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PTSD in women is associated with a block in conversion of progesterone to the GABAergic neurosteroids allopregnanolone and pregnanolone measured in plasma

S.L. Pineles[a,b,*], Y.I. Nillni[a,b], G. Pinna[c], J. Irvine[d], A. Webb[d], K.A. Arditte Hall[a,b], R. Hauger[e,f], M.W. Miller[a,b], P.A. Resick[g], S.P. Orr[h], A.M. Rasmusson[a,b]

[a] National Center for PTSD, VA Boston Healthcare System, Boston, MA, 02130, USA
[b] Department of Psychiatry, Boston University School of Medicine, Boston, MA, 02118, USA
[c] The Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL, 60612, USA
[d] The Charles Stark Draper Laboratory, Inc., Cambridge, MA, 02139, USA
[e] Center of Excellence for Stress and Mental Health, San Diego VA Healthcare System, San Diego, CA, 9216, USA
[f] Department of Psychiatry, University of California, San Diego, La Jolla, CA, 92093, USA
[g] Department of Psychiatry and Behavioral Sciences, Duke University, Durham, NC, 27701, USA
[h] Psychiatry Department, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, 02129, USA

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ABSTRACT

There is a need to identify new and more effective treatments for posttraumatic stress disorder (PTSD). Allopregnanolone and its stereoisomer pregnanolone (together termed ALLO) are metabolites of progesterone that positively and allosterically modulate GABA effects at $GABA_A$ receptors, thereby reducing anxiety and depression. Previous research revealed that women with PTSD had low cerebrospinal fluid (CSF) ALLO levels and a low ratio of ALLO to the allopregnanolone precursor 5α-DHP, consistent with deficient activity of the ALLO synthetic enzyme 3α-hydroxysteroid dehydrogenase (3α-HSD). The current study examined ALLO and the ratio of ALLO to 5α-DHP in *plasma* at rest and in response to psychophysiological stressors in trauma-exposed, medication-free women with and without PTSD. Participants were examined twice in random order during the early follicular phase (eFP) and mid-luteal phase (mLP) of the menstrual cycle. Plasma neurosteroids were measured using gas chromatography-mass spectrometry. Results indicate that the ALLO to 5α-DHP ratio in plasma increases between the eFP and mLP. In addition, women with PTSD have a lower ratio of ALLO to 5α-DHP than trauma-exposed healthy women, as well as blunted increases in this ratio in response to a moderately stressful laboratory procedure, i.e., differential fear conditioning, across the menstrual cycle. Clinically feasible testing for 3α-HSD dysfunction is critical to translating this line of research into clinical care. Measurement of this ratio in plasma could facilitate patient stratification in clinical treatment trials, as well as precision medicine targeting of treatments that address ALLO synthesis deficits in women with PTSD.

1. Introduction

There are few effective psychopharmacological interventions for posttraumatic stress disorder (PTSD). Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed medications for PTSD (American Psychiatric Association, 2004; Foa et al., 2005; The Management of Post-Traumatic Stress Working Group, 2010). However, recent meta-analyses reveal small-to-medium effect sizes for the efficacy of SSRIs, and complete remission of PTSD occurs in only 20–30% of individuals treated with SSRIs (Hoskins et al., 2015; Watts et al., 2013). In order to develop more effective psychopharmacologic treatments for PTSD, there is a need to identify mechanism-related biomarkers that can guide development and targeting of novel as well as currently available pharmaceuticals for PTSD (Krystal et al., 2017). Deficiency in the synthesis of the neuroactive steroids allopregnanolone and pregnanolone has been associated with increased PTSD symptoms in humans and PTSD-like behaviors in rodents, and constitutes a target for potential new PTSD treatments (Pibiri et al., 2008, Pinna and Rasmusson, 2014; Rasmusson et al., 2006; Zhang et al., 2014).

Allopregnanolone and pregnanolone (together termed ALLO) are metabolites of progesterone produced by 5α-reductase I or II and 3α-hydroxysteroid dehydrogenase (3α-HSD) (Fig. 1). ALLO positively,

* Corresponding author at: VA Boston Healthcare System (116B-3), Boston, MA, 02130, USA.
E-mail address: Suzanne.Pineles@va.gov (S.L. Pineles).

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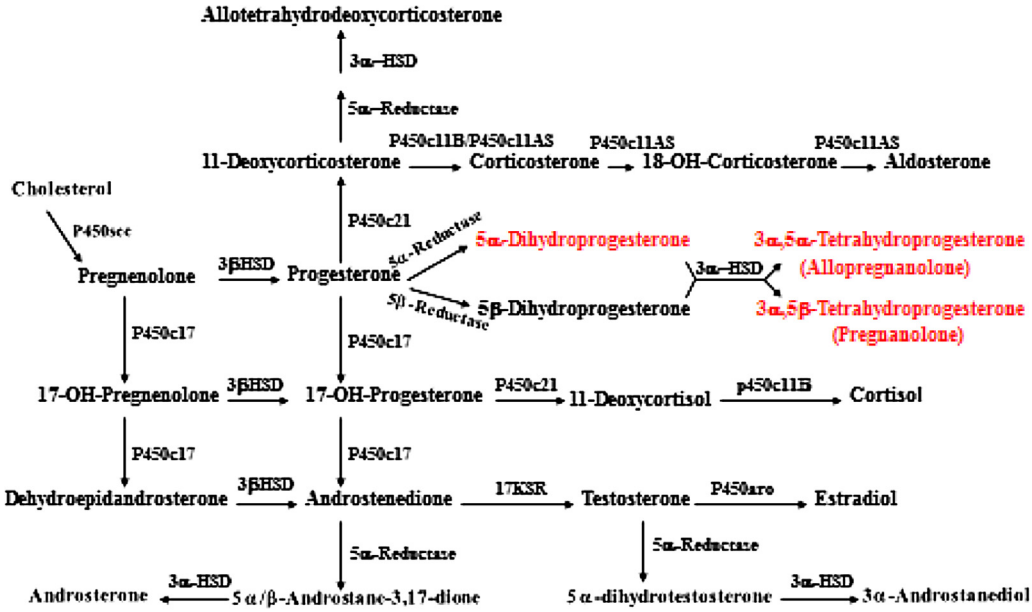

Fig. 1. Steroid Synthetic Pathways. Enzymes involved in steroidogenesis are positioned immediately above or below the arrows. Acronyms from top to bottom, left to right: 3α-HSD (3α-hydroxysteroid dehydrogenase); P450c11AS (P450c11 aldosterone synthase); P450scc (P450 side-chain cleavage enzyme); 17KSR (17-ketosteroid reductase); P450aro (P450 aromatase). Steroids in red were the focus of this investigation. This figure is a modified version of a figure that previously was used in an article titled Neuroactive Steroids and PTSD treament (Rasmusson et al., 2017).

allosterically, and potently modulates the effect of gamma aminobutyric acid (GABA) at $GABA_A$ receptors, resulting in anxiolytic, antidepressant, anti-aggressive and analgesic effects (Crowley and Girdler, 2014; Lambert et al., 2003; Puia et al., 1990; Schüle et al., 2014; Smith et al., 1998). Exposure to prolonged social isolation in male mice and ovariectomized, testosterone-treated female mice reduces 5α-reductase I gene expression (Fig. 1), resulting in reduced brain levels of allopregnanolone in association with increases in anxious and aggressive behaviors, as well as enhanced contextual fear conditioning and deficits in contextual fear extinction and extinction retention (Pibiri et al., 2008, Pinna and Rasmusson, 2014). Exposure to single-prolonged stress (an analog model of PTSD in rodents) also substantially reduces allopregnanolone in the frontal cortex and serum of male rats in association with enhanced contextual fear conditioning (Zhang et al., 2014). In normal cycling female rodents, infusion of finasteride (which inhibits 5α-reductase I and II; see Fig. 1) or 17-phenyl-(3α,5α)-androst-16-en-3-ol (an allopregnanolone antagonist) into the bed nucleus of the stria terminalis was associated with enhanced context, but not cue-specific, fear conditioning (Nagaya et al., 2015). Furthermore, the administration of allopregnanolone or a synthetic analog of allopregnanolone (ganaxolone), as well as neurosteroidogenic drugs that normalize brain allopregnanolone levels have normalized these PTSD-like behaviors in rodents (Pibiri et al., 2008; Pinna and Rasmusson, 2014; Zhang et al., 2014; see Rasmusson et al., 2017, for a review).

Complementing this preclinical research, Rasmusson and colleagues demonstrated low cerebrospinal fluid (CSF) ALLO levels in premenopausal women with PTSD, compared to healthy controls, which is apparently due to dysfunction of 3α-HSD (Fig. 1) (Rasmusson et al., 2006). CSF ALLO levels in women with PTSD were only 39% of healthy group levels and correlated strongly and negatively with PTSD re-experiencing and negative mood symptoms (Rasmusson et al., 2006). More recently, Inslicht et al. (2014) found that increases in plasma allopregnanolone in response to metyrapone challenge, which increases ACTH and progesterone levels, tended to be lower in women and men with PTSD. As ALLO potentiates the effects of GABA at $GABA_A$ receptors, low ALLO levels or deficient ALLO responses to stress in women with PTSD may be particularly problematic. Several studies have revealed a relationship between low plasma GABA levels and reactivity to stress or risk for PTSD (Trousselard et al., 2016; Vaiva et al., 2004; Vaiva et al., 2006). In healthy women, GABA levels decrease in the brain between the follicular and luteal phases of the menstrual cycle (Epperson et al., 2002; Harada et al., 2011) as progesterone and ALLO levels increase, perhaps to help preserve net normal GABAergic inhibitory transmission.

Additional work is needed to advance translation of this line of research into the clinical care of women with PTSD. Factors that have impeded extension of this line of research include the resources and expertise needed to: a) recruit participants willing to undergo lumbar puncture (LP), b) effectively control for possible menstrual cycle effects on ALLO levels or the capacity for ALLO synthesis, and c) implement mass spectrometry to accurately assay neurosteroids in the ALLO synthesis pathway (Cheney et al., 1995). In addition, a single CSF sample cannot discriminate resting from stress reactive ALLO levels, as undergoing an LP is inherently stressful. Plasma sampling would be preferable; however, it is unclear whether measurement of ALLO and other steroids in the ALLO synthesis pathway in plasma, instead of CSF, can be used to effectively predict PTSD or identify specific enzymatic blocks in ALLO synthesis. For example, different neurobiological factors initiate the synthesis of ALLO in the central nervous system (CNS) and periphery. Recent work by Izumi et al. (2013) suggests that NMDA receptor activation initiates ALLO synthesis in brain neurons, while adrenocorticotropic hormone (ACTH) and luteinizing hormone (LH) do so in the adrenal gland and ovary, respectively. In addition, the contribution of 5α or 5β-reductase to ALLO synthesis may differ between the CNS and periphery. Finally, a differential impact of stress on ALLO synthesis may confound discrimination of PTSD and healthy groups' capacity for ALLO synthesis. Low CNS ALLO levels in PTSD would be expected to potentiate hypothalamic-pituitary-adrenal (HPA) axis reactivity to a stressor (Patchev et al., 1994, 1996; Barbaccia et al., 2001; Inslicht et al., 2014) and raise peripheral ALLO, perhaps more so than in healthy individuals with higher CNS ALLO levels. Measuring peripheral ALLO levels at a single time point without good control over whether participants are in a true resting, i.e., low stress, state could obscure actual resting state group differences. Given resting state differences between PTSD and healthy individuals, stress would only be expected to magnify such differences in the *ratio* of ALLO to steroid precursors that precede a PTSD-related enzyme block in ALLO synthesis (e.g., 5α-DHP).

The present study was undertaken to determine whether plasma sampling, which is less invasive and more feasible to implement in the clinic than an LP, can be used effectively to identify ALLO synthesis deficits associated with PTSD in women. The study was specifically designed to ascertain whether: a) low *resting* and/or *stress-reactive increases* in ALLO synthesis characterize PTSD in premenopausal women