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Termination of pseudopregnancy in the rat produces an anxiogenic-like response that is associated with an increase in benzodiazepine receptor binding density and a decrease in GABA-stimulated chloride influx in the hippocampus

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Abstract

The neurosteroid, 3α-OH-5α-pregnan-20-one (allopregnanolone) is a potent positive modulator of the $GABA_A$ receptor complex. Its pharmacological spectrum of action is shared by the benzodiazepines and alcohol, and includes anxiolytic, anticonvulsant, ataxic, and hypnotic effects. Discontinuation from chronic exposure to allopregnanolone or other neuroactive steroids has been shown to elicit behavioral effects that are typically seen in benzodiazepine or alcohol withdrawal. In this series of experiments, the effects of an endogenous elevation of ovarian steroids on brain $GABA_A$ receptor function was examined by inducing pseudopregnancy. In female rats, pseudopregnancy did not affect behavior in the elevated plus-maze, despite a persistent increase in circulating levels of allopregnanolone. Pseudopregnancy was associated with a decrease in the maximal binding density of ^{3}H-flunitrazepam in the cerebral cortex and cerebellum; however, GABA-stimulated chloride influx in cerebral cortical, hippocampal, and cerebellar synaptoneurosomes remained unaffected during pseudopregnancy. Termination of pseudopregnancy by ovariectomy precipitated an anxiogenic-like effect in the elevated plus-maze. The withdrawal from elevated ovarian steroid levels also increased the number of benzodiazepine receptors and decreased GABA-stimulated chloride influx in the hippocampus.

Keywords: Neurosteroid; Allopregnanolone; Tolerance; Withdrawal; Anxiety; Benzodiazepine receptor

1. Introduction

A number of pregnane steroids have been show to elicit a pharmacological spectrum of action that is shared by benzodiazepines, barbiturates, and alcohol. With increasing doses, these compounds reliably elicit anxiolytic, anticonvulsant, ataxic, and hypnotic effects. Progesterone is an ovarian hormone that has been found to elicit many of these effects. In turn, the effects of progesterone have been attributed to the formation of its reduced metabolite, 3α-OH-5α-pregnan-20-one (allopregnanolone), a potent positive modulator of the $GABA_A$ receptor [1,2,4,5,7,8,14,15]. Allopregnanolone is one of a number of neurosteroids synthesized in brain de novo from cholesterol as a result of stimulation of a benzodiazepine receptor located on mitochondrial membranes of glial cells [31,32,36]. Another source of allopregnanolone is from the metabolism of progesterone secreted from the ovaries and adrenal glands.

In addition to the similarity in acute drug effects, withdrawal from allopregnanolone exposure produces responses that are similar to those seen after termination from chronic treatment with benzodiazepines or alcohol. For example, discontinuation of exogenous progesterone

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administration in the rat elicited an anxiogenic-like response [17], and treatment of progesterone-dependent rats with finasteride, a 5α-reductase inhibitor that prevents the formation of allopregnanolone from progesterone, was shown to have a pro-convulsant effect [16,25]. Progesterone withdrawal decreased the anxiolytic-like and sedative effects of benzodiazepines [6,24], and also eliminated the benzodiazepine-induced potentiation of GABA-stimulated chloride currents in dissociated hippocampal neurons [11,33,34].

The purpose of this study was to determine if an endogenous elevation in ovarian hormone levels would elicit changes in $GABA_A$ receptor function. Thus, we studied the effects of pseudopregnancy on behavior in the elevated plus-maze, on benzodiazepine binding, and on GABA-stimulated Cl^- influx response in vitro. We hypothesized that pseudopregnancy ought to be associated with a desensitization of the $GABA_A$ receptor, thus reflecting a pharmacodynamic tolerance that results from prolonged exposure to the GABAergic neurosteroid. In addition, we examined the effects of pseudopregnancy termination in order to test the hypothesis that a precipitous loss of ovarian steroids would trigger a withdrawal syndrome reflecting the pharmacodynamic changes that occurred in response to the persistent elevated ovarian steroid levels.

2. Materials and methods

2.1. Animals

Female Long-Evans rat pups (14 days of age) and their lactating mothers were purchased (Harlan Sprague–Dawley, Indianapolis, IN). Pups were weaned at 21 days of age and housed in groups of three (each pup from a different litter) in standard Plexiglas cages with corn cob bedding in a temperature and humidity controlled vivarium under a reversed 12 h light/dark cycle (lights off at 06:00 h). Food and water were available ad libitum. The experimental protocol was consistent with ethical standards established in the "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1996) and was approved by the Institutional Animal Care and Use Committee of the College of the Holy Cross.

2.2. Induction of pseudopregnancy and pseudopregnancy termination

At 28 days of age, females were injected with either 50 IU of pregnant mare serum gonadotropin (PMSG) or with 0.1% gelatin physiological saline vehicle (0.1 ml, s.c.). Forty-eight hours later, the experimental group received an injection of 25 IU of human chorionic gonadotropin (hCG); control animals received an additional injection of the gelatin-saline vehicle [10,19]. This was considered day 0 of pseudopregnancy.

Pseudopregnancy was terminated on day 10 by ovariectomy using ketamine HCl (60 mg/kg) and xylazine HCl (12 mg/kg) as anesthetics. Ovariectomy was also performed on vehicle-injected control animals that were not pseudopregnant.

2.3. Elevated plus-maze

Animals were tested once in the elevated plus-maze for 10 min. The elevated plus-maze consisted of two 'open' arms (50 cm × 10 cm) and two 'closed' arms (50 cm × 10 cm) with walls 40 cm high. The maze was elevated 50 cm from the floor. All trials were recorded by closed circuit television, and data were acquired and analyzed using Ethovision (Noldus Inc., Netherlands). The number of open and closed arm entries and the time spent in the open and closed arms were determined. An anxiogenic-like effect was indicated by a decreased proportion of number of open arm entries or time spent in the open arms; and anxiolytic-like effect was noted as an increase in these measures [26].

2.4. Steroid radioimmunoassay

Levels of estrogen and progesterone were determined in blood serum samples using radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, CA). For estrogen, the assay sensitivity was 0.02 ng/ml; the intra-assay and inter-assay reliability were 5.8 and 7.4%, respectively. For progesterone, the assay sensitivity was 8 pg/ml; the intra-assay and inter-assay coefficients of variance were 4.9 and 5.7%, respectively. Circulating levels of allopregnanolone were determined using RIA procedures previously described [27]. The sensitivity of the assay was 100 pg/ml; the intra-assay and inter-assay reliability were 8.0 and 11.3%, respectively.

2.5. Homogenate radioligand binding

Benzodiazepine receptor binding density and affinity were determined using ^{3}H-flunitrazepam (specific activity, 83.1 Ci/mmol) in the presence or absence of 10 μM clonazepam in thoroughly washed membrane fractions. Brain regions were prepared by homogenizing (Polytron setting 3 for 10 s) in 10 volumes of 50 mM Tris buffer (pH 7.4) and centrifugation at 30,000 × *g* for 20 min. The pellet was resuspended in 10 volumes of Tris buffer. The homogenization and centrifugation cycle was repeated three times. A 75 μl aliquot of the homogenate (approximately 0.1 mg of protein) was incubated with one of six concentrations of ^{3}H-flunitrazepam (0.25–25 nM) in a total of 500 μl reaction mixture for 60 min at 4 °C. The reaction was terminated by vacuum filtration using a Brandel harvester over GF/B filters. A 3-ml aliquot of Ecoscint A was added to the dried filters and counting was made using standard liquid scintillation spectrometry. The data were standardized for protein content, which was determined in all aliquots using the Bio-Rad protein assay (microassay procedure). Computer-assisted linear regression analysis yielded equilibrium dissociation constant (K_D) and