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Short communication

Discriminative stimulus effects of the endogenous neuroactive steroid pregnanolone

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Abstract

Naive male Sprague-Dawley rats were trained to discriminate the endogenous neuroactive steroid pregnanolone (5.6 mg/kg) from saline. Three positive modulators of the $GABA_A$ receptor complex substituted for pregnanolone: the neuroactive steroid allopregnanolone (1.0–10.0 mg/kg), the barbiturate pentobarbital (3.0–17.0 mg/kg), and the benzodiazepine diazepam (0.3–3.0 mg/kg). In contrast, buspirone, a $5\text{-}HT_{1A}$-mediated anxiolytic, failed to substitute up to rate-suppressing doses (1.0–5.6 mg/kg). The present experiment demonstrated the ability of an endogenous neuroactive steroid to function as a discriminative stimulus. Moreover, these results suggest that the discriminative stimulus effects of pregnanolone are mediated via positive modulation of $GABA_A$ receptors. © 1997 Elsevier Science B.V.

Keywords: Pregnanolone; Neurosteroid; Neuroactive steroid; Drug discrimination; Behavior

1. Introduction

Neuroactive steroids are positive modulators of the $GABA_A$ Cl^- channel receptor complex (Gee et al., 1995; Paul and Purdy, 1992). Their selective interaction with a unique binding site on the $GABA_A$ receptor complex results in the potentiation of inhibitory neurotransmission in the central nervous system (CNS). The neuroactive steroids pregnanolone (3α-hydroxy-5β-pregnan-20-one; 3α,5β-P) and allopregnanolone (3α-hydroxy-5α-pregnan-20-one; 3α,5α-P) are endogenous metabolites of progesterone.

Many behavioral effects of neuroactive steroids are thought to be due to positive modulation of $GABA_A$ receptors. Specifically, pregnanolone and allopregnanolone have demonstrated anticonvulsant, anxiolytic and sedative behavioral effects (Belelli et al., 1989; Bitran et al., 1991; Wieland et al., 1991, 1995; Zimmerberg et al., 1994). These effects are similar to those exhibited by other positive allosteric modulators of the $GABA_A$ receptor complex such as barbiturates and benzodiazepines (Colpaert et al., 1976; Harvey, 1985; Saano, 1987).

Neuroactive steroids have been shown to share discriminative stimulus effects with other drugs acting via the $GABA_A$ receptor complex (Ator et al., 1993; Deutsch and Mastropaolo, 1993; Grant et al., 1996). Although the discriminative stimulus effects of neuroactive steroids have been characterized in procedures using various drugs trained as discriminative stimuli, studies with a neuroactive steroid trained as a discriminative stimulus have not yet been published. The present study characterized the discriminative stimulus effects of pregnanolone in rats trained to discriminate pregnanolone from saline.

2. Materials and methods

2.1. Subjects

Naive male rats (Sprague-Dawley, Harlan Sprague-Dawley, San Diego, CA, USA) weighing approximately 280 g were individually housed in polycarbonate cages containing sterilized bedding material (Sani-Chips, P.J. Murray, Montville, NJ, USA) in a room maintained at 23.0°C (±2.5°C) and on a 12 h/12 h light/dark cycle. Food (Harlan Teklad, Madison, WI, USA) was restricted to post-session supplements (approximately 10 g per day) sufficient to maintain stable body weights (±5%) and

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behavioral performance. Sessions were conducted generally 5 days/week and rats were fed 10–15 g food per day on days with no session (i.e., weekends and holidays). Water was freely available in the home cage.

2.2. Apparatus

For experimental sessions, rats were placed in sound-attenuating chambers ($30 \times 24 \times 33$ cm; Coulbourn Instruments; Lehigh Valley, PA, USA) equipped with two response levers with associated stimulus lights. A magazine for the sucrose dipper and its associated light were located between the two levers. Med-Associates (East Fairfield, VT, USA) computer software and interface controlled stimulus events and recorded lever presses.

2.3. Procedure

Rats ($n = 10$) were trained to discriminate 5.6 mg/kg pregnanolone from saline in a two-lever, sucrose-reinforced (110 g sucrose/l water) drug discrimination paradigm. Experimental sessions lasted 20 min or until 50 reinforcers were earned. Rats were injected with either saline (1.0 ml/kg i.p.) or pregnanolone (5.6 mg/kg, i.p.). Fifteen minutes following injection, rats were placed in the operant chambers and the session began with stimulus lights lit above both levers. The pre-session injection determined the appropriate response lever. A saline injection indicated that 10 consecutive presses (fixed ratio 10; FR 10) on the right lever would be reinforced with sucrose availability, whereas an injection of pregnanolone indicated that 10 consecutive presses on the left lever would be reinforced. Sucrose availability occurred for 4 s during which time the stimulus lights were turned off and responding had no consequence. Responses on the inappropriate lever during the course of the session reset the response requirement on the appropriate lever. In addition 10 consecutive presses on the inappropriate lever resulted in a 10 s time-out in which stimulus lights were turned off and responding had no consequence. Pregnanolone (P) and saline (S) were administered in a fixed daily sequence (SPPSSPSSPP). Training was continued until rats reached a testing criterion of at least 80% injection-appropriate responses before the first reinforcer and at least 90% injection-appropriate responses over the entire session for at least 7 of 8 consecutive sessions.

After acquisition of the discrimination, test sessions (T) were conducted according to the daily sequence (STPSTPTSPT) as long as performance in the intervening training sessions remained at or above the testing criterion level. If performance of a rat failed to reach criterion, then the rat would return to the fixed daily sequence without testing until testing criterion again was achieved. Test sessions were identical to training sessions except that a test drug or its vehicle was administered and both levers were active such that 10 consecutive responses on either lever produced sucrose. Again, experimental sessions lasted 20 min or until 50 reinforcers were earned. A dose–response function for pregnanolone was determined first, followed by substitution tests. Drugs were tested with a subgroup of 5 rats except the training drug which was tested with 8 rats.

2.4. Data analysis

The means and standard errors of the percentage of total responses occurring on the pregnanolone-appropriate lever during test sessions were calculated. Rate of responding on both levers during test sessions was calculated as a percent of vehicle test for each rat. Means and standard errors of the percents were then calculated. If a rat failed to earn at least one reinforcer in any test session, the data from that session were not included in the calculation for pregnanolone-appropriate responses, but were included for the rate of responding measure. A test drug was considered to have substituted for the discriminative stimulus effects of pregnanolone in any individual rat if, at some dose, it engendered at least 80% pregnanolone-appropriate responding over the entire session. Response rates were considered to be increased if the percent of control rate engendered by the test dose exceeded 150% and to be decreased if it fell below 50%.

2.5. Drugs

A vehicle of 50% hydroxypropyl-β-cyclodextrin (50% distilled water) was used to dissolve pregnanolone synthesized by AKZO-Diosynth (Oss, Netherlands) and allopregnanolone synthesized by CoCensys (Irvine, CA, USA). Diazepam, pentobarbital and buspirone were purchased from Sigma (St. Louis, MO, USA) and were dissolved in 10% Tween 80 (90% distilled water), distilled water and 0.9% saline, respectively. 0.9% Saline was chosen instead of 50% hydroxypropyl-β-cyclodextrin as the non-drug training condition due to cost, but all vehicles were determined in test sessions not to be discriminated from saline. All drugs were administered i.p. in a volume of 1.0 ml/kg 15 min before the session, except pentobarbital which was administered 30 min before the session.

3. Results

The neuroactive steroid pregnanolone at 5.6 mg/kg was trained as a discriminative stimulus in 10 rats. All of the rats reached criteria to test. The mean number of sessions required for training to test criteria was 73.5 sessions (range = 41–111). Only 8 of the 10 rats, however, maintained relatively good stimulus control and were tested regularly. The other 2 rats failed to maintain adequate behavioral stability to complete testing of the dose–re-

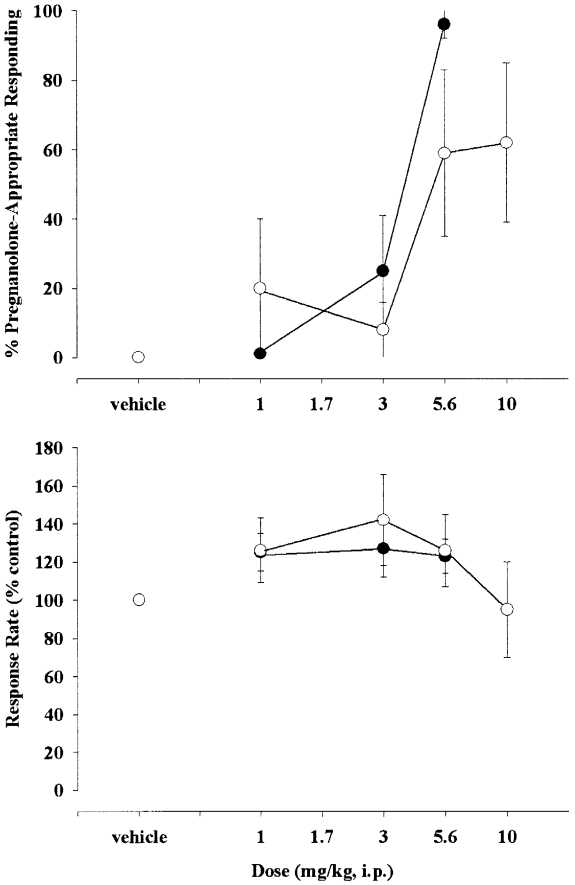

Fig. 1. Dose–response functions for pregnanolone (●) and allopregnanolone (○) in rats trained to discriminate pregnanolone (5.6 mg/kg) from saline. Percent pregnanolone-appropriate responding (upper panels) and response rate (lower panels) are shown as a function of dose. Vehicle control values are also shown. Each point represents the mean of the data collected ($n = 8$ for prenanolone, $n = 5$ for allopregnanolone). Vertical bars represent standard errors.

sponse function of the training drug and were thus excluded from further analysis. Vehicle injections engendered no pregnanolone-appropriate responding. Pregnanolone engendered a dose-related increase in pregnanolone-appropriate responding with 96% occurring at the training dose (Fig. 1). Rates of responding after vehicle injections ranged from 1.37 to 4.04 responses/s under the FR 10 schedule of reinforcement (2.35 responses/s $\pm$ 0.25; mean rate $\pm$ standard error). Overall, pregnanolone showed no change in response rate compared to vehicle (1.0 mg/kg, 2.84 $\pm$ 0.33; 3.0 mg/kg, 2.89 $\pm$ 0.33; 5.6 mg/kg, 2.95 $\pm$ 0.30).

Allopregnanolone fully substituted for pregnanolone in all rats tested, but substitution occurred at different doses in different rats (Fig. 1). Thus, the grouped data show only partial substitution at any given dose with large standard errors. Two rats exhibited substitution at 5.6 mg/kg, whereas another two exhibited substitution at 10.0 mg/kg. Pregnanolone generalized to both 5.6 mg/kg and 10.0 mg/kg allopregnanolone in the fifth rat tested. Similar to pregnanolone, allopregnanolone revealed no overall change in rate of responding (vehicle, 1.89 $\pm$ 0.14; 1.0 mg/kg, 2.45 $\pm$ 0.5; 3.0 mg/kg, 2.81 $\pm$ 0.68; 5.6 mg/kg, 2.46 $\pm$ 0.51; 10.0 mg/kg, 1.97 $\pm$ 0.58).

Pentobarbital (3.0–17.0 mg/kg) substituted for pregnanolone in all rats tested (Fig. 2). Response rate increased at 3.0 and 5.6 mg/kg and decreased at 17.0 mg/kg pentobarbital (vehicle, 1.97 $\pm$ 0.26; 3.0 mg/kg, 3.0 $\pm$ 0.48; 5.6 mg/kg, 3.06 $\pm$ 0.45; 10.0 mg/kg, 2.27 $\pm$ 0.42; 17.0 mg/kg, 0.38 $\pm$ 0.25). Similarly, diazepam (0.3–3.0 mg/kg) substituted for pregnanolone in all rats tested (Fig. 2►). Diazepam, however, had little effect on mean response rates at the doses tested (vehicle, 2.73 $\pm$ 0.66; 0.3 mg/kg, 3.27 $\pm$ 0.56; 1.0 mg/kg, 2.24 $\pm$ 0.51; 3.0 mg/kg, 2.31 $\pm$ 0.64).

Buspirone (1.0–5.6 mg/kg) failed to substitute for pregnanolone (Fig. 2). A dose of 1.0 mg/kg engendered pregnanolone-appropriate responding in one of four rats, producing the 25% partial substitution. Higher doses, however, failed to engender substitution in any rat tested and decreased mean rate of responding (vehicle, 2.68 $\pm$ 0.59; 1.0 mg/kg, 2.57 $\pm$ 0.58; 3.0 mg/kg, 0.28 $\pm$ 0.25; 5.6 mg/kg, 0).

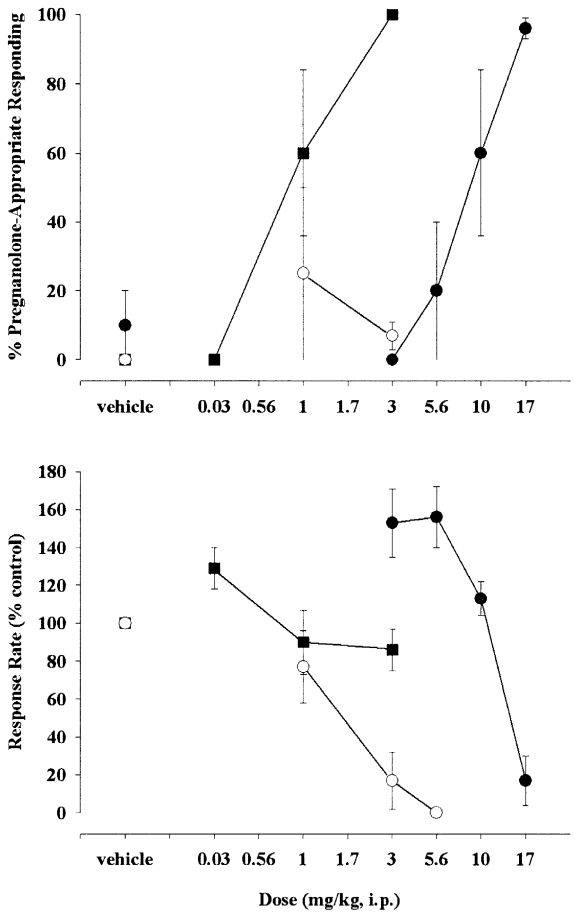

Fig. 2. Dose–response functions for pentobarbital (●), diazepam (■) and buspirone (○). Other details are as in Fig. 1 ($n = 5$).

4. Discussion

The present study is the first to report a neuroactive steroid trained as a discriminative stimulus. The discriminative stimulus effects of pregnanolone were established with 5.6 mg/kg in a two-lever, sucrose-reinforced operant paradigm in rats. The discrimination, however, was difficult to establish, requiring an average of 73.5 sessions to meet testing criteria. This compares poorly to a study of the degree of discriminability of a variety of drugs in a T-maze procedure in which the cut-off point was 60 sessions (Overton, 1982). It is likely that the discriminative stimulus effects of pregnanolone were mediated by positive modulation of the $GABA_A$ receptor complex. Three $GABA_A$ positive modulators, including a second neuroactive steroid, allopregnanolone, the barbiturate, pentobarbital, and the benzodiazepine, diazepam, substituted for pregnanolone as a discriminative stimulus. This was the case even though neuroactive steroids bind to molecular sites on the $GABA_A$ receptor complex distinct from the benzodiazepine and barbiturate sites (Gee et al., 1988; Paul and Purdy, 1992; Turner et al., 1989).

The present findings are consistent with previous studies showing that neuroactive steroids share discriminative stimulus effects with other $GABA_A$ positive modulators trained as discriminative stimuli (Ator et al., 1993; Deutsch and Mastropaolo, 1993; Grant et al., 1996). In fact, allopregnanolone, shown to substitute for pregnanolone in the present experiment, has substituted previously for diazepam and pentobarbital (Ator et al., 1993) both of which also substituted for pregnanolone in the present study. In addition, although not all benzodiazepines and barbiturates show cross-generalization, diazepam and pentobarbital do generalize to each other (Ator and Griffiths, 1989).

Pregnanolone has been shown to have anxiolytic-like effects (Bitran et al., 1991; Wieland et al., 1995). These effects are shared by other $GABA_A$ positive modulators (Crawley et al., 1986; Harvey, 1985). It is unlikely, however, that anxiolysis per se mediates the discriminative stimulus effects of pregnanolone. Buspirone, a $5\text{-}HT_{1A}$ agonist, with anxiolytic effects (Taylor et al., 1985; Yocca, 1990) failed to substitute for pregnanolone up to rate suppressing doses. This is consistent with previous studies demonstrating lack of substitution of buspirone for benzodiazepines and barbiturates (Ator and Griffiths, 1986; Hendry et al., 1983; Young et al., 1987).

Due to the endogenous nature of neurosteroids and the potential therapeutic usefulness of these and other, synthetic, neuroactive steroids (Gee et al., 1995), it is important to thoroughly understand the pharmacology of this class of compounds. The present study furthers the pharmacological characterization of neuroactive steroids by demonstrating that a neuroactive steroid can function as a discriminative stimulus. Further, the present results are consistent with allosteric positive modulation of $GABA_A$ receptors mediating the discriminative stimulus effects of neuroactive steroids.

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