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Rewarding Effect of the Neuroactive Steroid 3α-hydroxy-5α-pregnan-20-one in Mice

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FINN, D. A., T. J. PHILLIPS, D. M. OKORN, J. A. CHESTER AND C. L. CUNNINGHAM. *Rewarding effect of the neuroactive steroid 3α-hydroxy-5α-pregnan-20-one in mice.* PHARMACOL BIOCHEM BEHAV **56**(2) 261–264, 1997.—The $GABA_A$-receptor agonist neuroactive steroid 3α-hydroxy-5α-pregnan-20-one (3α,5α-P) has anxiolytic and locomotor stimulant effects and shares some subjective properties with benzodiazepines, barbiturates and ethanol, but there have been no studies of its reinforcing or rewarding effects. The present study examined the rewarding properties of 3α,5α-P using the conditioned place preference paradigm. Male DBA/2J mice received four pairings of a distinctive floor stimulus with 3α, 5α-P (3.2, 10 or 17 mg/kg, IP) in an unbiased conditioning procedure. On alternate days a different distinctive floor was paired with vehicle. At the lowest dose (3.2 mg/kg), there was no difference between conditioning subgroups in preference for the drug-paired floor type, indicating an absence of place conditioning. However, a dose-dependent conditioned preference was evident at the higher doses as shown by the greater amount of time spent on the floor paired with 3α,5α-P. In addition, 3α,5α-P produced a dose-dependent increase in locomotor activity, which was significant following the 17 mg/kg dose. A control study showed no effect of the β-cyclodextrin vehicle on place conditioning in the absence of neurosteroid. These results provide the first demonstration that 3α,5α-P, an endogenous modulator of $GABA_A$ receptor function, possesses rewarding properties using the conditioned place preference paradigm.

Conditioned place preference Reward Locomotor activity Neuroactive steroid $GABA_A$-receptor agonist Inbred mice

THE discovery that some neuroactive steroids interact with the $GABA_A$ receptor complex (GRC) to potentiate GABA-stimulated chloride conductance provides evidence for rapid membrane actions of these steroid metabolites in a manner distinct from the genomic action of "classical" steroid hormones. The reduced A-ring metabolites of progesterone (i.e., 3α-hydroxy-5α-pregnan-20-one or 3α,5α-P) and deoxycorticosterone (i.e., 3α,21-dihydroxy-5α-pregnan-20-one or 5α-THDOC) are potent modulators of the GRC (see reviews 10,16). When administered exogenously, 3α,5α-P produces anxiolytic, locomotor stimulant, ataxic, hypnotic and anticonvulsant effects (10,16). These behavioral effects are reminiscent of those produced by ethanol, benzodiazepines (BZs) and barbiturates. In addition, plasma and brain 3α,5α-P can reach levels that are within the range of concentrations previously shown to potentiate the *in vitro* action of GABA at the GRC (16), which suggests that it may be a physiologically significant neuromodulator.

The behavioral actions of 3α,5α-P and 5α-THDOC have been further characterized by studies comparing their discriminative-stimulus and response rate effects with those of classic sedative/hypnotic drugs. Administration of 3α,5α-P and 5α-THDOC produced complete generalization in rats trained to discriminate pentobarbital, ethanol or diazepam from the no-drug condition (1). These results suggest that 3α,5α-P and 5α-THDOC may produce some subjective effects which are similar to drugs with abuse liability. Collectively, the drug discrimination results and marked locomotor stimulant and anxiolytic properties of 3α,5α-P and 5α-THDOC raise the possibility that these endogenous steroids are rewarding, and may also play a role in modulating the rewarding effects of abused drugs.

There are no published data addressing the issue of whether $GABA_A$-receptor agonist neuroactive steroids are rewarding. Therefore, the potential rewarding properties of 3α,5α-P were examined using the conditioned place preference paradigm,

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which assesses a drug's motivational value by measuring an animal's tendency to approach a stimulus that was previously paired with drug in a Pavlovian conditioning paradigm (3). Place conditioning has the advantage of allowing one to evaluate a drug's hedonic effects in the absence of drug, which avoids the possible confounding influence of the drug's effects on performance of the target behavior (3). This model has been used successfully to demonstrate the rewarding effect of other GRC agonists, such as diazepam (19). In addition, the specific apparatus, experimental design and procedure used in the present study have been used successfully many times in studies of the rewarding effects of ethanol and morphine (2,4–8,17,18).

METHOD

Subjects

Adult male DBA/2J mice (Jackson Laboratory; Bar Harbor, ME) were shipped at 6 weeks of age and allowed to acclimate to the animal colony for 2 weeks before training. The mice (n = 96) were housed in groups of four with water and lab chow available at all times in the home cage. The experiment was conducted during the light phase of a 12 hr light-dark cycle (lights on at 0700), but no light was present during conditioning and testing.

Apparatus

Twelve identical place conditioning chambers (30 ×15 ×15 cm) made from acrylic and aluminum were enclosed in separate ventilated, light- and sound-attenuating enclosures (Coulbourn Instruments Model E10-20). Six sets of infrared light sources and photodetectors were mounted opposite each other at 5-cm intervals on the long walls of each box, 2.2 cm above the floor. Occlusion of the infrared light beams was used both as a measure of general activity and to detect the animal's position within the box. Total activity counts and amount of time (0.01 s resolution) spent on each side of the chamber were recorded every minute by microcomputer (5). Each box was placed over a floor with interchangeable halves made of one of two textures.

The "grid" floor was constructed using 2.3-mm stainless steel rods mounted 6.4-mm apart in acrylic rails. The "hole" floor was made from perforated stainless steel (16 GA) with 6.4-mm round holes on 9.5-mm staggered centers. This combination of floor textures was selected on the basis of several previous studies showing that vehicle-treated control groups spend about half their time on each floor type during preference tests (e.g., 2, 4–6). The floors and inside of the box were wiped with a damp sponge and the litter paper beneath the floors was changed before each subject was placed in the chamber.

Procedure

The experiment involved three phases: habituation (one session), conditioning (eight sessions), and testing (one session). Sessions were conducted 5 days a week with a 2-day break between the first four and second four conditioning sessions. Each mouse was weighed and injected (IP) immediately before being placed in the center of the apparatus for each session.

The habituation session was intended to reduce the novelty and stress associated with handling, injection and exposure to the apparatus. All mice were injected with vehicle [20% 2-hydroxypropyl-β-cyclodextrin (β-cyclodextrin); Research Biochemicals International, Natick, MA] at a volume of 0.01 ml/gm body weight and placed in the conditioning chamber on a smooth floor covered with paper for 5 min. Subjects were not exposed to the distinctive floor textures at this time in order to avoid latent inhibition (15).

During the conditioning phase, the mice were randomly assigned to one of three 3α,5α-P dose groups: 3.2, 10 or 17 mg/kg (0.32, 1.0 or 1.7 mg/ml solution in 20% β-cyclodextrin mixed in sterile distilled water) and then further assigned to one of two conditioning subgroups (n = 16/group). On alternate days, mice in the GRID+ subgroups received steroid prior to placement on the grid floor (CS+ trial), and vehicle prior to placement on the hole floor (CS− trial). In contrast, mice in the GRID− subgroups received vehicle before placement on the grid floor (CS− trial) and steroid before placement on the hole floor (CS+ trial). Four 15-min conditioning trials of each type were counterbalanced for order of exposure to CS+ and CS− and given over an 8-day period. On each conditioning trial, subjects had access to both sides of the apparatus and floor texture was homogeneous. In this discriminative conditioning design, the two counterbalanced subgroups within each dose group were matched for overall exposure to each floor type, steroid, and vehicle, and differed only in the floor-steroid contingency. Because differences between the subgroups during preference testing are presumably due to learning based on the Pavlovian relationship between the CS+ and neurosteroid (cf. 3), statistical analysis of test performance focused on comparisons between the GRID+ and GRID$^-$ subgroups within each dose condition.

The floor preference test was given 24 h after the last conditioning trial. All subjects received a vehicle injection before placement in the apparatus with half grid floor and half hole floor. Therefore, testing occurred in a drug-free state. Relative position of the floors (i.e., left vs. right) was counterbalanced within each subgroup. The primary dependent variable was the amount of time spent on the grid floor during the 60-min test session.

Control Experiment

To determine whether the β-cyclodextrin vehicle had effects on place preference in the absence of neurosteroid, an additional 24 DBA/2J mice were used in a control experiment in which the CS+ floor was paired with injection of β-cyclodextrin and the CS− floor was paired with injection of saline. Thus, both CSs were paired with handling and injection, but only the CS+ was associated with β-cyclodextrin. Because previous research has indicated that handling and injection of saline are not sufficient to produce place conditioning in this strain (7), group differences created by this conditioning procedure would presumably reflect motivational changes induced by β-cyclodextrin. As in the main experiment, mice were randomly assigned to GRID+ or GRID− subgroups (n = 12/group) and exposed to an habituation session, eight conditioning sessions and a preference test using temporal parameters identical to those described earlier.

Statistical Analysis

Activity and preference test data were analyzed by analysis of variance (ANOVA) using a 0.05 alpha level. Due to an equipment malfunction, the activity data on Conditioning Trial 1 were lost for one subject in the 10 mg/kg group. This

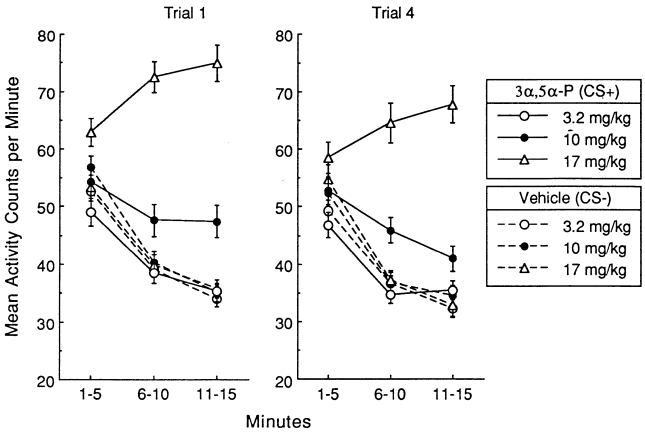

FIG. 1. Mean activity counts per min (± SEM) during consecutive 5-min sample periods on the first (left panel) and last (right panel) place conditioning trials. Solid lines depict activity after injection of the assigned dose of 3α,5α-P on CS+ trials, whereas dashed lines depict activity after vehicle (β-cyclodextrin) injection on CS− trials. Group labels refer to the neurosteroid dose received on CS+ trials.

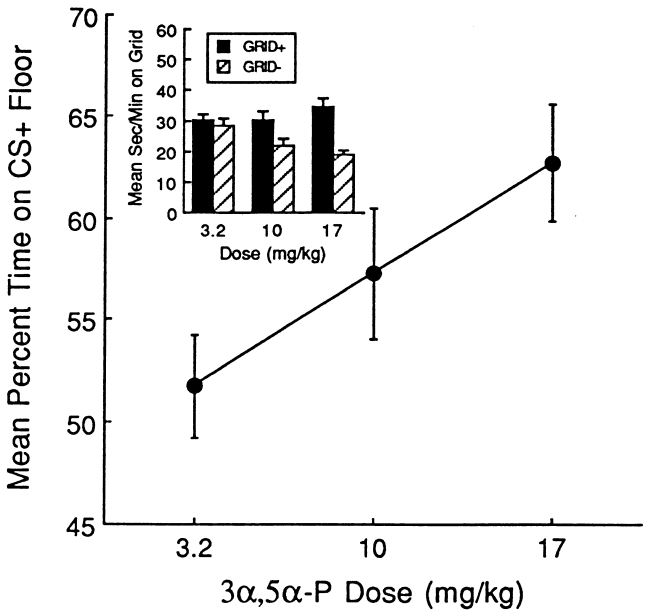

FIG. 2. Mean percent (± SEM) of the 60-min place preference test spent on the CS+ floor by mice in each of the three neurosteroid dose groups (collapsed over GRID+ and GRID−). The inset shows the raw data (s/min on the grid floor) for both of the conditioning subgroups at each dose. Mice in the GRID+ subgroups had previously received four pairings of the grid floor with 3α,5α-P and four pairings of the hole floor with vehicle. The floor-steroid contingency was reversed for mice in the GRID− conditioning groups.

subject was excluded from conditioning trial analyses, but was included in analyses of the place preference test.

RESULTS

Figure 1 depicts mean activity counts per minute during successive 5-min periods of the first and last conditioning trials. In general, 3α,5α-P produced a dose-dependent increase in locomotor activity, relative to the activity of vehicle treated mice, over the dose range studied in this experiment. Activity increased steadily over the 15-min period after injection at the highest dose (17 mg/kg), but declined over time at the two lower doses and after vehicle injection on CS− trials. The decline in activity over trials for both CS+ and CS− was consistent with the development of habituation to the apparatus and procedure. Analysis of activity on CS+ trials (Dose × Trial × Time mixed ANOVA) yielded significant main effects of Dose [$F(2, 92) = 67.2$, $p < 0.0001$], Trial [$F(1, 92) = 7.0$, $p < 0.01$], and Time [$F(2, 184) = 9.6$, $p < 0.001$], and a significant Dose × Time interaction [$F(4, 184) = 32.4$, $p < 0.0001$]. A similar analysis of activity on CS− trials showed significant main effects of Trial [$F(1, 92) = 6.1$, $p < 0.02$] and Time [$F(2, 184) = 357.8$, $p < 0.0001$].

Figure 2 depicts the mean percentage of the 60-min test that mice in each dose group spent on their CS+ floor (collapsed across GRID+ and GRID− subgroups). As can be seen, there was a dose-dependent preference for the neurosteroid-paired floor. Because place conditioning in this counterbalanced design is indicated by the difference between the GRID+ and GRID− conditioning subgroups at each dose, the figure inset shows the mean times (s per min) spent by each group on the grid floor during the preference test (mean times spent on the hole floor can be derived by subtracting these scores from 60 s). The lack of difference between the GRID+ and GRID− subgroups given 3.2 g/kg 3α,5α-P, indicates an absence of place conditioning. However, place preference was evident at the higher conditioning doses as shown by the greater time spent on the grid floor by mice that had previously received grid floor paired with 3α,5α-P (GRID+) compared to mice that had previously received grid floor paired with vehicle (GRID−). Two-way ANOVA (Dose × Conditioning Group) applied to the raw data shown in the figure inset yielded a significant main effect of Conditioning Group [$F(1, 90) = 20.2$, $p < 0.0001$] and a significant Dose × Conditioning Group interaction [$F(2, 90) = 3.9$, $p < 0.03$]. Planned comparisons between conditioning subgroups at each dose revealed a significant conditioned preference at 10 mg/kg [$F(1, 30) = 5.6$, $p < 0.03$] and 17 mg/kg [$F(1, 30) = 21.6$, $p < 0.0001$], but not at 3.2 mg/kg [$F < 1$].

Activity during the test session did not differ among dose groups [$F < 1$]. Mean activity counts per min (± SEM) were 33.2 ± 1.0, 32.8 ± 0.8 and 32.8 ± 1.2 for the 3.2, 10 and 17 mg/kg conditioning groups, respectively.

Control Experiment

Mice in the control experiment spent 47.3% (± 2.8) time on the CS+ floor during the preference test, indicating that β-cyclodextrin had little effect on place conditioning in the absence of neurosteroid. Statistical comparison of the number of s per min spent on the grid floor by the GRID+ (28.0 ± 2.5) and GRID− (31.2 ± 2.3) conditioning subgroups confirmed this conclusion [$F(1,22) = 0.9$]. Analyses of activity on conditioning trials showed no effect of β-cyclodextrin on the first three trials (all Fs < 1). However, mean activity rate on the fourth β-cyclodextrin (CS+) trial (36.9 ± 2.8) was slightly lower than that on the fourth saline (CS−) trial (40.1 ± 2.2) [$F(1, 23) = 5.5$, $p < 0.05$]. Overall, the control experiment offers little support for the suggestion that 3α,5α-P produced conditioned place preference by blocking an aversive effect of the β-cyclodextrin vehicle.

DISCUSSION

The present results represent the first demonstration of a dose-dependent conditioned place preference to the neuroactive steroid 3α,5α-P. In conjunction with recent work suggesting that 3α,5α-P shares some subjective properties with ethanol, BZs and barbiturates (1), the present results support the conclusion that 3α,5α-P possesses positive motivational effects.

Locomotor activity during the first conditioning trial indicated that 3α,5α-P had stimulant effects. Furthermore, the dose of 3α,5α-P with the greatest stimulant effect (i.e., 17 mg/kg) also produced the largest conditioned place preference. These data are consistent with recent work indicating that administration of 17 mg/kg 3α,5α-P produced locomotor stimulation and anxiolysis in DBA mice (9).

Although plasma 3α,5α-P was not measured in the present study, the doses of 3α,5α-P that were effective in producing place conditioning (i.e., 10 and 17 mg/kg) probably lead to plasma 3α,5α-P in excess of endogenous levels. Recent work found that plasma 3α,5α-P attained following exogenous administration of 5 mg/kg (11) was 2–3 fold higher than endogenous levels following swim stress or during pregnancy (i.e., ⩾30 ng/ml or 100 nM; ref. 16). However, DBA mice are relatively resistant to 3α,5α-P's anxiolytic and locomotor stimulant effects when compared with other genotypes (9). Therefore, it is possible that lower doses of 3α,5α-P, which more closely reflect endogenous 3α,5α-P under some circumstances, may possess rewarding properties in other genotypes.

The neurochemical systems involved in mediating the rewarding effect of 3α,5α-P are open to speculation. One possibility is that, as hypothesized for sedative/hypnotic drugs with a $GABA_A$-receptor agonist pharmacological profile (e.g., ethanol, BZs, barbiturates), 3α,5α-P produces a rewarding effect through its agonist action at the GRC. Alternatively, its rewarding action may depend on direct or GABA-mediated changes in central dopaminergic system activity (cf. 14). At present, however, the effects of 3α,5α-P on brain dopamine levels are unknown.

Based on the demonstration that an endogenous steroid possesses positive motivational effects, it is tempting to speculate that fluctuations in 3α,5α-P levels might participate in the propensity of animals to administer drugs of abuse. Endogenous 3α,5α-P increases to pharmacologically relevant plasma concentrations following swim and restraint stress in male rodents and during pregnancy and the estrus cycle in female rats (12,16). Depending on genotype, acute and chronic ethanol also alter plasma 3α,5α-P (12,13). Therefore, these fluctuations in endogenous 3α,5α-P might modulate the rewarding effects of ethanol. Alternatively, the rewarding effects of abused drugs might interact with stress-induced increases in 3α,5α-P. These possibilities warrant further investigation.

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