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Short communication

Self-administration of progesterone and synthetic neuroactive steroids by male rhesus monkeys

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ABSTRACT

Background: Progesterone-derived neuroactive steroids have shown promise clinically (*e.g.*, anti-seizure medications) but, as with other $GABA_A$ receptor modulators (*e.g.*, benzodiazepines), may have the potential for abuse.
Methods: We evaluated the reinforcing effects of progesterone, a steroid precursor of endogenous neuroactive steroids, with and without pretreatments with the neuroactive steroid synthesis inhibitor, finasteride, in rhesus monkeys trained under a progressive-ratio (PR) schedule of i.v. midazolam injection. We also assessed reinforcing effects of the short-acting neuroactive steroid alphaxolone and the long-acting neuroactive steroid ganaxolone in comparison with the short-acting benzodiazepine triazolam and the long-acting benzodiazepine clonazepam.
Results: At least one dose of progesterone, alphaxolone, and ganaxolone was self-administered significantly above vehicle levels in all monkeys tested (n = 4 for progesterone, n = 3 for alphaxolone and ganaxolone). The 5α-reductase inhibitor finasteride attenuated progesterone self-administration, consistent with the reinforcing effects of progesterone being mediated by the *in vivo* synthesis of neuroactive steroids. The comparison drugs, triazolam and clonazepam, were self-administered significantly above vehicle by all monkeys. Although the maximum number of injections/session maintained by the neuroactive steroids was below that maintained by the midazolam training dose, analysis of break points (*i.e.*, highest response requirement achieved) suggested modest differences in relative reinforcing effectiveness for neuroactive steroids compared with benzodiazepines.
Conclusions: Our results are consistent with endogenous and synthetic neuroactive steroids having reinforcing effects similar to that of benzodiazepines, with reinforcing effectiveness possibly lower for the neuroactive steroids compared with benzodiazepines based on some measures.

1. Introduction

Neuroactive steroids are cholesterol-based compounds that can act as positive allosteric modulators of γ-aminobutyric acid (GABA) type A receptors (Lambert et al., 2009; Carver and Reddy, 2013). Many neuroactive steroids are synthesized endogenously from the progestogen steroid progesterone, which is typically identified as a female reproductive hormone but exists in both females and males. Both exogenous and endogenous progesterone have been evaluated as mediators of the addictive effects of drugs (*e.g.*, Evans and Foltin, 2011; Babalonis et al., 2011); however, the extent to which progesterone has abuse-related effects itself is unknown.

Progesterone is converted in the CNS *via* 5α-reductase to neuroactive steroids such as pregnanolone, which we have shown previously to be self-administered by male rhesus monkeys (Rowlett et al., 1999; Fischer and Rowlett, 2011). In the present study, we evaluated the reinforcing effects of progesterone, based on the hypothesis that it might be self-administered due to conversion to neuroactive steroids. Because only pregnanolone has been evaluated in our self-administration procedure, we investigated the potential reinforcing effects of two synthetic neuroactive steroids alphaxalone and ganaxolone in order to confirm the generality of neuroactive steroid self-administration. For comparison purposes, we additionally included tests with the relatively short-

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acting benzodiazepine triazolam and the relatively long-acting benzodiazepine clonazepam.

2. Material and methods

2.1. Animals

Four experimentally-naïve, adult male rhesus monkeys (*Macaca mulatta*), weighing 8–9 kg, were housed individually and maintained on a 12-h lights-on/12-h lights-off cycle (lights on at 7:00 AM). Temperature and humidity were controlled automatically and water was available continuously. Monkeys received Teklad monkey diet, supplemented with fruits and vegetables, at least 2 h prior to the daily sessions. One monkey, Mm-68-08, was brought in to provide data for progesterone only and then was switched to another previously-planned study. Animals were maintained in accordance with the guidelines of the Committee on Animals of Harvard Medical School and the Guide for Care and Use of Laboratory Animals (8th edition, 2011). Research protocols were approved by the Harvard Medical School Institutional Animal Care and Use Committee. Monkeys were prepared with a chronic indwelling venous catheter according to previously described procedures (Platt et al., 2011).

2.2. Drugs

Alphaxalone, progesterone, ganaxolone and finasteride (Tocris Biosciences, Bristol, UK) were dissolved in 45% (w/v) 2-hydroxypropyl-β-cyclodextrin and then diluted in sterile water. Midazolam (5.0 mg/ml; Henry Schein, Dublin, OH) was diluted in sterile saline. Clonazepam and triazolam (both from Sigma-Aldrich, St. Louis, MO) were dissolved in 100% propylene glycol and diluted to a 50/50% propylene glycol/water mixture. Doses were based on our previous research (Fischer and Rowlett, 2011) or dose-ranging pilot studies.

2.3. Self-administration procedure

Monkeys were trained to self-administer (in the home cage) the benzodiazepine midazolam (0.03 or 0.056 mg/kg/infusion) under a PR schedule of i.v. drug injection (for details, see Fischer and Rowlett, 2011). Briefly, sessions consisted of 5 components made up of 4 trials each. Each trial consisted of a response requirement signaled by white stimulus lights available for 30 min or until the response requirement was completed. Each trial was separated by a 30-min timeout. The response requirement remained constant for each of the 4 trials within a component and doubled during each successive component in the following series: 40, 80, 160, 320, and 640 responses per injection. The session ended when a maximum of 20 injections were delivered or when the response requirement was not completed for two consecutive trials. Midazolam or saline was made available on alternating days until self-administration was stable (>11 midazolam injections/session and <5 saline injections/session). Test sessions (T) were added to the alternating sequence of midazolam (M) and saline (S) sessions according to the following sequence: MTSMTSTMST. Drugs were tested in the order of presentation shown below, but doses within a drug were counterbalanced across monkeys.

After dose-response functions were obtained for all drugs, we conducted a study with progesterone using the 5α-reductase inhibitor, finasteride, which blocks the rate-limiting conversion of progesterone to neuroactive steroids (Finn et al., 2006). Finasteride was given *via* the i.v. catheter 5 min before a test session with a peak reinforcing dose of progesterone (individually determined).

2.4. Data analysis

Test sessions with progesterone resulted in a notable degree of variability, although orderly data were obtained for individual monkeys. To adjust for variance in the progesterone tests, we evaluated progesterone and all other drugs in this study computing a 95% confidence interval based on (a) the last three sessions of midazolam availability and (b) three vehicle tests for each monkey. These data were graphed for individual monkeys in panels for each drug. Any individual subject's data point above the upper level of the 95% confidence interval (represented as error bars) of each monkey's vehicle control was considered to represent significant reinforcing effects. The 95% confidence intervals constructed for midazolam training conditions also were plotted. For analyses of group data, separate one-way repeated-measures analyses of variance (ANOVA) were used to evaluate the injection per session data for each drug. Differences from vehicle were determined by Bonferroni *t*-tests ($p \leq 0.05$).

To more accurately compare the relative reinforcing effectiveness of the neuroactive steroids with the benzodiazepines triazolam and clonazepam, we calculated BP_{max} values, which are the highest break points (*i.e.*, last response requirement completed) obtained irrespective of dose for a given drug. The BP_{max} measure provides an index of reinforcing effectiveness that takes into account individual differences in peak BP values. These data were analyzed with the nonparametric Friedman's ANOVA on ranks ($p \leq 0.05$).

3. Results

3.1. Self-administration of progesterone and neuroactive steroids

The data for individual monkeys for progesterone and the two neuroactive steroids are shown in separate panels in Fig. 1 with group data summarized in the bottom right panel. Analysis of individual-subject data with progesterone (Fig. 1, top left panel; note that symbols obscured the error bars in some instances) revealed evidence for significant self-administration with different peak doses evident across monkeys (*e.g.*, compare Mm-68-08 with Mm-243-03). For all monkeys, self-administration dose-response functions were biphasic (*i.e.*, approximated a characteristic inverted U-shaped function) with at least one dose self-administered above vehicle levels and peak doses in most cases significantly lower than the midazolam training dose. Due to the variability observed with the progesterone data, the dose range was extended to 0.0001 and 0.01 mg/kg/injection for 2 monkeys in order to obtain complete dose-response functions with ascending and descending "limbs" of the curves.

We repeated self-administration tests in monkeys Mm-243-03 and Mm-122-05 using their peak progesterone dose (0.001 and 0.003 mg/kg/injection, respectively), after 5-min pre-treatments with finasteride. Pretreatment with finasteride dose-dependently decreased the number of progesterone injections/session to the levels maintained under conditions of vehicle availability. For Mm-243-03, 0.001 mg/kg/injection progesterone maintained 10 injections/session that was reduced to 6 and 2 injections/session by 0.01 and 0.03 mg/kg, i.v., of finasteride, respectively. Similarly, for Mm-122-05, 0.003 mg/kg/injection progesterone maintained 12 injections/session, that was reduced to 8 and 1 injections/session by 0.01 and 0.03 mg/kg, i.v., respectively.

For the neuroactive steroid alphaxolone, the individual-subject analysis using the 95% confidence intervals did reveal that the majority of monkeys self-administered alphaxolone above vehicle levels for at least 0.003–0.03 mg/kg/injection (Fig. 1, top right panel). Except for Mm-233-97, peak doses of alphaxolone were