ORIGINAL RESEARCH—BASIC SCIENCE

Motivated Behaviors and Levels of 3α,5α-THP in the Midbrain Are Attenuated by Knocking Down Expression of Pregnane Xenobiotic Receptor in the Midbrain Ventral Tegmental Area of Proestrous Rats

Cheryl Anne Frye, PhD,*†‡§¶ Carolyn J. Koonce, BS,* Alicia A. Walf, PhD,§ and Jamie C. Rusconi, PhD†

*Department of Psychology, The University at Albany-SUNY, Albany, NY, USA; †Department of Biological Sciences, The University at Albany-SUNY, Albany, NY, USA; ‡The Centers for Neuroscience, The University at Albany-SUNY, Albany, NY, USA; §Life Science Research, The University at Albany-SUNY, Albany, NY, USA; ¶Department of Chemistry, The University of Alaska–Fairbanks, IDeA Network of Biomedical Excellence (INBRE), Fairbanks, AK, USA

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ABSTRACT

Introduction. Progesterone (P_4) and its product, 5α-pregnan-3α-ol-20-one (3α,5α-THP), act in the midbrain ventral tegmental area (VTA) to alter motivated behaviors, such as mating, and motor and anxiety behavior. Of interest is whether 3α,5α-THP formation requires the pregnane xenobiotic receptor (PXR), which is expressed in the midbrain of rats.

Aim. The role of PXR in the midbrain for 3α,5α-THP formation, which precedes modulation of motivated behaviors, was investigated.

Methods. Rats had estrous cycle phase determined and were assessed when they were in diestrus or proestrus. Diestrous and proestrous rats were infused with control or antisense oligodeoxyribonucleotides (AS-ODNs) targeted against PXR to the VTA.

Main Outcome Measures. In pilot studies, PXR gene and protein expression in the midbrain were determined with quantitative reverse transcriptase polymerase chain reaction and Western blotting, respectively. Diestrous and proestrous rats infused with control or AS-ODNs to the VTA were tested for anxiety (open field and plus maze), social (social interaction), and sexual (paced mating) behavior. Expression of PXR in the midbrain was verified with Western blotting. Plasma estradiol, P_4, dihydroprogesterone (DHP), and 3α,5α-THP levels, and brain P_4, DHP, and 3α,5α-THP levels were measured. We predicted that proestrous rats infused with PXR AS-ODNs would have decreased anti-anxiety, social, and sexual behavior, lower midbrain expression of PXR, and lower midbrain levels of 3α,5α-THP compared with controls.

Results. Results supported the hypothesis that formation of 3α,5α-THP requires PXR and may be important for motivated behaviors. PXR AS-ODN, compared with control, infusions to the VTA reduced PXR expression and 3α,5α-THP levels in the midbrain and attenuated sexual receptivity of proestrous rats.

Conclusions. Knockdown of PXR in the midbrain reduces 3α,5α-THP levels and sexual receptivity of proestrous rats. Thus, PXR in the midbrain may be required for the observed increase in 3α-5α-THP during proestrus, which has subsequent effects on motivated, reproductive behaviors. **Frye CA, Koonce CJ, Walf AA, and Rusconi JC. Motivated behaviors and levels of 3α,5α-THP in the midbrain are attenuated by knocking down expression of pregnane xenobiotic receptor in the midbrain ventral tegmental area of proestrous rats. J Sex Med 2013;10:1692–1706.**

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Introduction

A focus on the functional role of progesterone (P_4) has been on its pregnancy-maintaining effects; however, it is clear that P_4 and its products have effects beyond pregnancy for motivated behaviors, encompassing social interaction, mating, and other reproduction-relevant behaviors. One brain target of progestogens, such as P_4 and 5α-pregnan-3α-ol-20-one (3α,5α-THP), is the midbrain ventral tegmental area (VTA). Natural elevations of P_4 and 3α,5α-THP across the estrous cycle coincide with increases in motivated behaviors, such as mating and reproduction-relevant behaviors (e.g., social interaction and anxiety), during proestrus [1–7]. Moreover, the effects of exogenous administration of progestogens support the role that they play for motivation. For example, administration of 3α,5α-THP to ovariectomized rats enhances mating, prosocial behavior, and anti-anxiety-like responses [4]. Results from other animal models corroborate these findings that progestogens can alter motivated processes. For instance, 3α,5α-THP produces a place preference [2,8,9] and rats will preferentially self-administer 3α,5α-THP over water [10]. Progestogens have effects to amplify motivated processes, such as those observed with cocaine. In support, proestrous rats have greater responses to cocaine than do diestrous rats [11]. Among ovariectomized rats, P_4 produces sex-dependent effects on behavioral responses to cocaine [12,13] and sequential administration of estradiol (E_2) and P_4 increases cocaine self-administration and tends to increase behavioral sensitivity to cocaine [14]. Thus, progestogens play a role in motivated behaviors.

Determining the source of 3α,5α-THP is critical to investigating the role and mechanisms of 3α,5α-THP for anxiety, social, and reproductive processes. 3α,5α-THP is formed via activation of a metabolic and/or biosynthetic pathway. For example, in the VTA, ovarian P_4 is readily metabolized to dihydroprogesterone (DHP) by 5α-reductase and DHP is converted to 3α,5α-THP by 3α-hydroxysteroid dehydrogenase (3α-HSD). Blocking P_4's metabolism to 3α,5α-THP pharmacologically or with knockout mouse models inhibits progestogens' facilitating effects on reproductive, social, and anti-anxiety behaviors, and 3α,5α-THP replacement can produce the opposite effects [1,15–18]. For female rats, engaging in mating promotes biosynthesis or neurosteroidogenesis of 3α,5α-THP from the precursor, cholesterol in the midbrain VTA, as well as regions important for reward, emotion, and cognition (i.e., striatum, hippocampus, and cortex) [4,16,19]. The 18-kDa translocator protein (TSPO; formerly known as the peripheral-type benzodiazepine receptor) is essential for neurosteroidogenesis in that it transports cholesterol to cytochrome P450-dependent side chain cleavage enzymes (P450scc) [20–22]. The steroidogenic acute regulatory protein is important for stimulating this action, but the precise mechanisms are unknown. Inhibiting formation of 3α,5α-THP through any of these metabolic pathways in the VTA attenuates these neuroendocrine and behavioral effects [1,16,18,23]. Thus, the effects of 3α,5α-THP on reproductive, social, and anxiety behaviors occur following its generation from the metabolism of ovarian P_4, which infiltrates the VTA, as well as from de novo synthesis from cholesterol within the brain.

It is generally accepted that progestogens are synthesized in the brain and peripheral nerves, but other factors that may regulate steroid biosynthesis are poorly understood. The pregnane xenobiotic receptor (PXR), a promiscuous nuclear receptor, binds 3α,5α-THP and can mediate transcription of cytochrome (CYP) enzymes [24–26]. CYP enzymes are involved in biosynthesis of steroids. The rodent PXR is analogous to the steroid and xenobiotic receptor in humans, also referred to as the human PXR. Whether behavioral and/or neuroendocrine variations in midbrain 3α,5α-THP occur concomitant with and/or require PXR is of interest.

Aims and Hypotheses

Our hypothesis is that formation of 3α,5α-THP requires PXR in the midbrain. First, we determined that there is expression of PXR gene and protein in the proestrous rat midbrain with quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and Western blotting, respectively. Second, to determine whether PXR may underlie some of 3α,5α-THP's actions in the midbrain VTA, diestrous and proestrous rats were administered placebo control or PXR antisense oligodeoxyribonucleotides (AS-ODNs) via infusions to the midbrain VTA. Following infusions to the midbrain, motivated and socially relevant behaviors (open field, elevated plus maze, social interaction, and paced mating) of rats were assessed. For the open field and elevated plus maze, measures of anti-anxiety behavior were