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Pregnenolone-progesterone-allopregnanolone pathway as a potential therapeutic target in first-episode antipsychotic-naïve patients with schizophrenia

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ABSTRACT

Neurosteroids are both endogenous and exogenous steroids that rapidly alter neuronal excitability through interactions with ligand-gated ion channels and other cell surface receptors. They are originated from cholesterol and have important implications for schizophrenia (SZ) pathophysiology and treatment strategies. Specifically, pregnenolone (PREG), progesterone (PROG) and allopregnanolone (ALLO) exhibit similar psychotropic properties. Using enzyme immunoassay, we compared the neurosteroids in PREG downstream pathways in plasma between healthy controls (HC, n = 43) and first-episode antipsychotic-naïve patients with SZ (FEAN-SZ, n = 53) before antipsychotic drug (APD) treatment. Comparisons were also made particularly along PREG-PROG-ALLO pathway in the same FEAN-SZ patients across multiple time points following initiation of treatment for 12 months (m). Firstly, at baseline, levels of PREG were significantly higher and those of ALLO were lower in FEAN-SZ than in HC, whereas PROG, cortisol, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) were not different. Consequently, the molar ratios of ALLO/PREG and ALLO/PROG in FEAN-SZ were significantly reduced. Secondly, in response to APD at 1 month, ALLO levels in FEAN-SZ were markedly elevated, whereas PREG and PROG levels decreased. Thirdly, among FEAN-SZ, lower levels of PROG (reflecting higher conversion to ALLO) at baseline may predict better therapeutic outcome after 1 month of APD treatment. These findings point to the perturbations of the PREG-PROG-ALLO pathway early in psychosis, and further study of this pathway may inform alternative and innovative therapeutic targets for SZ.

1. Introduction

Neuroactive steroids are both endogenous and exogenous steroids, which can alter brain excitability by binding to ligand-gated ion channels as well as cell surface receptors, e.g., γ-aminobutyric acid$_A$ ($GABA_A$) receptor (Lan and Gee, 1994). They are synthesized from cholesterol, which is converted into pregnenolone (PREG) and then into other endogenous steroids (Fig. 1). Although locally synthesized neurosteroids play a major role as signaling molecules in the brain, the mechanisms underlying the regulation of neurosteroid biosynthesis remain to be elucidated. Since neurosteroids are highly lipophilic and readily taken up across the blood-brain barrier, those neurosteroids and their precursors that are produced by an endocrine gland in the periphery can also exert their biological functions by subsequently reaching the brain through the bloodstream. A previous study (Mensah-Nyagan et al., 1999) indicated that the expression of several key steroidogenic enzymes in the brain may be regulated by adrenal and gonadal steroids, suggesting a putative association between peripheral and brain neurosteroids. Furthermore, their findings suggest that neurosteroids in the periphery may serve as a proxy or surrogate marker utility for regulation of neurosteroids in the brain. In fact, positive correlations between plasma and brain levels of neurosteroids have been demonstrated in rats (Barbaccia et al., 1997, 2001). A similar correlation has also been observed between plasma and CSF in humans

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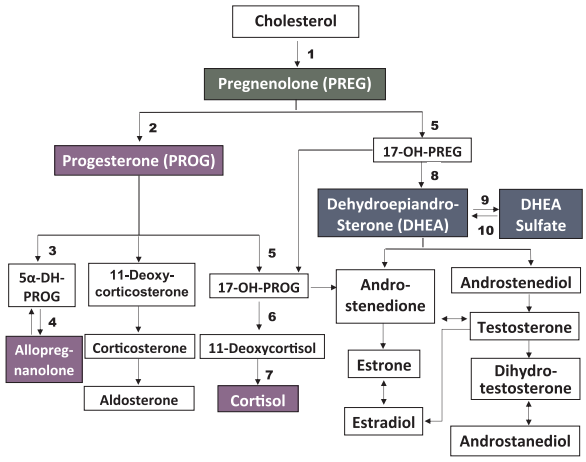

Fig. 1. Pregnenolone downstream pathways. Metabolites identified by colored background were determined in the present study. Abbreviations: DH, dihydro; 1, cytochrome P450scc enzyme (or CYP11A1); 2, 3β-hydroxysteroid dehydrogenase; 3, 5α-reductase; 4, 3α-hydroxysteroid dehydrogenase; 5, 17α-hydroxylase; 6, 21β-hydroxylase; 7, 11β-hydroxylase; 8, 17,20 lyase; 9, sulfotransferase; 10, sulfatase; 11, 3β-hydroxysteroid dehydrogenase 2/Δ5-4 isomerase.

(Kim et al., 2000). Among the neurosteroids, PREG, progesterone (PROG), allopregnanolone (ALLO), cortisol (CORT), and dehydroepiandrosterone (DHEA) are involved in different aspects of biological functions that have been implicated in the pathophysiology of some psychiatric disorders.

Converging evidence indicates a pattern of reduced PREG and its related endogenous steroids in schizophrenia (SZ). Low levels of serum PREG have been found in SZ patients compared to healthy controls (HC) (Ritsner et al., 2007). Levels of plasma CORT in male SZ patients with moderate negative symptoms were significantly higher than HC (Shirayama et al., 2002). Also, significant correlations between levels of plasma CORT and severity of negative symptom were observed in male SZ patients (Shirayama et al., 2002), suggesting that this endogenous steroid may serve as a biological marker for the severity of negative symptoms in SZ patients. In another study, following metabolic stress induced by injection of 2-deoxyglucose, SZ patients exhibited a significantly greater increase in plasma PROG compared to HC (Breier and Buchanan, 1992).

Moreover, findings from a proof-of-concept trial with adjunctive PREG treatment suggested that increases in serum PREG and ALLO levels may predict cognitive outcome after 8 weeks (Marx et al., 2009). In a recent follow-up clinical trial, this same group of investigators further concluded that PREG improved functional capacity in SZ patients, but did not improve cognitive symptoms after 8 weeks of treatment, suggesting that neurosteroids offer partial promise as a new therapeutic agent for SZ (Marx et al., 2014).

In several pharmacologic interventions in rodents, increases of PROG and ALLO levels in the brain were observed after clozapine and haloperidol administration (Khisti et al., 2002); interestingly, intracerebroventricular administration of ALLO produced inhibition of amphetamine-induced motor hyperactivity (Khisti et al., 2002). Progesterone and ALLO administered intraperitoneally and intracerebroventricularly, respectively, also inhibited the conditioned avoidance response in rodents (Ugale et al., 2004), an effect that was also observed following intraperitoneal injection of olanzapine, risperidone, and haloperidol (Ugale et al., 2004; Wadenberg et al., 2001). These psychotropic-like effects of progesterone and ALLO are likely due to interactions between these steroids, the GABAergic system and the dopaminergic system (Jaworska-Feil et al., 1998; Lewis et al., 2004).

Thus far, the bulk of the evidence for the psychotropic-like effects of neuroactive steroids like PROG and ALLO has come from animal models or human studies of antipsychotic drug (APD)-treated patients with chronic SZ. We hypothesize that 1) a homeostatic imbalance in PREG-PROG-ALLO pathway exists early in the course of SZ, and 2) the PREG-PROG-ALLO pathway may be a therapeutic target for antipsychotic treatment. To address these hypotheses in the present study, we compared six key plasma neurosteroids including PREG, PROG, ALLO, CORT, DHEA, and DHEA sulfate (DHEAS) between HC and first-episode antipsychotic-naïve patients with schizophrenia (FEAN-SZ) before initiation of APD treatment (baseline). Comparisons of parameters at baseline were also made in the same FEAN-SZ individuals across multiple time points following APD treatment.

2. Materials and methods

2.1. Participants

Blood samples were obtained after overnight fasting from 53 patients who were recruited in their first-episode of psychosis after they met DSM-IV criteria for SZ, schizophreniform or schizoaffective disorder based on the Structured Clinical Interview for DSM Diagnosis (SCID), and 43 age- and gender-matched HC subjects drawn from the same communities as where the patients were recruited. The follow-up blood samples were also obtained in the same patient individuals at 1, 6 and 12 months (m) after initiation of treatment with one or more of the following antipsychotic drugs: risperidone, olanzapine, quetiapine, aripiprazole and haloperidol. Approximately, 81% of patients were treated with risperidone either as a single drug (74%) or as an adjunctive (26%).

2.2. Clinical assessments

Results of the initial diagnostic assessments were reviewed and final research diagnoses were determined at consensus diagnostic conferences reviewing SCID and all clinical data, attended by research faculty and staff, and chaired by one of the authors (MSK or DM). All subjects provided signed informed consent after a full explanation of the study. The study was approved by the Institutional Review Boards of both the University of Pittsburgh and the VA Pittsburgh Healthcare System. Clinical symptomatology and global clinical status were rated by experienced research clinicians at all time points using the Brief Psychiatric Rating Scales (BPRS; Overall and Gorham, 1962), the Schedules for the Assessment of Positive (SAPS) and Negative Symptoms (SANS) (Andreasen and Olsen, 1982), the Global Assessment Scale (GAS; Endicott et al., 1976); the Hamilton Depression Rating Scale (HDRS; Williams, 1988), and the Clinical Global Impression (CGI) severity scale (Guy, 1976).

2.3. Plasma neurosteroid assays

To reduce the variance associated with circadian rhythm effects (Brambilla et al., 2009), blood samples were drawn around 8:00 am on the morning of each session. In addition, since the steroid hormones can change drastically throughout the menstrual cycle, for female subjects, we inquired about the date of their last menstrual bleeding and restricted the sampling time to the period within the follicular phase (Day 1–13). Briefly, freshly drawn blood with anticoagulant citrate dextrose was centrifuged at 750*g* for 7 min to remove RBC and stored at −80 °C. Aliquots of banked samples that have not previously thawed were used for assay.

Plasma neurosteroid concentrations were analyzed in duplicate by quantitative sandwich enzyme linked immunoassay (ELISA) using commercially available kits. Pregnenolone and DHEAS were purchased from BioVender (Modrice, Czech Republic). Dehydroepiandrosterone was acquired from Eagle Biosciences (Nashua, NH, USA). Progesterone and CORT were provided by Cayman Chemical (Ann Arbor, MI, USA). Allopregnanolone was obtained from Arbor Assays (Ann Arbor, MI, USA) and its original sensitivity was 0.13 ng/mL as indicated in the