Pharmacology, Biochemistry and Behavior 103 (2013) 568–572

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

RU486 blocks effects of allopregnanolone on the response to restraint stress

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ARTICLE INFO

Article history:
Received 23 July 2012
Received in revised form 11 September 2012
Accepted 30 September 2012
Available online 6 October 2012

Keywords:
Lordosis behavior
Proceptivity
Progesterone metabolites
Progesterone receptors
Female rats

ABSTRACT

These experiments were designed to provide information about the potential involvement of progesterone receptors in the ability of allopregnanolone (3α-hydroxy-5α-pregnan-20-one) to reduce the lordosis-inhibiting effects of restraint stress. Ovariectomized Fischer rats were hormonally primed with 10 μg estradiol benzoate and 4 mg/kg allopregnanolone or vehicle. One hour before allopregnanolone, rats were injected with the progesterone receptor antagonist, RU486 (11β-(4-dimethylamino)phenyl-17β-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one), or vehicle. Four hours after allopregnanolone or vehicle, sexual behavior was examined before and after a 5-min restraint stress. Lordosis behavior of rats primed only with estradiol benzoate declined after the 5 min of restraint while allopregnanolone prevented this decline. RU486 attenuated the ability of allopregnanolone to prevent the restraint-induced decline in lordosis behavior. These findings are consistent with earlier suggestions that progesterone receptors are involved in allopregnanolone's ability to reduce the effects of restraint stress.

1. Introduction

Female rat sexual behavior is temporally coordinated with ovulation to maximize reproductive fitness (Blaustein, 2008; Sodersten, 1981). Both estrogens and progestins influence this reproductive synchrony (Auger, 2004; Mani et al., 1997; Pfaff, 1970) so that reproductive behavior abruptly ceases following ovariectomy. However, sexual behavior can be restored in ovariectomized rats by exogenous treatment with gonadal hormones (Auger, 2004; Pfaff, 2005). The full repertoire of female sexual behavior includes appetitive, precopulatory and copulatory activities and gonadal hormones appear to differentially influence these various events (Blaustein, 2008; Erskine, 1989; Frye, 2007). The lordosis reflex (required for copulation) is thought to depend exclusively on estradiol (Auger, 2004; Blaustein, 2008). Progesterone is not required, but the probability and frequency of lordosis behavior can be increased by progesterone; and progesterone may be required for the occurrence of precopulatory activity such as hopping and darting behavior (Erskine, 1989; Frye and Vongher, 1999; Sodersten, 1981). Progesterone also reduces measures of anxiety (Auger and Forbes-Lorman, 2008; Gomez et al., 2002) and we have previously suggested that progesterone's anxiolytic action contributes to its facilitation of female rat sexual behavior (Truitt et al., 2003; Uphouse et al., 2008; White and Uphouse, 2004).

When ovariectomized Fischer inbred rats are hormonally primed with 10 μg estradiol benzoate, lordosis behavior is comparable to that obtained following hormonal priming with estradiol benzoate and progesterone. However, when subjected to a mild 5-min restraint stress, rats primed only with estradiol benzoate show disruption of lordosis behavior (Hassell et al., 2011; White and Uphouse, 2004). The lordosis disruption is transient, lasting 5–10 min after the restraint experience (Uphouse et al., 1993; White and Uphouse, 2004). The restraint experience amplifies the negative effect of the serotonin 1A (5-HT_{1A}) receptor agonist, 8-OH-DPAT [(+/−)-8-hydroxy-2-(di-n-propylamino)tetralin] on lordosis behavior (Uphouse et al., 2007) but is attenuated by the 5-HT_2 receptor agonist DOI [(+/−)-2,5-dimethoxy-4-iodophenyl-2-aminopropane HCl]. We have suggested that this dual regulation by 5-HT is important to the female's continuity of mating in the presence of a mild stressor. Increased 5-HT release, precipitated by stress (Chaouloff, 2000), can activate all 5-HT receptors. Activation of the higher affinity 5-HT_{1A} receptors produces a rapid decline in lordosis behavior; activation of slower acting, lordosis facilitating 5-HT_2 receptors attenuates the decline in behavior and allows mating to continue (Uphouse, 1997; Wolf et al., 1998, 1999). Progesterone attenuates this response to stress by reducing the lordosis-inhibitory effects of 5-HT_{1A} receptor activation, in part by reducing extracellular 5-HT (Farmer et al., 1996; Maswood et al., 1995, 1999; Truitt et al., 2003; White and Uphouse, 2004). Therefore, one of the effects of progesterone in the modulation of female rat sexual behavior includes its attenuation of the negative effects of mild stress on lordosis behavior. However, the relative roles of the parent molecule and its metabolites in the mechanisms leading to this attenuation are unknown.

Progesterone modulates reproductive and nonreproductive behaviors through classical progesterone-receptor-mediated mechanisms that can be initiated via both ligand-dependent and ligand-independent pathways (Conneely et al., 2003; Dressing et al., 2011; Mani et al.,

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0091-3057/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pbb.2012.09.024

1997; Pluchino et al., 2009). Progesterone can also be metabolized by 5α-reductase into 5α-dihydroprogesterone and then by 3α-hydroxysteroid dehydrogenase (3α-HSD) into allopregnanolone (Rupprecht, 2003; Schule et al., 2011). Progesterone metabolites, such as allopregnanolone, contribute to the regulation of female rat sexual behavior (Blaustein, 2008; Frye et al., 1998; Mani and Portillo, 2010) and are especially important mediators of progesterone's anxiolytic effects (Dubrovsky, 2006; Eser et al., 2008). However, these progesterone metabolites do not appear to be required for progesterone's attenuation of the negative effects of 5 min restraint on lordosis behavior (Hassell et al., 2011; Miryala et al., 2011).

In previous studies, we questioned if progesterone's reduction of the lordosis-inhibiting effect of restraint stress resulted from the parent molecule, progesterone, or if progesterone metabolites were responsible (Hassell et al., 2011; Miryala et al., 2011). Progesterone's attenuation of the response to restraint stress (a) was mimicked by the nonmetabolizable progestin, medroxyprogesterone; (b) was not attenuated by the 5α-reductase inhibitor, finasteride, or the 3α-HSD inhibitor, indomethacin; but (c) was attenuated by the progesterone/glucocorticoid receptor antagonist, RU486, and by the more selective progesterone receptor antagonist, CDB4124, that is devoid of glucocorticoid receptor action (Hassell et al., 2011; Miryala et al., 2011; Uphouse and Hiegel, submitted for publication). These data, therefore, were consistent with a requirement for progesterone-receptor mediated events in progesterone's attenuation of the lordosis-inhibiting effect of restraint. However, the progesterone metabolite, allopregnanolone, was also effective in reducing the effects of restraint (Miryala et al., 2011). Since inhibition of progesterone metabolism with finasteride or indomethacin did not eliminate progesterone's ability to attenuate the negative response to restraint, allopregnanolone's ability to reduce the response to restraint could mean that redundant mechanisms (e.g. progesterone-receptor mediated or progesterone-metabolite initiated events) were capable of reducing the lordosis-inhibiting effect of restraint. However, this seems unlikely since blocking progesterone receptors blocked progesterone's attenuation of restraint while blocking progesterone metabolism did not (Hassell et al., 2011; Miryala et al., 2011). An alternative explanation is that allopregnanolone is also able to lead to activation of progesterone receptors.

Allopregnanolone does not bind directly to intracellular progesterone receptors (Raynaud et al., 1974; Smith et al., 1974) but may indirectly activate progesterone receptors through a variety of intracellular signaling pathways (Etgen et al., 2006; Frye and Walf, 2008; Gonzalez-Flores et al., 2010; Mani et al., 2000). PKC, MAPK, and Src kinase-dependent signaling are modulated by allopregnanolone (Etgen and Acosta-Martinez, 2003; Frye and Walf, 2008; Gonzalez-Flores et al., 2006; Mani et al., 2000) and each of these mechanisms can contribute to ligand-independent activation of progesterone receptors (Boonyaratanakornkit et al., 2007; Gonzalez-Flores et al., 2006; Li and Shang, 2007; Mani and Portillo, 2010; Tetel, 2009). Such an indirect activation of progesterone receptors by allopregnanolone has been suggested previously (Auger and Forbes-Lorman, 2008; Beyer et al., 1995; Gonzalez-Flores et al., 2006, 2010; Miryala et al., 2011). Moreover, at least 1 h of priming by allopregnanolone was required for the progesterone metabolite to reduce the lordosis-inhibiting effect of restraint (Miryala et al., 2011). Therefore, the collective data are consistent with the hypothesis that progesterone receptors contribute to allopregnanolone's attenuation of the lordosis-inhibiting effect of restraint. If so, RU486 should be able to block the effect of allopregnanolone. The following experiment was designed to test this hypothesis.

2. Methods

2.1. Materials

Estradiol benzoate, allopregnanolone (3α-hydroxy-5α-pregnan-20-one), RU486 (11β-(4-dimethylamino)phenyl-17β-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one), dimethyl sulfoxide (DMSO) and sesame seed oil were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Propylene glycol was obtained from Eastman Kodak Company (Rochester, NY). Isoflurane (AErrane®) was purchased from Butler Schein Animal Health (Dublin, OH). Decapicone® restrainers were from Braintree Scientific, Inc. (Braintree, MA). Other supplies came from Fisher Scientific (Houston, TX).

2.2. Animals, housing and surgical procedures

Adult Fischer (F-344) female rats were purchased from Charles River Laboratories (Wilmington, MA) and were housed in polycarbonate shoebox cages in a colony room with lights on from 12 midnight to 12 noon. Food and water were available ad lib. After a two week acclimation to the animal facility, when 60 to 90 days of age, females were anesthetized with AErrane® and ovariectomized as previously described (White and Uphouse, 2004). Two weeks after ovariectomy, rats were injected subcutaneously (sc) with 10 μg estradiol benzoate. Two days later, rats were injected sc with vehicle (13.5% DMSO + propylene glycol) or the progesterone receptor antagonist, RU486 (5 mg/kg). One hour later, rats were injected sc with vehicle (sesame seed oil) or 4 mg/kg allopregnanolone. This led to 4 treatment conditions: estradiol benzoate, vehicle, vehicle (EB/VEH rats); estradiol benzoate, RU486, vehicle (EB/RU486 rats); estradiol benzoate, vehicle, allopregnanolone (EB/ALLO rats); and estradiol benzoate, RU486, allopregnanolone (EB/RU486/ALLO rats). Testing occurred 4 h after allopregnanolone or vehicle. All procedures were approved by the TWU IACUC committee in accordance with the PHS Guide.

2.3. Testing for sexual behavior

Four hours after sesame seed oil or allopregnanolone treatment, females were pretested for sexual behavior. Pretesting took place in the home cage of a sexually active male and behavior was monitored until the male had achieved 10 mounts or for a maximum of 30 min. Sexual receptivity (L/M ratio; number of lordosis responses divided by number of male mounts) and lordosis quality (measured by the magnitude of the lordosis response) were scored as previously described (White and Uphouse, 2004). Proceptivity (defined as the presence of hopping and darting) and resistance (defined as fighting, boxing, rolling over, trying to escape the cage) were measured as present or absent. When females showed at least one hop/dart sequence or one fighting/boxing sequence, that behavior was categorized as present.

2.4. Restraint procedures

Immediately after completion of the pretest, females were restrained for 5 min as previously described (White and Uphouse, 2004). The female was placed head first into a Decapicone® so that her nose was flush with the small opening at the tip of the cone. The base of the cone was gathered around the female's tail and secured tightly with tape. Immediately after the restraint experience, females were placed back into a male's cage for 15 consecutive min of behavioral testing, as described in Section 2.3.

2.5. Statistical procedures

Data for L/M ratios and lordosis quality were grouped into 5 min intervals and were evaluated by two-way repeated measures ANOVA with time relative to restraint as the repeated factor and with RU486 and allopregnanolone as independent factors. Post-hoc comparisons were made with Tukey's test. Proceptivity and resistance were compared by Chi-Square and Fisher's Exact Test procedures. Data were analyzed with SPSS and the statistical reference was Zar (1999).