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Modulation of Corticosterone Does Not Affect the Acquisition or Expression of Ethanol-Induced Conditioned Place Preference in DBA/2J Mice

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CHESTER, J. A. AND C. L. CUNNINGHAM. *Modulation of corticosterone does not affect the acquisition or expression of ethanol-induced conditioned place preference in DBA/2J mice.* PHARMACOL BIOCHEM BEHAV **59**(1) 67–75, 1998.—Several recent studies have implicated the stress hormone corticosterone in modulating the rewarding properties of abused drugs, including amphetamine and ethanol. The present experiments examined a role for corticosterone in modulating the rewarding effects of ethanol in the place conditioning paradigm. Male DBA/2J mice were subjected to a Pavlovian conditioning procedure in which a distinctive floor stimulus (CS+) was paired four times with ethanol (2 g/kg). On intervening days, a different floor stimulus was paired with saline (CS−). In the first experiment, the steroid synthesis inhibitor, aminoglutethimide (AMG), administered prior to conditioning trials with ethanol, did not alter the acquisition of place preference. However, during conditioning trials, ethanol-stimulated locomotor activity in the AMG-treated group was significantly higher relative to the vehicle-treated group, suggesting that corticosterone may normally inhibit ethanol-stimulated activity. Plasma corticosterone levels in AMG-treated mice were significantly lower than in vehicle-treated mice, showing that AMG effectively suppressed corticosterone release on CS+ trials. The second experiment examined the effect of AMG on the expression of conditioned ethanol place preference. AMG administration prior to the preference test did not alter the magnitude of ethanol place preference. Corticosterone levels in the AMG-treated groups were significantly reduced relative to vehicle-treated groups, which showed a higher level of corticosterone during the preference test. These findings show that manipulation of corticosterone levels in a physiological range does not alter the acquisition or expression of ethanol-induced conditioned place preference in DBA/2J mice. © 1998 Elsevier Science Inc.

Alcohol Stress Reward Drug abuse Inbred mice

STRESS is thought to be a significant factor that contributes to substance abuse and addictive behavior [see reviews (15, 22,26,31,32)]. The primary physiological stress response of the organism is activation of the hypothalamic–pituitary–adrenal (HPA) axis, resulting in the release of the endogenous hormone corticosterone. It has been suggested that the rise in corticosterone produced by both stressors and abused drugs may be an important factor in the development of addictive behavior [see review (28)].

Several recent studies have demonstrated a role for the HPA axis and specifically, corticosterone in modulating the reinforcing properties of several psychostimulant drugs, including cocaine (14,30), morphine (5,41), and amphetamine (29). For example, previous exposure to stressors (27) and prolonged activation of the HPA axis due to chronic social stress conditions (20) has been shown to increase amphetamine self-administration in rats, presumably by increasing the reinforcing potency of amphetamine. Other studies have indicated that individual differences in HPA axis functioning and the adrenocortical response to novelty and environmental stressors may be a significant factor in the susceptibility to develop drug-seeking behavior. For example, rats with a longer

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duration of corticosterone secretion in response to a novel environment showed an enhanced acquisition and maintenance of amphetamine self-administration (29).

A number of studies suggest that corticosterone also plays a significant role in modulating the reinforcing properties of ethanol consumption. However, unlike psychostimulants, the relationship between endogenous corticosterone levels and ethanol self-administration is less clear. In general, these studies indicate that removal of endogenous corticosterone decreases ethanol consumption in ethanol-preferring rats (9,12, 21,23) and chronic corticosterone treatment via subcutaneous pellets potentiates ethanol drinking in both adrenalectomized and adrenally intact rats (11). Stressor-induced corticosterone levels are also found to potentiate ethanol consumption in rats and monkeys (17,25). In addition, individual differences in adrenocortical functioning and the corticosterone response to stressors, including ethanol, may differentially affect neural substrates mediating ethanol reward (33).

The relatively few studies that have examined specific manipulations of endogenous corticosterone levels on ethanol reward-related behaviors have used oral self-administration as a measure of ethanol's motivational properties. However, a potential problem in interpreting the effects of corticosterone on ethanol's rewarding properties is that corticosterone may be affecting mechanisms involved in ingestive behavior rather than affecting a mechanism modulating ethanol reward. For example, corticosterone may alter taste reactivity to ethanol. Indeed, adrenal corticosteroids have been shown to alter sensory processes such as taste reactivity (16).

The present experiments use the place-conditioning paradigm to examine corticosterone effects on the rewarding properties of ethanol. In mice, the place-conditioning procedure appears to be a useful tool for studying the rewarding properties of ethanol. Several inbred and selectively bred lines of mice have shown a reliable and robust place preference for the environment paired with ethanol [e.g., (2–4)]. One advantage of the place conditioning paradigm over the oral self-administration paradigm is that it does not involve ingestive behavior. Thus, it avoids interpretive problems related to possible nonspecific effects of an agonist or antagonist on consumption, rather than a selective effect on ethanol reinforcement or reward. In addition, this paradigm is useful for examining the effects of pharmacological manipulations on the direct rewarding properties of ethanol during acquisition of place preference and on ethanol's conditioned motivational aspects during a drug-free preference test, which may represent anticipation or craving for ethanol.

The rationale for the present study was based on the hypothesis that corticosterone released by handling and injection procedures and/or exposure to ethanol may ordinarily exert a rapid effect on the neural substrates mediating ethanol reward in the place-conditioning paradigm. The following experiments were designed to test the hypothesis that high endogenous corticosterone levels are involved in modulating the acquisition and expression of ethanol-induced conditioned place preference in mice. Experiment 1 examined the effect of inhibiting corticosterone release, via the steroid synthesis inhibitor aminoglutethimide (AMG), on the acquisition of ethanol place preference. Experiment 2 assessed the effect of AMG administered prior to the expression of ethanol place preference.

EXPERIMENT 1: EFFECTS OF AMG ON ACQUISITION OF CONDITIONED PLACE PREFERENCE

The purpose of Experiment 1 was to examine the effects of inhibition of corticosterone release with AMG during ethanol conditioning trials on the acquisition of ethanol place preference. It was hypothesized that a rise in corticosterone levels following ethanol exposure (18,42), as well as handling and injection procedures, is an important factor modulating ethanol's rewarding effects during conditioning. Based on this hypothesis, AMG administration prior to conditioning trials was expected to reduce the magnitude of place preference, as revealed in the preference test without AMG.

AMG has been shown to effectively inhibit restraint stressor-induced release of corticosterone in mice (38). However, the effects of AMG blockade on corticosterone levels in the presence of ethanol have not been studied. Thus, a control experiment was conducted to determine plasma corticosterone levels and confirm that the AMG dose used in the place conditioning study was effective in suppressing corticosterone synthesis and release in the presence of ethanol.

METHOD

Subjects

Subjects in both experiments were adult male inbred mice (DBA/2J) obtained from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. Mice were housed in polycarbonate cages (27.9 × 9.5 × 12.7 cm) in groups of four. Continuous access to food and water was provided and animals were allowed to acclimate to the colony room for 12–14 days before training. Ambient temperature was maintained at 21 ± 1°C. Experimental procedures were conducted during the light phase of a 12:12 light:dark cycle (lights on at 0700 h). Experimental procedures began 2–3 h after the onset of the light cycle.

Apparatus

Twelve identical acrylic and aluminum boxes (30 × 15 × 15 cm) were separately enclosed in ventilated, light and sound-attenuating chambers (Coulbourn Model E10-20). Six sets of infrared light sources and photodetectors were mounted opposite each other at 5-cm intervals along the length of each box, 2.2 cm above the floor. Occlusion of the infrared light beams was used to measure general activity and location of the animal (left or right) within the box. Total activity counts were recorded every minute by computer (10-ms resolution). The floor of each box consisted of interchangeable halves of one of two distinct textures. "Grid" floors consisted of 3.2-mm rods mounted 6.4 mm apart in acrylic rails. "Hole" floors consisted of perforated 16 gauge stainless steel with 6.4-mm round holes on 9.5-mm staggered centers. This combination of floor textures was selected on the basis of previous studies showing that drug-naive mice spend approximately equal time on each floor type during drug-free preference tests (1–3). The floors and the inside of the boxes were wiped with a damp sponge and the litter paper beneath the floors was changed between animals.

Drugs

Ethanol (20% v/v) was prepared from a 95% stock solution using saline as the vehicle. A dose of 2 g/kg ethanol was administered intraperitoneally (IP) in an injection volume of 12.5 ml/kg. This dose has previously been shown in mice to produce a strong preference for the paired tactile stimuli [e.g., (4)]. AMG was dissolved in a 20% w/v solution of 2-hydroxy-β-cyclodextrin [(β-cyclodextrin); Research Biomedicals International, Natick, MA] and saline. A dose of 50 mg/kg was administered IP in an injection volume of 10 ml/kg. This dose of

AMG has been found to maximally inhibit restraint stressor-induced release of corticosterone but does not impair motor behavior (38). A previous study has shown that administration of the β-cyclodextrin vehicle alone has no effect on place conditioning in DBA/2J mice (13).

Procedure

The place-conditioning study involved one habituation session, eight conditioning sessions, and one test session. For the habituation session, mice received an injection of saline immediately before being placed in the conditioning box for 5 min on a smooth paper floor.

For conditioning, mice were randomly assigned to one of three groups: AMG (n = 31), ETOH (n = 32), and AMG/ETOH (n = 32). The AMG group served as a control for the possible rewarding or aversive effects of AMG alone. Within each of the three experimental groups, mice were randomly assigned to one of two conditioning subgroups (G+ or G−) and exposed to a Pavlovian differential conditioning procedure. During conditioning trials, all mice had access to both sides of the apparatus on a homogeneous floor type. All mice received two IP injections before each conditioning session. On alternating days (CS+ sessions), G+ subjects in the AMG and AMG/ETOH group received an injection of AMG 2 h before the conditioning session, and the ETOH group received an injection of the vehicle β-cyclodextrin. This 2 h pretreatment interval was chosen because it is within an effective range in which AMG produces a maximal inhibition of stressor-induced release of corticosterone in mice (38). A saline (AMG group) or ethanol injection was given immediately before a 5 min session on the grid floor. On intervening days (CS− sessions), these mice received β-cyclodextrin and saline paired with the hole floor. Conversely, G− subjects received AMG/saline, vehicle/ethanol, or AMG/ethanol injections paired with the hole floor and vehicle and saline paired with the grid floor. Conditioning groups were matched for overall exposure to CS type (grid or hole) and drug treatment, and the order of drug exposure was counterbalanced within groups. The 5 min session duration was chosen based on previous studies showing that it produced a stronger conditioned place preference with ethanol than did longer session durations (4).

Mice received an injection of vehicle 2 h before the 60 min preference test. A saline injection was given immediately prior to placement in the apparatus to match the cues during conditioning days. The floor of each box was half grid and half hole with left/right position counterbalanced within groups.

Control Experiment

Because blood sampling procedures might alter place conditioning, separate groups of naive mice were used to determine the effectiveness of AMG in suppressing corticosterone synthesis and release on CS+ trials. These mice were subjected to experimental procedures similar to those described above for the ETOH (n = 6) and AMG/ETOH (n = 6) groups. All mice received an acute injection of AMG or vehicle 2 h before an injection of ethanol (2 g/kg), and were immediately placed in the apparatus for 5 min. Following the 5 min session, each mouse was removed from the box and approximately 20 μl of tail blood was taken for corticosterone assay.

Corticosterone Radioimmunoassay

A sharp scalpel was used to nick the tip of the tail (2 mm) and approximately 20 μl of blood was collected into heparinized capillary tubes. The tubes were centrifuged at 2000 rpm for 5 min, 5 μl of plasma was removed and diluted in 100 μl sterile water, and the sample was stored at 4°C until assayed for corticosterone. Samples were immersed in boiling water to denature corticosterone binding globulin (24). Corticosterone radioimmunoassay was executed following a previously reported method (19) and utilized [^{125}I]-corticosterone from ICN Biomedicals and corticosterone antibody from Ventrex. The minimum concentration of corticosterone detectable within the 95% confidence interval was 0.2 μg/dl. The maximum detectable corticosterone concentration was 200 μg/dl. Intra-assay variability was less than 10%. Assay specificity was very high, with only 4% cross-reactivity to deoxycorticosterone, 1% cross-reactivity to 5β-pregnanedione, and less than 0.6% cross-reactivity to other adrenal steroids.

Statistical Analyses

Data were analyzed by analysis of variance (ANOVA) with the alpha level set at 0.05. Because the performance of the ETOH compared to the AMG/ETOH group was of primary interest, data from these two groups were included in one set of analyses. A separate set of analyses was conducted for the AMG control group to determine whether AMG alone altered locomotor activity or produced place conditioning.

RESULTS

Conditioning

Figure 1 shows the mean (±SEM) activity counts per min for the ETOH and AMG/ETOH groups during conditioning trials 1–4. Ethanol produced significant locomotor activation during the CS+ sessions relative to the CS− sessions with saline. As previously observed with DBA/2J mice [e.g., (3)], activity counts were significantly higher on the last CS+ session compared to the first CS+ session in both groups, indicating sensitization to the locomotor-activating effects of ethanol occurred across the four trials.

Initial analysis of CS+ session data (two-way ANOVA: drug treatment × trials) yielded a significant main effect of trials, $F(3,186) = 40.2$, $p < 0.001$, and a significant interaction of drug treatment × trials, $F(3, 186) = 3.0$, $p < 0.05$. Separate repeated measures ANOVAs (trials) conducted for each drug treatment group yielded a significant main effect of trials for

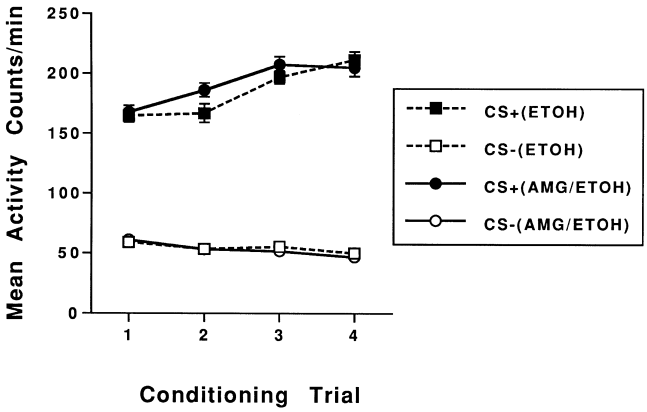

FIG. 1. Mean activity (±SEM) counts per min during CS+ and CS− sessions for the ETOH and AMG/ETOH groups during conditioning trials 1–4.

the ETOH, $F(3, 93) = 30.9$, $p < 0.001$, and AMG/ETOH group, $F(3, 93) = 14.8$, $p < 0.001$, confirming the development of sensitization to ethanol's stimulant effects in both ethanol-treated groups across the four trials. To further examine the nature of the two-way interaction, one-way ANOVAs (drug treatment) were conducted separately for each trial. These analyses yielded a significant main effect of drug treatment on trial 2 only, $F(1, 62) = 4.0$, $p < 0.05$. This effect was due to significantly higher activity counts for the AMG/ETOH group relative to the ETOH group and suggests that sensitization to ethanol may have developed more rapidly in AMG-treated animals.

Two-way ANOVA (drug treatment × trials) of CS− session data indicated significant effects of trials, $F(3, 186) = 14.1$, $p < 0.001$. Mean (±SEM) activity counts per min collapsed across drug treatment groups were 60.2 ± 1.1 on trial 1 and 48.1 ± 1.9 on trial 4, indicating habituation to experimental procedures. Overall, these analyses show that drug treatment on CS+ days did not affect group activity levels during CS− sessions.

Table 1 shows mean (±SEM) activity counts/min during CS+ and CS− sessions for the AMG control group on trials 1–4. Activity levels during CS+ sessions with AMG were higher relative to CS− sessions. A two-way repeated measures ANOVA (trials × drug type) conducted for the AMG group showed significant effects of trials, $F(3, 93) = 34.7$, $p < 0.001$, and drug type, $F(1, 31) = 8.1$, $p < 0.01$, but no interactions. This analysis indicates a slight activating effect of AMG on activity. Despite an overall decrease in activity across trials, activity counts during CS+ sessions remained significantly higher relative to CS− sessions. This suggests that mice habituated to experimental procedures across trials but did not become tolerant to the activating effect of AMG.

Preference Testing

Figure 2 shows the mean (±SEM) s/min spent on the grid floor by all conditioning subgroups during the 60 min preference test. To facilitate comparisons across drug treatment groups, the figure inset depicts the mean (±SEM) percent time spent on the ethanol-paired floor, averaged across conditioning subgroups. Mice in the G+ conditioning subgroup in ethanol-treated groups spent more time on the ethanol-paired grid floor than the G− subgroup, indicating a conditioned place preference for the grid floor.

The analysis of ethanol-treated groups (two-way ANOVA: drug treatment × conditioning group) yielded significant main effects of drug treatment, $F(1, 60) = 6.5$, $p = 0.013$, and conditioning group, $F(1, 60) = 56.1$, $p < 0.001$, but no interaction was found ($F < 1$). The significant main effect of conditioning group signifies a conditioned place preference for the ethanol-paired grid floor; however, the lack of a significant drug treatment × conditioning group interaction indicates no group differences in the magnitude of place preference. This analysis shows that AMG did not affect the development of place preference. Figure 2 suggests the drug treatment effect was due to less time spent on the grid floor by both conditioning subgroups in the AMG/ETOH group. This effect is possibly the result of a sampling error (i.e., a greater number of mice randomly assigned to the AMG/ETOH subgroups happened to show an unconditioned preference for the hole floor). Alternatively, AMG may alter tactile sensitivity and cause a shift (increase) in the amount of time spent on the hole floor in both conditioning subgroups. However, the separate one-way ANOVA (conditioning group) conducted for the AMG group showed no significant preference or aversion for the AMG-paired floor, $F(1, 29) = 1.2$, $p > 0.2$. Thus, these data show that AMG administered prior to conditioning trials does not alter the acquisition of ethanol-induced conditioned place preference. In addition, AMG administered alone does not cause a conditioned preference for either floor type (grid or hole).

Mean (±SEM) activity counts per min during the 60 min test session were 27.1 ± 1.0, 26.1 ± 1.8, and 24.1 ± 1.6 for AMG, ETOH, and AMG/ETOH groups, respectively. One-way ANOVA showed no effect of drug treatment (during conditioning) on activity levels during the preference test, $F(2, 92) = 1.0$, $p > 0.3$.

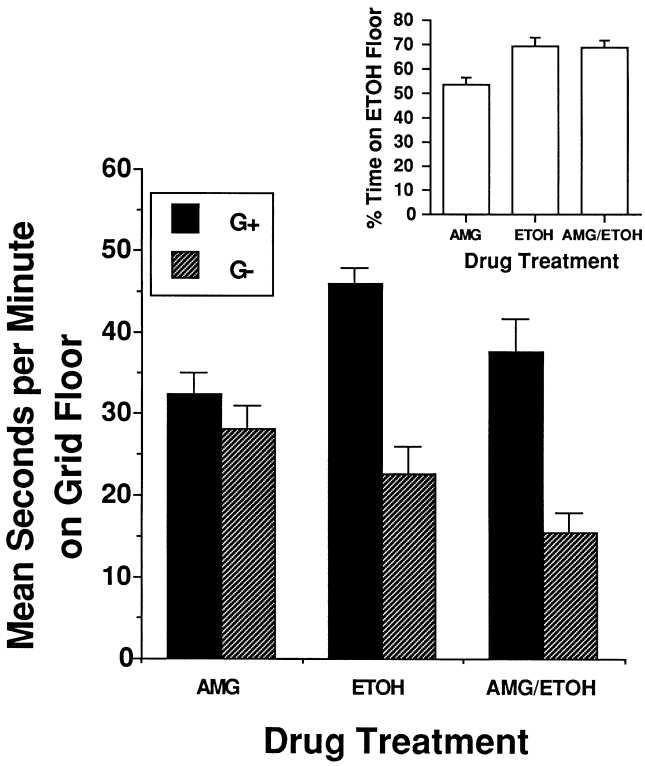

FIG. 2. Mean (±SEM) s/min spent on the grid floor by subjects in both conditioning subgroups of the AMG, ETOH, and AMG/ETOH groups during the preference test. During conditioning, G+ animals received AMG, ethanol, or both paired with the grid floor and vehicle and saline paired with the hole floor. G− animals received AMG, ethanol, or both paired with the hole floor and vehicle and saline paired with the grid floor. Data shown are collapsed across the 60 min session. The inset depicts the same data transformed to percent time spent on the floor paired with ethanol (ETOH) collapsed across G^+ and G^- subgroups within each drug treatment group.

TABLE 1

MEAN (±SEM) ACTIVITY COUNTS PER MIN DURING CS+ AND CS− SESSIONS FOR THE AMG GROUP ON TRIALS 1–4

CS Type	Trial 1	Trial 2	Trial 3	Trial 4
CS+	59.4 ± 2.7	45.6 ± 2.1	44.0 ± 2.1	40.8 ± 2.3
CS−	56.0 ± 2.2	42.3 ± 1.9	40.3 ± 2.1	35.8 ± 1.9

Control Experiment

AMG-treated animals showed significantly lower corticosterone levels following ethanol relative to vehicle-treated animals. Mean (±SEM) plasma corticosterone levels were 6.2 ± 0.3 μg/dl and 2.8 ± 0.4 μg/dl in the vehicle and AMG-treated groups, respectively, immediately following the 5 min session. One-way ANOVA showed a significant effect of drug treatment on corticosterone levels, $F(1, 10) = 44.4$, $p < 0.001$.

DISCUSSION

Although AMG-treated animals showed significantly lower levels of corticosterone on CS+ trials relative to vehicle-treated animals, AMG did not alter the acquisition of conditioned place preference with ethanol. Thus, this outcome does not support the hypothesis that a rise in corticosterone levels facilitates the conditioning of ethanol place preference by enhancing ethanol's rewarding effects. Moreover, this finding suggests that the acquisition of ethanol-induced place preference is independent of ethanol's corticosterone-elevating effects. These conclusions may be limited, however, because it is unclear whether ethanol produced a significant elevation in corticosterone during the 5 min conditioning session or AMG simply reduced the level of basal circulating corticosterone (control experiment). The AMG control group did not develop a preference or aversion for the drug-paired floor, showing that AMG does not possess any rewarding or aversive properties of its own in the place conditioning paradigm.

The corticosterone data suggest that an increase in corticosterone levels during conditioning trials does not normally influence the acquisition of ethanol place preference. However, even though the vehicle pretreated group had a significantly higher level of corticosterone than the AMG pretreated group, the level of corticosterone in the vehicle group was still within a nonstressed range [e.g., (34,40)]. This low level of corticosterone following ethanol is probably due to the fact that blood was sampled 5 min following the ethanol injection, but the peak in corticosterone following ethanol normally occurs after 30 min [e.g., (18)]. The addition of a vehicle/saline control group to compare to the vehicle/ethanol group would be helpful in determining the extent of ethanol's corticosterone-elevating effects during the 5 min conditioning session. It may be that the difference in corticosterone levels between the two groups during the 5 min conditioning sessions was not large enough to affect the magnitude of place preference. Perhaps this difference would have been larger if the experiment had been conducted during the dark phase of the light/dark cycle, because basal circulating corticosterone is higher during the dark cycle (35). Nonetheless, administration of AMG appeared to facilitate the development of locomotor sensitization across trials (i.e., AMG-treated animals showed increased activity counts on an earlier conditioning trial relative to vehicle-treated animals). One interpretation of this finding is that higher corticosterone levels may normally delay the development of sensitization. These data are in contrast to a previous study that showed a significant attenuation of ethanol-induced locomotor sensitization with administration of RU 38486, a glucocorticoid receptor antagonist (39).

In summary, blockade of corticosterone release by AMG did not affect the acquisition of ethanol-induced conditioned place preference. However, the results of this study suggest that AMG administration may facilitate the development of locomotor sensitization with repeated ethanol exposure. Thus, consistent with other recent studies using this paradigm (1,36,37), there was no relationship between the acute stimulant response to ethanol and the magnitude of conditioned place preference.

EXPERIMENT 2: EFFECTS OF AMG ON EXPRESSION OF CONDITIONED PLACE PREFERENCE

The results of Experiment 1 suggest that inhibition of corticosterone release does not alter the rewarding ethanol effects responsible for the acquisition of conditioned place preference. However, high corticosterone levels may still be an important factor modulating the expression and maintenance of conditioned ethanol reward. Handling and injection procedures prior to a preference test may result in a substantial release of corticosterone, or they may become conditioned stimuli (after repeated pairings with ethanol) that trigger a conditioned release of corticosterone. In addition, exposure to the floor CS may cause a conditioned corticosterone release, or there may be nonspecific arousal effects resulting in elevated corticosterone.

The purpose of Experiment 2 was to examine the effect of AMG on the expression of conditioned ethanol place preference. It was hypothesized that elevated corticosterone levels during a drug-free preference test may be important in activating or modulating mechanisms responsible for the expression of conditioned reward. Because the ability to detect such effects might vary with the strength of conditioned place preference, we examined the effects of AMG on the conditioning produced by two different doses of ethanol (1.5 and 2 g/kg). The lower dose of ethanol was expected to produce a smaller magnitude of preference. Because the 2 g/kg ethanol dose often produces a near-maximal preference in DBA/2J mice, we chose to include a group conditioned with 1.5 g/kg ethanol to optimize the possibility of observing an effect of AMG (increase or decrease in preference magnitude) on the expression of preference.

To obtain a measure of plasma corticosterone in each experimental group and confirm AMG's suppressive effect on corticosterone release, blood was taken from subjects in each group following the preference test for radioimmunoassay. Mice received either AMG or vehicle 2 h before the preference test. All mice received a saline injection immediately before the preference test. It was expected that AMG-treated groups would have significantly lower corticosterone levels relative to vehicle-treated animals.

Procedure

The experiment consisted of three phases: one habituation session, eight conditioning sessions, and one test session. Habituation and conditioning procedures were identical to those used in Experiment 1, except that AMG was not administered during the conditioning phase of the study.

For the conditioning phase, all subjects were randomly assigned to one of two ethanol dose groups: 1.5 g/kg and 2 g/kg. Within each of the experimental groups, mice were randomly assigned to G+ and G− conditioning subgroups ($n = 27$–30) and subjected to standard ethanol place conditioning procedures, as previously described.

For the 60 min test session, mice from each ethanol dose group were assigned to one of two AMG dose groups (0 or 50 mg/kg). An injection of either vehicle or AMG was administered IP (10 ml/kg) 2 h before the preference test. A saline injection was also given immediately before the test session to match the cues during conditioning days. During the test, the floor was half grid and half hole with left/right position counterbalanced within groups. Immediately following the test ses-

sion, approximately 20 μl of tail blood was taken from each mouse for corticosterone assay.

RESULTS

Conditioning

Figure 3 shows mean activity counts/min during conditioning trials 1–4 averaged across each ethanol dose group. Ethanol produced significant locomotor activation during CS+ sessions relative to CS− sessions in both the 1.5 and 2 g/kg dose group.

Two-way ANOVAs (ETOH dose × trials) were separately conducted for CS+ and CS− session data. The CS+ ANOVA revealed a significant effect of ETOH dose, $F(1, 113) = 9.8$, $p < 0.01$, and trials, $F(3, 339) = 21.9$, $p < 0.0001$, and a significant interaction, $F(3, 339) = 5.9$, $p < 0.001$. To further investigate the ETOH dose × trials interaction, one-way repeated measures ANOVAs were conducted for each ETOH Dose group (1.5 and 2 g/kg). A significant increase in activity across trials was found in the 2 g/kg dose group, $F(3, 168) = 24.1$, $p < 0.0001$, indicating the development of locomotor sensitization with repeated ethanol exposure. Significant locomotor sensitization did not occur across trials in the 1.5 mg/kg group, $F(3,171) = 2.8$, NS. The CS− ANOVA showed no significant main effects and no interactions (Fs < 1).

Preference Testing

Figure 4 shows the mean (±SEM) s/min spent on the grid floor by both conditioning subgroups in the four drug treatment groups during the 60 min preference test. The figure inset depicts the mean (±SEM) percent time spent on the ethanol-paired floor, averaged across conditioning subgroups. G+ subgroups in each drug treatment group spent significantly more time on the grid floor relative to G− subgroups, indicating the development of ethanol-induced preference for the grid floor.

Overall analysis of the data collapsed across the 60 min test session (three-way ANOVA: AMG group × ETOH dose × conditioning group) yielded a significant effect of conditioning group, $F(1, 107) = 34.9$, $p < 0.0001$, indicating a conditioned place preference for the ethanol-paired floor. No significant effects of AMG group, ETOH dose, or interactions were found. Although the magnitude of preference in the 1.5/AMG group appears to be reduced relative to the 1.5/VEH group (Fig. 4), there is no statistical support for concluding that AMG decreased the magnitude of place preference in this group. Thus, AMG did not affect expression of ethanol-induced place conditioning in either the 1.5 or 2 mg/kg dose groups.

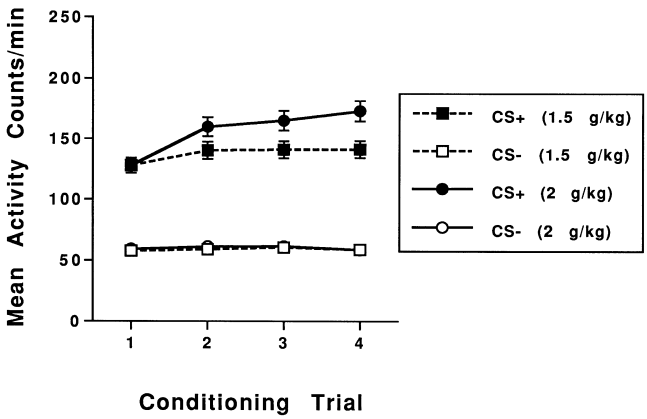

FIG. 3. Mean (±SEM) activity counts per min during CS+ and CS− sessions for the 1.5 and 2 g/kg ethanol dose groups during conditioning trials 1–4.

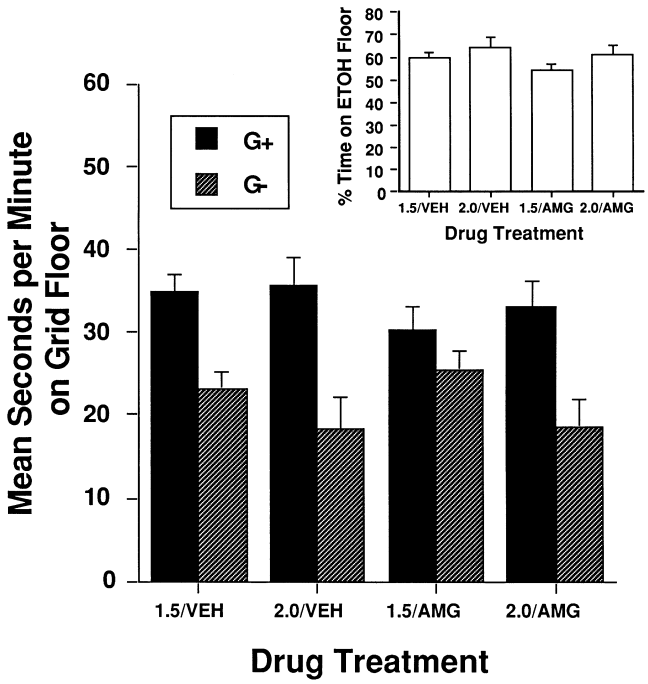

FIG. 4. Mean (±SEM) s/min spent on the grid floor by subjects in both conditioning subgroups of the four drug treatment groups during the preference test. During conditioning, G+ subjects received ethanol (1.5 or 2.0 g/kg) paired with the grid floor and saline paired with the hole floor and G− subjects received ethanol paired with the hole floor and saline paired with the grid floor. Two hours before the preference test, subjects received an injection of AMG or vehicle. Data shown are collapsed across the 60 min session. The inset depicts the same data transformed to percent time spent on the floor paired with ethanol (ETOH) collapsed across G^+ and G^- subgroups within each drug treatment group.

Activity levels during the preference test were higher in the 1.5 g/kg ETOH dose group relative to the 2.0 g/kg group. Activity levels were also higher in AMG-treated groups relative to vehicle-treated groups. Mean (±SEM) activity counts/min during the 60 min test were 34.3 ± 1.3, 29.4 ± 1.5, 39.0 ± 1.6, and 34.1 ± 1.6 for the 1.5/vehicle, 2.0/vehicle, 1.5/AMG, and 2.0/AMG groups, respectively. Two-way ANOVA (AMG group × ETOH dose) revealed a significant effect of AMG group, $F(1, 111) = 10.0$, $p < 0.01$, and ETOH dose, $F(1, 111) = 11.1$, $p < 0.01$, on activity levels during the test.

Corticosterone Assay

AMG-treated groups showed reduced corticosterone levels compared to vehicle-treated groups. Mean (±SEM) plasma corticosterone levels were 14.6 ± 1.1, 16.3 ± 1.6, 2.7 ± 0.2, and 3.6 ± 0.3 in the 1.5/vehicle, 2.0/vehicle, 1.5/AMG, and 2.0/AMG groups, respectively, immediately after the 60 min preference test. Three-way ANOVA (AMG group × ETOH dose × conditioning group) showed a significant effect of AMG on corticosterone levels, $F(1, 104) = 147.0$, $p < 0.0001$. No significant effect of ETOH dose or conditioning group on corticosterone levels was observed.

DISCUSSION

AMG administration did not affect the magnitude of ethanol place preference in the 1.5 or 2.0 g/kg ethanol dose groups. Thus, these results do not support the hypothesis that stressor-induced corticosterone levels influence the expression of ethanol place preference.

AMG-treated groups showed significantly higher activity levels during the preference test relative to vehicle-treated groups within the 1.5 and 2.0 g/kg ETOH Dose groups. This finding is consistent with the conditioning trial activity data from Experiment 1, which showed a locomotor-activating effect of AMG administered alone. Within the AMG and vehicle-treated groups, activity levels during the preference test were also higher in the 1.5 g/kg ETOH dose group relative to the 2.0 g/kg group. This is possibly due to a conditioned suppression of activity levels in the 2.0 g/kg group relative to the 1.5 g/kg group. Alternatively, this effect could be due to sampling error.

Corticosterone levels in AMG-treated groups were significantly lower relative to vehicle-treated groups. Vehicle-treated groups showed a higher level of corticosterone, which supports the hypothesis that corticosterone is normally elevated during a preference test. The level of plasma corticosterone levels in these groups was comparable to those observed 30 min following novelty stress or 1.5 mg/kg corticosterone (~13–15 μg/dl) (6). Elevated corticosterone in the vehicle-treated groups could be due to a conditioned corticosterone release triggered by handling and injection cues or exposure to the floor CS. In addition, general arousal from exposure to the testing apparatus could account for an increase in corticosterone. However, reducing the level of corticosterone with AMG did not alter the expression of place preference. Overall, these data suggest that corticosterone does not modulate the expression of ethanol-induced conditioned place preference.

GENERAL DISCUSSION

The present experiments examined a role for corticosterone in modulating the rewarding effects of ethanol in the place conditioning paradigm. The results of Experiment 1 show that an acute rise in corticosterone on CS+ trials does not facilitate the acquisition of place preference. Consistent with previous studies [e.g., (1,36)], these data also suggest a dissociation between ethanol's rewarding and locomotor effects. The results of Experiment 2 suggest that elevated plasma corticosterone levels do not modulate the expression of ethanol-induced conditioned place preference.

The mechanism by which AMG increases ethanol-stimulated locomotor activity is unclear. Although the difference in locomotor activity between AMG- and vehicle-treated animals was rather small, these data suggest that corticosterone may normally inhibit ethanol-stimulated locomotor sensitization. If so, the concentration of corticosterone necessary for an inhibitory effect is not very high because plasma corticosterone in the vehicle-treated group (~6 μg/dl) was only slightly elevated relative to the AMG-treated group (~3 μg/dl). Alternatively, the increase in ethanol-stimulated activity in AMG-treated animals could be due to an effect of AMG not related to its effect on corticosterone release. AMG blocks the synthesis and release of corticosterone by inhibiting the conversion of cholesterol to pregnenolone, the first step in the adrenal steroid synthesis pathway (7). Because pregnenolone is the precursor for every adrenally derived steroid (44), AMG also inhibits the synthesis of many other steroids, such as mineralocorticoids, androgens, and estrogens, which could be important in modulating locomotor activity. The present data, however, are not consistent with another study that found adrenalectomy significantly decreased stimulated locomotor activity in response to 1.5 g/kg ethanol in female mice (43). The discrepancy between these studies may be due to different physiological effects of adrenalectomy vs. AMG. For example, the biochemical deficits produced by adrenalectomy are permanent and probably more severe than the effects of acute administration of AMG. In addition, because AMG does not completely eliminate adrenally derived steroids, including corticosterone, it may allow a more normal physiological state (38).

The results of Experiments 1 and 2 suggest that corticosterone is not involved in the acquisition or expression of ethanol-induced conditioned place preference. In addition to corticosterone, it is possible that other adrenal steroids might have rapid neural effects that alter behavior. Because AMG inhibits all other steroids derived from the adrenal cortex, these findings suggest that a reduction in other circulating steroids (e.g., aldosterone, which is synthesized from corticosterone) may not alter the acquisition or expression of ethanol place preference. Many of these steroids have a short half-life (approximately 20 min) and would be expected to be significantly reduced 2 h following AMG administration (44). It might be useful to coadminister AMG with a corticosterone replacement to disentangle effects due to changes in corticosterone from changes in other steroids.

Overall, the present experiments do not support the hypothesis that corticosterone is important in modulating ethanol's unconditioned and conditioned rewarding properties by exerting a rapid effect on the neural substrate mediating the acquisition or expression of ethanol-induced conditioned place preference. In general, these data are inconsistent with previous studies that demonstrated a facilitatory effect of corticosterone on the rewarding properties of amphetamine (29) and ethanol (9,11). There are several possible reasons for the discrepancies between the previous and present studies. For example, the facilitatory effect of corticosterone on the rewarding properties of amphetamine and ethanol may be specific to the self-administration paradigm. This could be due to different routes of administration and time course of effects (acute vs. chronic exposure) of corticosterone in the IV and oral self-administration paradigm relative to the place conditioning paradigm. Furthermore, the level of corticosterone achieved during the 5 min conditioning session in Experiment 1 may be significantly lower than the level of corticosterone shown in previous studies to facilitate the rewarding effects of amphetamine and ethanol in rats. Moreover, the absence of a vehicle/saline group to compare to the vehicle/ethanol group (control experiment) limits the interpretation of AMG's effects on an ethanol-induced rise in corticosterone levels during acquisition of place preference.

It is possible that subject vs. experimenter control over exposure to these drugs may be an important factor in determining corticosterone's facilitatory effect. Thus, corticosterone may specifically interact with neural pathways that mediate the reinforcing and rewarding properties of amphetamine and ethanol in self-administration paradigms, and these pathways may be distinct from those mediating the rewarding effects of ethanol in the place conditioning paradigm. Another possibility is that the effect of corticosterone in the previous studies may be unique to rats that show a predisposition for self-administration. Several studies suggest this predisposition is due to individual differences in dopaminergic reactivity to these drugs (8,10) and dopaminergic reactivity may also be influenced by a differential corticosterone response to these drugs (28). These individual differences may be due to genetic

variability in the rats strains that were utilized. In the present studies, the inbred DBA/2J mouse strain was used because these mice are highly sensitive to ethanol's rewarding properties in the place conditioning paradigm. In addition, because these mice are genetically identical, they exhibit a stable phenotype to examine the neuropharmacological basis of ethanol reward. However, the findings in the present studies may not be generalizable to other strains of mice or other species, such as rats. Consequently, corticosterone may indeed facilitate the rewarding effects of abused drugs in other species, as suggested by the findings of other studies (9,11,12,29). Nevertheless, the present studies suggest that corticosterone does not modulate the unconditioned and conditioned rewarding properties of ethanol in DBA/2J mice in the place conditioning paradigm.

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REFERENCES

1. Cunningham, C. L.: Localization of genes influencing ethanol-induced conditioned place preference and locomotor activity in BXD recombinant inbred mice. Psychopharmacology (Berlin) 120:28–41; 1995.
2. Cunningham, C. L.; Niehus, D. R.; Malott, D. H.; Prather, L. K.: Genetic differences in the rewarding and activating effects of morphine and ethanol. Psychopharmacology (Berlin) 107:385–393; 1992.
3. Cunningham, C. L.; Noble, D.: Conditioned activation induced by ethanol: Role in sensitization and conditioned place preference. Pharmacol. Biochem. Behav. 43:307–313; 1992.
4. Cunningham, C. L.; Prather, L. K.: Conditioning trial duration affects ethanol-induced conditioned place preference in mice. Anim. Learn. Behav. 20:187–194; 1992.
5. Deroche, V.; Piazza, P. V.; Le Moal, M.; Simon, H.: Social isolation-induced enhancement of the psychomotor effects of morphine depends on corticosterone secretion. Brain Res. 640:136–139; 1994.
6. Deroche, V.; Piazza, P. V.; Maccari, S.; Le Moal, M.; Simon, H.: Repeated corticosterone administration sensitizes the locomotor response to amphetamine. Brain Res. 584:309–313; 1992.
7. Dexter, R. N.; Fishman, L. M.; Ney, R. L.; Liddle, G. W.: Inhibition of adrenal corticosteroid synthesis by aminoglutethimide: Studies of the mechanism of action. J. Clin. Endocrinol. 27:473–480; 1967.
8. Engel, J. A.; Enerback, C.; Fahlke, C.; Hulthe, P.; Hård, E.; Johannessen, K.; Svensson, L.; Soderpalm, B.: Serotonergic and dopaminergic involvement in ethanol intake. In: Naranjo, C. A.; Sellers, E. M., eds. Novel pharmacological interventions for alcoholism. Berlin: Springer; 1992:68–82.
9. Fahlke, C.; Engel, J. A.; Eriksson, C. J. P.; Hård, E.; Soderpalm, B.: Involvement of corticosterone in the modulation of ethanol consumption in the rat. Alcohol 11:195–202; 1994.
10. Fahlke, C.; Hård, E.; Eriksson, C. J. P.; Engel, J. A.; Hansen, S.: Amphetamine-induced hyperactivity: Differences between rats with high or low preference for alcohol. Alcohol 12:363–367; 1995.
11. Fahlke, C.; Hård, E.; Eriksson, C. J. P.; Engel, J. A.; Hansen, S.: Consequence of long-term exposure to corticosterone or dexamethasone on ethanol consumption in the adrenalectomized rat, and the effect of type I and type II corticosteroid receptor antagonists. Psychopharmacology (Berlin) 117:216–224; 1995.
12. Fahlke, C.; Hård, E.; Thomasson, R.; Engel, J. A.; Hansen, S.: Metyrapone-induced suppression of corticosterone synthesis reduces ethanol consumption in high-preferring rats. Pharmacol. Biochem. Behav. 48:977–981; 1994.
13. Finn, D. A.; Phillips, T. J.; Okorn, D. M.; Chester, J. A.; Cunningham, C. L.: Rewarding effect of the neuroactive steroid 3α-hydroxy-5α-pregnan-20-one in mice. Pharmacol. Biochem. Behav. 56:261–264; 1997.
14. Goeders, N. E.; Geurin, G. F.: Noncontingent electric foot shock facilitates the acquisition of intravenous cocaine self-administration in rats. Psychopharmacology (Berlin) 114:63–70; 1994.
15. Grunberg, N. E.; Baum, A.: Biological commonalities of stress and substance abuse. In: Shiffman, S. W.; Wills, T. A., eds. Coping and substance abuse. Orlando: Academic Press, Inc.; 1985:25–62.
16. Henkin, R. I.: The role of adrenal corticosteroids in sensory processes. In: Greep, R. O.; Astwood, E. B., eds. Handbook of physiology. Washington, DC: American Physiological Society; 1975: 209–230.
17. Higley, J. D.; Hasert, M. F.; Suomi, S. J.; Linnoila, M.: Nonhuman primate model of alcohol abuse: Effects of early experience, personality, and stress on alcohol consumption. Proc. Natl. Acad. Sci. USA 88:7261–7265; 1991.
18. Kakihana, R.; Noble, E. P.; Butte, J. C.: Corticosterone response to ethanol in inbred strains of mice. Nature 218:360–361; 1968.
19. Keith, L. D.; Winslow, J. R.; Reynolds, R. W.: A general procedure for estimation of corticosteroid response in individual rats. Steroids 31:523–531; 1978.
20. Lemaire, V.; Deminiere, J.; Mormede, P.: Chronic social stress conditions differentially modify vulnerability to amphetamine self-administration. Brain Res. 649:348–352; 1994.
21. Mardones, J.: Experimentally induced changes in the free selection of ethanol. Int. Rev. Neurobiol. 2:41–76; 1960.
22. Marlatt, G. A.; Baer, J. S.: Addictive behaviors: Etiology and treatment. Annu. Rev. Psychol. 39:223–252; 1988.
23. Morin, L. P.; Forger, N. G.: Endocrine control of ethanol intake by rats or hamsters: Relative contributions of the ovaries, adrenals and steroids. Pharmacol. Biochem. Behav. 17:529–537; 1982.
24. Murphy, B. P.; Engelberg, W.; Pattee, C. J.: Simple method for the determination of plasma corticoids. J. Clin. Endocrinol. 23: 293–300; 1963.
25. Nash, J. F. J.; Maickel, R. P.: Stress-induced consumption of ethanol by rats. Life Sci. 37:757–765; 1985.
26. Newcomb, M. D.; Harlow, L. L.: Life events and substance abuse among adolescents: Mediating effects of perceived loss of control and meaningless in life. J. Pers. Soc. Psychol. 51:564–577; 1986.
27. Piazza, P. V.; Deminière, J. M.; Le Moal, M.; Simon, H.: Stress- and pharmacologically induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Res. 514:22–26; 1990.
28. Piazza, P. V.; Deminière, J.; Maccari, S.; Le Moal, M.; Mormede, P.; Simon, H.: Individual vulnerability to drug self-administration: Action of corticosterone on dopaminergic systems as a possible pathophysiological mechanism. In: Willner, P.; Scheel-Kruger, J., eds. The mesolimbic dopamine system: From motivation to action. New York: John Wiley & Sons, Ltd.; 1991:473–495.
29. Piazza, P. V.; Maccari, S.; Deminière, J.; Le Moal, M.; Mormede, P.; Simon, H.: Corticosterone levels determine individual vulnerability to amphetamine self-administration. Proc. Natl. Acad. Sci. USA 88:2088–2092; 1991.
30. Piazza, P. V.; Marinelli, M.; Jodogne, C.; Deroche, V.; Rouge-Pont, F.; Maccari, S.; Le Moal, M.; Simon, H.: Inhibition of corticosterone synthesis by metyrapone decreases cocaine-induced locomotion and relapse of cocaine self-administration. Brain Res. 658:259–264; 1994.
31. Pohorecky, L. A.: Interaction of ethanol and stress: Research with experimental animals—An update. Alcohol Alcohol. 25: 263–276; 1990.
32. Pohorecky, L. A.: Stress and alcohol interaction: An update of human research. Alcohol. Clin. Exp. Res. 15:438–459; 1991.
33. Prasad, C.; Prasad, A.: A relationship between increased volun-

tary alcohol preference and basal hypercorticosteronemia associated with an attenuated rise in corticosterone output during stress. Alcohol 12:59–63; 1995.
34. Reul, J. M. H. M.; de Kloet, E. R.: Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. Endocrinology 117:2505–2511; 1985.
35. Reul, J. M. H. M.; van den Bosch, F. R.; de Kloet, E. R.: Differential response of type I and type II corticosteroid receptors to changes in plasma steroid level and circadian rythmicity. Neuroendocrinology 45:407–412;1987.
36. Risinger, F. O.; Dickinson, S. D.; Cunningham, C. L.: Haloperidol reduces ethanol-induced motor activity stimulation but not conditioned place preference. Psychopharmacology (Berlin) 107: 453–456; 1992.
37. Risinger, F. O.; Malott, D. M.; Prather, L. K.; Niehus, D. R.; Cunningham, C. L.: Motivational properties of ethanol in mice selectively bred for ethanol-induced locomotor differences. Psychopharmacology (Berlin) 116:207–216; 1994.
38. Roberts, A. J.; Gallaher, E. J.; Keith, L. D.: Dissociation of the effect of aminoglutethimide on corticosterone biosynthesis from ataxic and hypothermic effects in DBA and C57 mice. Neuroendocrinology 58:303–309; 1993.
39. Roberts, A. J.; Lessov, C. N.; Phillips, T. J.: Critical role for glucocorticoid receptors in stress- and ethanol-induced locomotor sensitization. J. Pharmacol. Exp. Ther. 275:790–797; 1995.
40. Sapolsky, R. M.; Krey, L. C.; McEwen, B. S.: Stress downregulates corticosterone receptors in a site-specific manner in the brain. Endocrinology 114:287–292; 1984.
41. Shaham, Y.; Alvares, K.; Nespor, S. M.; Grunberg, N. E.: Effect of stress on oral morphine and fentanyl self-administration in rats. Pharmacol. Biochem. Behav. 41:615–619; 1992.
42. Thiagarajan, A. B.; Mefford, I. N.; Eskay, R. L.: Single-dose ethanol administration activates the hypothalamic–pituitary–adrenal axis: Exploration of the mechanism of action. Neuroendocrinology 50:427–432; 1989.
43. Wallis, C. J.; Anton, R. F.; Randall, C. L.: Adrenalectomy reduces alcohol-stimulated activity: Blood and brain alcohol content. Pharmacol. Biochem. Behav. 20:883–886; 1984.
44. White, P. C.; Pescovitz, O. H.; Cutler, G. B. J.: Synthesis and metabolism of corticosteroids. In: Becker, K. L.; Bilezikian, J. P.; Bremner, W. J.; Hung, W.; Kahn, C. R.; Loriaux, D. L.; Nylen, E. S.; Rebar, R. W.; Robertson, G. L.; Wartofsky, L., eds. Principles and practice of endocrinology and metabolism. Philadelphia: J. B. Lippincott Company; 1995:647–662.