extent to which positive modulators affect ethanol intake.

Unlike positive modulators of GABA_A receptors, negative modulators have thus far been reported to affect ethanol intake in a much more predictable manner. For example, inverse agonists at the benzodiazepine receptor site on the GABA_A receptor complex, as well as GABA receptor antagonists, have been shown to decrease both voluntary ethanol consumption and operant responding for ethanol (Balakleevsky et al., 1990; Boyle et al., 1993; Rassnick et al., 1993; Samson et al., 1987; Wegelius et al., 1994). In particular, administration of DHEA, 15 minutes prior to 30-minute preference sessions, dose-dependently decreased voluntary consumption of 18% ethanol in adult male rats irrespective of their adolescent ethanol history (Gurkovskaya et al., 2009).

As indicated by a number of investigators (Barbaccia et al., 1999; Morrow et al., 1999; Morrow et al., 2001), there is strong evidence to support both a molecular and behavioral interaction between ethanol and neurosteroids that positively modulate the GABA_A receptor complex. For instance, allopregnanolone and allotetrahydrodeoxycorticosterone (THDOC) are increased in the plasma and brain following acute ethanol injection in rats (Barbaccia et al., 1999). Rats undergoing ethanol withdrawal show increased sensitivity to the anti-convulsant effects of allopregnanolone and to the anxiolytic effects of alphaxalone, also a neurosteroid and positive GABA_A receptor modulator (Cagetti et al., 2004; Devaud et al., 1996). In addition, 18 to 30 mg/kg of allopregnanolone and THDOC have been shown to substitute for the discriminative stimulus effects produced by 1.5 g/kg of ethanol in rats (Ator et al., 1993).

The present data are important in that they strengthen existing evidence for an interaction between alcohol and neurosteroids that act as negative modulators, as they suggest that the neurosteroid DHEA may have the capacity to reduce ethanol preference prior to an individual's first drinking episode. These data complement existing data from our laboratory showing that DHEA (Gurkovskaya et al., 2009) and 7-keto DHEA (Worrel et al., 2011) can significantly reduce home-cage drinking, and support data with other neurosteroids. For instance, the negative GABA_A receptor modulator pregnenolone sulfate, which inhibits muscimol-stimulated chloride uptake (Majewska & Schwartz, 1987), and DHEA and DHEA sulfate, which inhibit GABA-mediated chloride uptake (Imamura & Prasad, 1998), have been shown to prevent ethanol-induced memory impairment (Melchior & Ritzmann, 1996). Furthermore, tolerance to the anxiolytic effect of ethanol was attenuated by chronic DHEA-S administration in rats (Sharma et al., 2007).

Another commonality between ethanol and the neurosteroids is their capacity for altering the expression of different GABA_A receptor subunits. The alpha-4 subunit, for example, has been shown to be particularly sensitive to changing levels of neurosteroids (Smith et al., 2007), and was upregulated after chronic administration of progesterone (a precursor to the positive GABA_A receptor modulator allopregnanolone) as well as during withdrawal. This subunit is also of interest because it is upregulated following chronic treatment with benzodiazepines and its expression decreases the sensitivity of the GABA_A receptor complex to benzodiazepines (Holt et al., 1996). For this reason, GABA_A receptors containing an alpha-4 subunit are often referred to as "benzodiazepine insensitive" receptors. The capacity of GABA_A receptor modulators to modify GABA_A receptor subunit expression is, therefore, a putative mechanism by which adolescent administration of

lorazepam and DHEA could alter ethanol preference later in life. In other words, if DHEA increases the expression of GABA_A receptors containing an alpha-4 subunit, it may be concomitantly reducing the central effects of the benzodiazepines and alcohols.

The present data are also of interest because they show the long-term effects of adolescent GABA_A receptor modulation on ethanol preference in adulthood and show those effects on competing reinforcers (i.e., ethanol and saccharin). One advantage of the present study over previous preference studies conducted in this laboratory was that ethanol preference was not assessed after a saccharin-fading procedure, which could have overshadowed a pre-existing ethanol preference resulting from adolescent administration of ethanol (Gurkovskaya et al., 2009), as these procedures are designed to train subjects to consume alcohol. Therefore, the present experiment not only omitted the saccharin-fading procedure, but also presented multiple solutions to assess the effect of each adolescent treatment on multiple reinforcers (Rodd-Henricks et al., 2001).

In summary, the present study clearly demonstrates that lorazepam administration during adolescence increases adult preference for ethanol, while DHEA administration decreases adult preference for ethanol compared to vehicle administration. These findings also demonstrate that multiple periods of ethanol availability with an associated ethanol deprivation were helpful in assessing the development of an adult ethanol preference. Finally, these findings highlight the importance of drug history in establishing the liability for drug abuse and the long-term effects of GABA_A receptor modulation during adolescence. However, future investigations will need to focus on further establishing the mechanism of these effects, information that could be useful in understanding the role of neurosteroids in brain development and in understanding the long-term effects of adolescent drug use.

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Fig. 1.
Timeline of manipulations for the different treatment groups during adolescence and adulthood.

Fig. 2.

Effects of chronic intermittent administration of lorazepam, DHEA, or vehicle during adolescence on food intake under two different food-access conditions. Top panel: food intake 60 minutes following injection of 3.2 mg/kg lorazepam, 56 mg/kg DHEA, or vehicle compared to intake on days without injections when 25–30 g of food was available daily. Middle panel: food intake 60 minutes following injection of 3.2 mg/kg lorazepam, 56 mg/kg DHEA, or vehicle compared to intake on days without injections when 18–20 g of food was available daily. Bottom panel: food intake 60 minutes following injection of 5.6 mg/kg lorazepam, 100 mg/kg DHEA, or vehicle compared to intake on days without injections when 18–20 g of food was available daily. Pound signs indicate significant differences between injection and non-injection days. Letters indicate significant differences across treatments on injection and non-injection days (e.g., ‘a’ is significantly different from ‘b’).

Fig. 3. Ethanol, saccharin or water intake in adult rats treated with vehicle (top panel), lorazepam (middle panel), or DHEA (bottom panel) during adolescence. The first determination represents the mean for the initial 3-day access period, whereas the second determination represents the mean for the first 3 days of a 12-day access period when the same ethanol concentration (5%) was presented. Asterisks indicate significant differences from water and pound signs indicate differences from saccharin. A cross indicates a significant difference between determinations for a given solution.

Fig. 4.

Ethanol, saccharin, or water intake in adult rats when increasing concentrations of ethanol were presented during a 12-day access period. Groups of subjects treated with vehicle, lorazepam, or DHEA during adolescence are shown in the top, middle and bottom panels, respectively. Asterisks indicate a significant difference from water intake and pound signs indicate a significant difference from saccharin intake; letters indicate differences within a solution (e.g., 'a' is significantly different from 'b', but not significantly different from 'a,b').

Table 1

Ethanol Preference and Ethanol Dose Consumed

Ethanol preference (ethanol intake/total fluid intake $\times$ 100) and ethanol dose consumed (g/kg) for each treatment group when each concentration of ethanol was available. Each value represents the average $\pm$ standard error of the mean.

Ethanol/Saccharin Concentration	Vehicle-Treated		Lorazepam-Treated		DHEA-Treated	
	Ethanol Preference	Ethanol Dose (g/kg)	Ethanol Preference	Ethanol Dose (g/kg)	Ethanol Preference	Ethanol Dose (g/kg)
0.05/5%	49.37 $\pm$ 11.32	3.54 $\pm$ 0.84	76.26 $\pm$ 4.56	4.87 $\pm$ 0.13	54.99 $\pm$ 13.26	3.31 $\pm$ 0.83
0.05/10%	46.21 $\pm$ 5.13	4.59 $\pm$ 0.83	61.97 $\pm$ 3.33	5.64 $\pm$ 0.58	37.93 $\pm$ 10.31	3.51 $\pm$ 1.12
0.05/18%	27.22 $\pm$ 3.52	4.55 $\pm$ 0.83	37.46 $\pm$ 5.04	5.29 $\pm$ 0.76	26.28 $\pm$ 6.53	3.57 $\pm$ 0.93
18%	18.41 $\pm$ 3.78	3.50 $\pm$ 0.83	17.76 $\pm$ 2.71	2.61 $\pm$ 0.36	16.25 $\pm$ 4.27	2.64 $\pm$ 0.83