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Dehydroepiandrosterone Is an Anxiolytic in Mice on the Plus Maze

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MELCHIOR, C. L. AND R. F. RITZMANN. *Dehydroepiandrosterone is an anxiolytic in mice on the plus maze.* PHARMACOL BIOCHEM BEHAV 47(3) 437–441, 1994.—Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) are neurosteroids that have been shown to interact with the GABA system. The present study examined the effects of these compounds in mice on motor activity and behavior in the elevated plus maze. Doses of 0.5 mg/kg and above of DHEA reduced motor activity. This effect was blocked by diazepam, RO15-1788, pentylenetetrazole (PTZ), and ethanol. Both DHEA and DHEAS showed anxiolytic activity in the plus maze test, with DHEA being effective over a very wide range of doses (5 μg/kg to 1.0 mg/kg). Both RO15-1788 and PTZ blocked the anxiolytic effect of DHEA, there was no interaction with diazepam, and ethanol enhanced the anxiolytic effect of DHEA. At 1.0 mg/kg, DHEAS blocked the anxiolytic effect of ethanol. These results support the hypothesis that neurosteroids could be involved in the termination of a stress response.

Dehydroepiandrosterone Dehydroepiandrosterone sulfate GABA Benzodiazepine Anxiety Motor activity Ethanol Mice

DEHYDROEPIANDROSTERONE (DHEA) and its sulfate metabolite (DHEAS) are steroids that are formed both in the periphery and in the brain (5). The biological function of these compounds has not yet been defined (17,24,27).

Several steroids have been shown to act centrally at the $GABA_A$ receptor complex as agonists (22,24,27). Many of them have subsequently been shown to have anxiolytic effects in a variety of testing paradigms (2,3,6,31), thus supporting the hypothesis that they may function endogenously to terminate a stress response (15,24,26). Unlike the neuroactive steroids that have GABA agonist activity and demonstrate anxiolytic effects, DHEA and its sulfate (DHEAS) have been shown to have GABA antagonist activity in radioligand binding or electrophysiological studies (4,8,18). Both of these compounds have proconvulsant effects when administered in high doses (12). This suggests that DHEA and DHEAS would be more likely to be anxiogenic than anxiolytic, since other excitatory compounds with GABA antagonist activity have demonstrated anxiogenic actions (23,25). In contrast, another line of evidence suggests that DHEA may attenuate the stress response. DHEA has immunomodulatory activity (7). One mechanism for this activity is that DHEA counteracts the immunosuppressive effects of glucocorticoids, which are elevated during stress (1,7,14,20). Furthermore, DHEA attenuates the elevation in corticosterone produced by subjecting rats to a cold stress (1).

The purpose of this study was to determine whether or not DHEA or DHEAS have anxiogenic or anxiolytic activity and to examine interactions in this regard with some compounds that interact with the GABA receptor complex to determine if any action of DHEA on anxiety is via a GABA-mediated action.

METHOD

Subjects

Male C57BL/6 mice purchased from Harlan and weighing 20–25 g were used in these studies. The animals were housed five per cage in temperature (22 ± 1°C)- and light (0600–1800 h)-controlled rooms. Mice were housed in the animal facility for at least 7 days prior to the initiation of any experiment.

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Drugs

The benzodiazepine diazepam (DZ; Hoffmann-La Roche, Nutley, NJ), the benzodiazepine antagonist RO15-1788 (RO; Hoffmann-La Roche), the GABA channel blocker pentylenetetrazole (PTZ; Sigma Chemical Co., St. Louis, MO) and dehydroepiandrosterone (DHEA; Sigma Chemical Co.) were prepared for an intraperitoneal (IP) injection of 0.1 ml/10 g body weight by suspending them in a vehicle of 0.4% Tween 80 and saline. DHEA was sonicated for 30 min prior to use. Dehydroepiandrosterone sulfate (DHEAS; Sigma Chemical Co.) was dissolved in saline. Ethanol, a compound that activates GABA-stimulated chloride channels (11), was prepared as a 20% w/v solution from 95% ethanol and saline. The control injections for each compound were the respective vehicle solutions.

Activity

Since sedative to convulsant effects have been reported for many neuroactive steroids (12,17), and such activity could interfere with measurements of anxiety (below), mice were tested for alterations in motor activity. To evaluate the drug effects on motor activity, an individual animal was placed in a large plastic cage (25 × 48 × 16 cm, W × L × H), which was placed on a platform of an activity monitor (Stoelting Co., Wood Dale, IL). After 20 min, subjects were injected and activity was recorded for 20 min.

Plus Maze

Mice were tested on the elevated plus maze (13,30) immediately after completion of the activity test.

The plus maze is made of black Plexiglas consisting of two opposite-facing open arms (30 × 5 cm) and two opposite-facing closed arms (30 × 5 × 15 cm). The walls of the closed arms are clear Plexiglas and are separated by a central area that is 5 × 5 cm. The whole plus maze is mounted on a base, raising it 38 cm above the floor. Testing involved placing a mouse at one end of one of the open arms (30). The time the mouse took to leave the start position (the first 10 cm of the open arm) was recorded. The time it took until the mouse entered half way into one of the closed arms was also recorded. At this time the 3-min test session was begun. The number of times the mouse enters into the open arms was recorded. An entry was defined as placing at least 2 paws into the open arm.

Statistical Analysis

Specific group comparisons were made with *t*-tests. Data were considered significant at $p < 0.05$. There were 6–12 mice per group, except for the control group, where pooling of the controls run on each day of testing resulted in an $N = 29$. (Controls did not differ across days.) Appropriate controls were run on each day of testing. Each mouse was used only once.

RESULTS

DHEA: Activity

The effect of DHEA in doses ranging from 0.0005 to 20 mg/kg is shown in Fig. 1. High doses from 0.5 to 20 mg/kg all produced a significant reduction in locomotor activity compared to vehicle-injected controls. Doses of 0.05 mg/kg or less did not alter locomotor activity.

Figure 1 shows the interaction of several compounds with 0.5 mg/kg DHEA. RO15-1788 (0.5 mg/kg), a benzodiazepine antagonist that did not alter motor activity by itself, inhibited the decrease caused by DHEA. The benzodiazepine diazepam, at a dose that did not affect motor activity (0.2 mg/kg), also blocked the decrease caused by DHEA. An interaction occurred with pentylenetetrazole (PTZ). At 15 mg/kg, PTZ significantly decreased motor activity. However, the combination of PTZ and DHEA resulted in activity that was not different from control levels. A dose of 1.5 g/kg ethanol decreased motor activity. The combination of ethanol and DHEA was not different from ethanol alone but was greater than DHEA alone.

DHEA: Plus Maze

As shown in Fig. 1, DHEA exhibited an inverted U-shaped dose-response curve for the number of entries into the open arms of the plus maze. At 0.5 μg/kg, DHEA-treated mice were not different from controls. However, between 0.005 and 1.0 mg/kg, DHEA-treated mice entered the open arms more often than controls. At higher doses, the number of entries was reduced, probably due to effects on motor activity. These data indicate that DHEA has anxiolytic effects rather than anxiogenic actions.

While 0.5 mg/kg of RO15-1788 by itself had no effect, it blocked the increase in number of entries into the open arm caused by both 0.2 mg/kg diazepam and 0.5 mg/kg DHEA. Both DHEA and diazepam increased number of entries, but both drugs together were not different from either drug alone. An interaction was again observed with PTZ. PTZ alone decreased the number of entries and coadministration of PTZ and DHEA resulted in numbers of entries that were not different from controls. A dose of 1.5 g/kg ethanol increased the number of open arm entries. When DHEA and ethanol were given together, a greater number of open arm entries was observed than with either drug alone. Thus, the anxiolytic effects of DHEA can be influenced by drugs acting at the GABA-benzodiazepine-chloride receptor complex (Fig. 1).

In the plus maze test, the only significant difference from controls in either start time or time to enter half way into one of the closed arms occurred with 20 mg/kg of DHEA, which increased the start time from 1.33 ± 0.48 min (mean ± SEM) for controls to 1.82 ± 0.90 min ($t(33) = 2.65$, $p < 0.05$). This appeared to be due to the decrease in motor activity that was noted in the activity test.

DHEAS: Activity

In doses of 0.05 to 1.0 mg/kg, DHEAS had no effect on motor activity. DHEAS failed to alter the decrease in activity caused by 1.5 g/kg ethanol (Fig. 2).

DHEAS: Plus Maze

Anxiolytic effects were observed with DHEAS in doses of 0.05 and 0.5 mg/kg. There were no significant interactions with the anxiolytic effect of ethanol except at the highest dose of DHEAS tested, 1.0 mg/kg, which reduced the animals performance to control levels (Fig. 2).

DISCUSSION

In a very wide range of doses, DHEA has anxiolytic effects in the plus maze. Over a narrower dose range, DHEAS shows a similar effect.

Finding that DHEA and DHEAS have anxiolytic effects

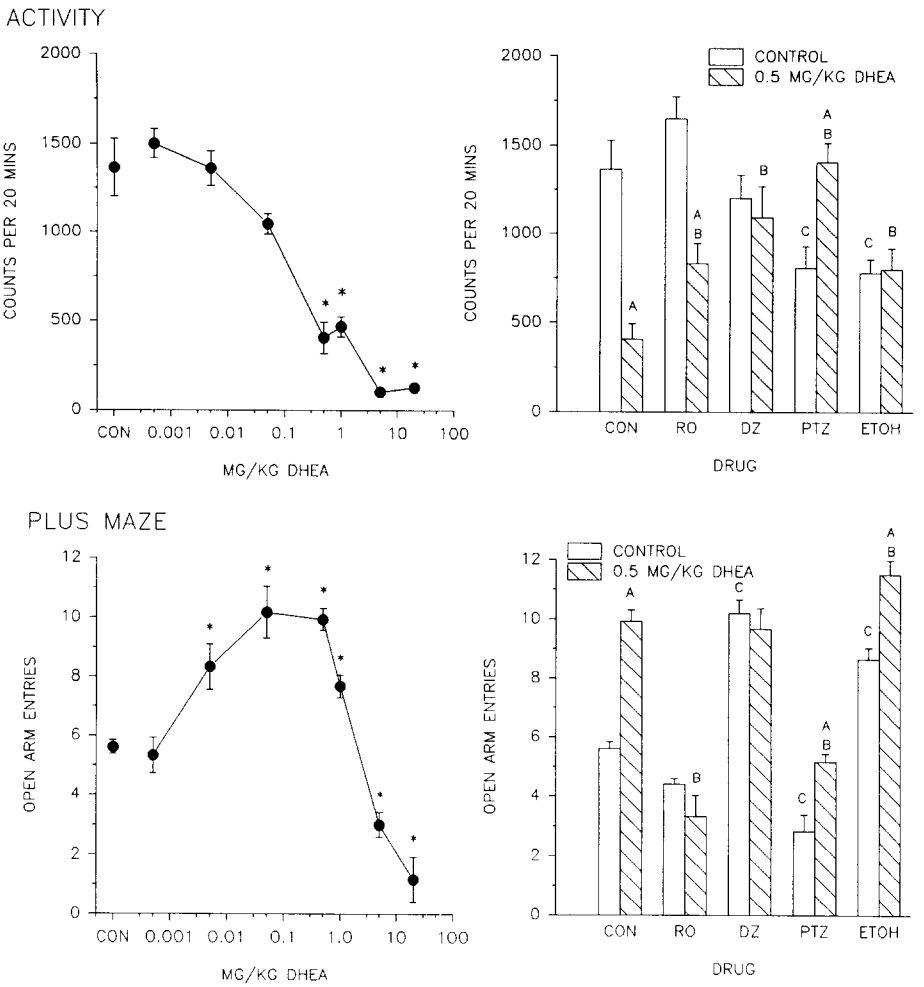

FIG. 1. Motor activity (top) and number of open arm entries on the plus maze (bottom) for a range of doses of dehydroepiandrosterone (DHEA; left) or DHEA in combination with various drugs (right). CON = vehicle solution; RO = 0.5 mg/kg RO15-1788; DZ = 0.2 mg/kg diazepam; PTZ = 15 mg/kg pentylenetetrazole; ETOH = 1.5 g/kg ethanol. N = 6–12 per group, except CONTROL/CON, in which N = 29. $^{*}p < 0.01$, t-test compared to control; $^{A}p < 0.01$, t-test compared to same drug without DHEA; $^{B}p < 0.01$, t-test compared to DHEA alone; $^{C}p < 0.01$, t-test compared to CONTROL/CON.

supports the hypothesis that these neurosteroids could play a role in the termination of a stress response (17,24,26). Utilizing a variety of tests for anxiety, neuroactive steroids such as 3α,5α-tetrahydrodeoxycorticosterone (THDOC), 3α-hydroxy-5α-pregnan-20-one (3α-OH-DHP), 3α,21-dihydroxy-5α-pregnan-20-one (alloTHDOC), pregnanolone (and not its 3β5α epimer), and alphaxalone have been shown to have anxiolytic activity (2,3,6,31). Additionally, Purdy et al. (26) demonstrated that 3α-OH-DHP and alloTHDOC were increased in brain and plasma of rats after exposure to ambient temperature swim stress. Finding that these steroids are increased during stress prompted Purdy et al. (26) to suggest that they may form a novel feedback loop for decreasing the enhanced activity of the hypothalamic–pituitary–adrenal axis after stress. Although DHEAS was reported to be increased after the stress of surgery (5), the GABA antagonist activities reported in electrophysiological studies for DHEA and in both electrophysiological and radioligand binding studies for its metabolite DHEAS (4,8,18) suggested that these neurosteroids might have the opposite action (17), and may be involved in the arousal associated with the early stages of stress (15). However, for DHEA and DHEAS, the anxiolytic effect found in this study, rather than anxiogenic action, provides a consistent biological picture with their positive effects on the immune system and the ability of DHEA to attenuate a stress-induced increase in corticosterone levels (1,7,14,20). Since the (peripheral) precursors and preponderant metabolites of DHEA do not have similar immune effects (7), DHEA appears to have a unique capacity to provide a coordinated effect on behavior and the immune system.

Some preliminary work was done to determine whether or not the effect of DHEA in the plus maze was related to its ability to alter increases in corticosterone. A dose of 0.5 mg/kg of DHEA did not affect the modest rise in corticosterone

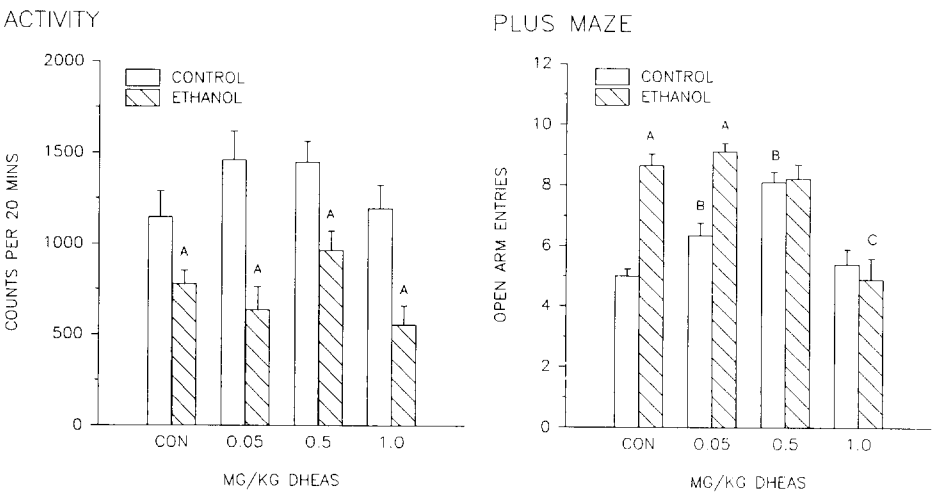

FIG. 2. Motor activity (left) and number of open arm entries on the plus maze (right) after various doses of dehydroepiandrosterone sulfate (DHEAS) with and without 1.5 g/kg ethanol. CON = vehicle solution. N = 8–10 per group. $^{A}p < 0.05$, t-test compared to same dose without ethanol; $^{B}p < 0.05$, t-test compared to CONTROL/CON; $^{C}p < 0.05$, t-test compared to ETHANOL/CON.

levels seen 20 and 60 min after taking mice from their home room and injecting them in another room (Melchior, Ritzmann, and McGivern, unpublished observations). Since much larger doses of DHEA (1.0 g/kg, SC, 3 days before, followed by 20 mg/kg, IP, 24 and 2 h before testing) were used to show an attenuation of corticosterone levels induced by a more severe stress (cold water) (1), the effect of DHEA in this regard may be dose dependent.

Both the very broad range of effective doses of DHEA and the exceedingly small amount required to demonstrate activity are characteristics that Flood and his colleagues (9,10,28) reported for DHEA when examining the positive effect this compound has on memory. The dose–response curves generated on the activity measure also indicate that effects on anxiety and activity can be separated. These features should be beneficial if this compound proves clinically useful, since they indicate that the drug would have a wide therapeutic window.

Testing for motor activity in a nonnovel environment revealed that higher doses of DHEA suppressed motor activity. All of the compounds tested in combination with DHEA inhibited this response, even those that caused a decrease in activity themselves, PTZ and ethanol. A different pattern of interaction occurred on the plus maze. Both RO15-1788 and PTZ blocked the anxiolytic effect of DHEA, there was no interaction with diazepam, and ethanol enhanced the anxiolytic effect of DHEA. Since the $GABA_A$ receptor is comprised of multiple subunits, and the subunit composition of the receptor not only varies across brain areas but also can alter the sensitivity of the receptor to a drug (24,29), it is not surprising to see differential drug interactions on two separate behavioral measures.

A previous study (21) examining the interaction of high doses of DHEA and DHEAS (5.0 mg/kg and above) with hypnotic doses of ethanol (3.5 g/kg) and pentobarbital (50 mg/kg) showed that DHEA but not DHEAS enhanced the duration of sleep time following both ethanol and pentobarbital. In the present study, different interactions with ethanol were again observed with DHEA and DHEAS. DHEA enhanced the anxiolytic effects of ethanol, while, at one dose (1.0 mg/kg), DHEAS blocked the anxiolytic effects of ethanol.

In administering drugs peripherally, the potential contribution of pharmacokinetic variables or metabolism cannot be ignored. Such factors may contribute to the differences observed between DHEA and DHEAS. One report of the effect of DHEA on other neurosteroids indicates that peripheral administration of DHEA does not alter brain DHEAS levels but does reduce brain levels of pregnenolone sulfate (32), an excitatory neurosteroid that has GABA antagonist properties (16,17,19). It is therefore possible that the anxiolytic effect of DHEA is due to a reduction in pregnenolone sulfate.

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