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Using drug combinations to assess potential contributions of non-$GABA_A$ receptors in the discriminative stimulus effects of the neuroactive steroid pregnanolone in rats☆

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HIGHLIGHTS

- Potential role was examined for non-$GABA_A$ receptors in effects of pregnanolone.
- Drug combinations were studied in rats discriminating pregnanolone or midazolam.
- Neither 5-HT_3 nor NMDA receptors are involved in these effects of pregnanolone.
- Non-$GABA_A$ receptors might contribute to the other effects of pregnanolone.

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ABSTRACT

Neuroactive steroids are increasingly implicated in the development of depression and anxiety and have been suggested as possible treatments for these disorders. While neuroactive steroids, such as pregnanolone, act primarily at γ-aminobutyric $acid_A$ ($GABA_A$) receptors, other mechanisms might contribute to their behavioral effects and could increase their clinical effectiveness, as compared with drugs acting exclusively at $GABA_A$ receptors (e.g., benzodiazepines). The current study examined the role of non-$GABA_A$ receptors, including N-methyl-D-aspartate (NMDA) and $serotonin_3$ (5-HT_3) receptors, in the discriminative stimulus effects of pregnanolone. Separate groups of rats discriminated either 3.2 mg/kg pregnanolone from vehicle or 0.32 mg/kg of the benzodiazepine midazolam from vehicle while responding under a fixed-ratio 10 schedule for food pellets. When administered alone in both groups, pregnanolone and midazolam produced ≥80% drug-lever responding, the NMDA receptor antagonists dizocilpine and phencyclidine produced ≥60 and ≥30% drug-lever responding, respectively, and the 5-HT_3 receptor agonist 1-(m-chlorophenyl)-biguanide (CPBG) and morphine produced <20% drug-lever responding up to doses that markedly decreased response rates. When studied together, neither dizocilpine, phencyclidine, CPBG nor morphine significantly altered the midazolam dose–effect curve in either group. Given that CPBG is without effect, it is unlikely that 5-HT_3 receptors contribute substantially to the discriminative stimulus effects of pregnanolone. Similarities across groups in effects of dizocilpine and phencyclidine suggest that NMDA receptors do not differentially contribute to the effects of pregnanolone. Thus, NMDA and 5-HT_3 receptors are not involved in the discriminative stimulus effects of pregnanolone.

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1. Introduction

The role of neuroactive steroids in a variety of affective disorders is becoming increasingly apparent [30]. Endogenous neuroactive steroids are synthesized from cholesterol and steroidal precursors, and the 3α-reduced metabolites of progesterone allopregnanolone and pregnanolone are important products of neurosteroid biosynthesis. A large number of clinical trials have shown that a reduction in circulating levels of these metabolites is associated with anxiety and depressive disorders, and one strategy for treating these conditions is to restore levels of endogenous neuroactive steroids by giving allopregnanolone or pregnanolone exogenously [30].

The mechanisms that contribute to these potential therapeutic effects of neuroactive steroids are not entirely clear. It is well established that allopregnanolone and pregnanolone positively modulate γ-aminobutyric $acid_A$ ($GABA_A$) receptors [19], and actions at these receptors play an important role in their behavioral effects, in general, and in their anxiolytic effects, in particular. Other positive $GABA_A$ modulators,

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primarily benzodiazepines, have long been used clinically for anxiety. Because of their similar actions on $GABA_A$ receptors, neuroactive steroids produce behavioral effects that are similar to those of benzodiazepines, including anxiolytic effects [33] as well as sedative and anticonvulsant effects [17,20,28,32]; however, benzodiazepines are not effective in treating depression, suggesting that an action of neuroactive steroids other than modulation of $GABA_A$ receptors accounts for their antidepressant effects. There are other important differences between neuroactive steroids and benzodiazepines that could provide a clinical advantage for neuroactive steroids. For example, tolerance does not develop to some effects of neuroactive steroids [18,24,27]. In contrast, tolerance develops readily to many effects of benzodiazepines [14,23,25]. Thus, despite similarities in their actions at $GABA_A$ receptors, the effects of neuroactive steroids and benzodiazepines are not identical, suggesting that other mechanisms might be involved in the behavioral effects of neuroactive steroids. While benzodiazepines act exclusively at benzodiazepine sites on $GABA_A$ receptors, neuroactive steroids act at distinct modulatory sites on $GABA_A$ receptors as well as on other receptors, such as N-methyl-D-aspartate (NMDA) and 5-hydroxytryptamine$_3$ (5-HT_3) receptors [8,29], and it might be their actions at these other receptors that account for differences between neuroactive steroids and benzodiazepines.

Drug discrimination is a behavioral procedure that has been used to examine the possible differences among positive $GABA_A$ modulators, including neuroactive steroids, benzodiazepines and barbiturates. Drugs acting at any of these distinct sites on $GABA_A$ receptors can be established as discriminative stimuli (e.g., [2,3,6,9]). Regardless of which training drug is used to establish the discrimination, positive $GABA_A$ modulators generally produce drug-lever responding, although some exceptions have been reported. For example, in rats, the benzodiazepine lorazepam produces pentobarbital-lever responding, although pentobarbital does not produce lorazepam-lever responding [1,2]. In addition, some subjects discriminating midazolam do not respond on the drug lever after administration of barbiturates or neuroactive steroids [11,13] whereas all subjects discriminating a neuroactive steroid respond on the drug lever after administration of benzodiazepines or barbiturates [10,12]. These differences support the notion that the discriminative stimulus effects of positive $GABA_A$ modulators are not identical. In addition, drug discrimination procedures have identified multiple mechanisms of action for another positive $GABA_A$ modulator, ethanol, including actions at $GABA_A$, NMDA and 5-HT_3 receptors [4,15].

This study used drug discrimination procedures to determine whether actions of pregnanolone at receptors other than $GABA_A$ receptors contribute to its discriminative stimulus effects in rats. That $GABA_A$ receptors have a predominant role in the discriminative stimulus effects of pregnanolone has been established [3,10,12]; however, a role of other receptors has not been clearly determined. Drugs with actions at 5-HT_3 and NMDA receptors have been studied previously in subjects discriminating pregnanolone, and the results have been inconsistent with 5-HT_3 receptor agonists and NMDA receptor antagonists producing pregnanolone-lever responding in some studies [9] and not in others [10,31]. The current study extends those findings by determining whether the 5-HT_3 receptor agonist 1-(m-chlorophenyl)-biguanide (CPBG) and the NMDA receptor antagonists phencyclidine and dizocilpine modify the discriminative stimulus effects of pregnanolone or midazolam. Two separate groups of rats were used with one group discriminating pregnanolone from vehicle and the second group discriminating midazolam from vehicle; experimental conditions were identical in the two groups except for the training drug. Because the midazolam discriminative stimulus is pharmacologically selective with only positive $GABA_A$ modulators producing effects similar to those of the training drug [3,21], drugs acting at other receptors would not be expected to produce midazolam-lever responding or to enhance the effects of either midazolam or pregnanolone (i.e., shift dose–effect curves for positive modulators to the left) in rats discriminating midazolam. In contrast, actions of neuroactive steroids at 5-HT_3 or NMDA receptors might contribute to their discriminative stimulus effects. Under those conditions, drugs acting at those other receptors might produce pregnanolone-lever responding, although the inconsistent results obtained in previous studies suggest that any role of 5-HT_3 or NMDA receptors in the discriminative stimulus effects of pregnanolone might be small, as compared with the role of $GABA_A$ receptors, so that drugs acting at these other receptors do not reliably mimic the effects of pregnanolone. To the extent that these other receptors contribute to the effects of pregnanolone, they would be expected to enhance the discriminative stimulus effects of positive $GABA_A$ modulators in rats discriminating pregnanolone, shifting their dose–effect curves leftward. This enhancement would be especially apparent when drugs acting at 5-HT_3 or NMDA receptors are studied in combination with midazolam, which acts only at $GABA_A$ receptors, as compared to the enhancement obtained when those drugs are studied with pregnanolone, which might already have actions at 5-HT_3 or NMDA receptors in addition to its actions at $GABA_A$ receptors.

2. Methods

2.1. Subjects

One group of male Sprague–Dawley rats discriminated pregnanolone from vehicle and the other group discriminated midazolam from vehicle. They received 45-mg grain-based food pellets (Bio Serv, Inc., Frenchtown, NJ) during experimental sessions with rodent chow (Harlan Teklad, Madison, WI) provided in the home cage in sufficient quantities to maintain weights between 320 and 330 g throughout the experiment. Rats had unlimited access to water in the home cage and were housed individually in a humidity- and temperature-controlled vivarium under a 12-h light/dark cycle with experiments conducted during the light cycle. Animals used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee at The University of Texas Health Science Center at San Antonio, and the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council [Department of Health, Education and Welfare, publication no. (NIH) 85-23, revised 2011].

2.2. Apparatus

Sessions were conducted in chambers enclosed within sound-attenuating cubicles. Each chamber was equipped with a houselight, two response levers, two stimulus lights, a pellet trough, a pellet dispenser and a fan for ventilation (MED Associates, Inc., St. Albans, VT). During sessions, white noise was present in the room to mask extraneous noise. An interface connected the chambers to a computer that controlled the experimental events and recorded data using Med-PC/Medstate Notation software (MED Associates, Inc., St. Albans, VT).

2.3. Procedure

One group of 13 rats discriminated 3.2 mg/kg pregnanolone from vehicle and the other group of 11 rats discriminated 0.32 mg/kg midazolam from vehicle while responding under a fixed-ratio 10 schedule of food presentation; other than the training drug, experimental sessions were identical in the two groups. These training doses were selected because previous studies indicated that the potency of pregnanolone and midazolam to produce drug-lever responding would be similar across the two groups with these training doses [3]. Sessions were divided into 15-min cycles and there could be up to 8 cycles in a session. Each cycle began with a 10-min timeout period, during which the chamber was dark and responding had no programmed consequence, and ended with a response period that could last up to 5 min. Response periods were signaled by illumination of the stimulus lights located above the levers. Under these stimulus conditions, 10 responses on the lever designated correct by the injection given during the first

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