REVIEW

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Pregnenolone, Dehydroepiandrosterone, and Schizophrenia: Alterations and Clinical Trials

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Neurosteroids, such as pregnenolone (PREG), dehydroepiandrosterone (DHEA), and their sulfates (PREGS and DHEAS) are reported to have a modulatory effect on neuronal excitability and synaptic plasticity. They also have many other functions associated with neuroprotection, response to stress, mood regulation, and cognitive performance. Furthermore, these neurosteroids have been linked to, and their levels are altered in, neuropsychiatric disorders. This review highlights what is currently known about the metabolism and mode of action of PREG and DHEA, as well as about alterations of these neurosteroids in schizophrenia. This review also provides substantial information about clinical trials with DHEA and PREG augmentation with of antipsychotic agents in schizophrenia.

Introduction

Schizophrenia is a chronic and disabling mental disorder characterized by positive, negative and mood symptoms, disturbed coping abilities with elevated distress and a significant decline in cognition, quality of life, and psychosocial functioning. Understanding the etiology and pathogenesis of schizophrenia is a major challenge facing psychiatry. Though recent studies have shown that several neurobiological alterations in domains of brain structure, physiology, and neurochemistry may reflect diverse pathophysiological pathways from the "genome to the phenome" (reviewed in [1,2]), a conclusive identification of specific etiological factors or pathogenic processes in the illness has remained elusive.

Treatment of schizophrenia patients typically includes a combination of pharmacotherapy with antipsychotic agents and psychosocial interventions. Antipsychotic agents ameliorate symptoms in the early phases of disease but become less effective over time, as the underlying disease progresses. Despite the effectiveness of antipsychotic medications in the treatment of schizophrenia, in a large scale multicenter study [3] about 74% of the patients discontinued study medication within the first 18 months: 64% of those assigned to olanzapine, 75% of those assigned to perphenazine, 82% of those assigned to quetiapine, 74% of those assigned to risperidone, and 79% of those assigned to ziprasidone. The majority of patients in each group discontinued their assigned treatment owing to inefficacy or intolerable side effects or for other reasons. Furthemore, about one-third of all patients with schizophrenia do not respond adequately to drug treatment. Although antipsychotic agents are an indispensable component of the treatment, the development of more effective treatments is an important and desirable goal.

A promising direction is the use of neuroactive steroids. Baulieu [4] discovered that pregnenolone (PREG) and dehydroepiandrosterone (DHEA) are produced in the brain and introduced the term "neurosteroids" in 1981. As described later, experimental and clinical observations suggested that PREG and DHEA have neuroprotective and neuropsychopharmacological properties, and might be involved in the pathophysiology of schizophrenia, and in some of its manifestations. This review begins with

short information about metabolism and mode of action of these neurosteroids, and it provides substantial information regarding alterations and clinical trials with PREG and DHEA in schizophrenia.

Steroidogenesis

Neurosteroids are synthesized in the central and peripheral nervous system, particularly but not exclusively in myelinating glial cells, from cholesterol or steroidal precursors imported from peripheral sources [4]. Do Rego et al. [5] summarize the current knowledge regarding the existence, neuroanatomical distribution and biological activity of the enzymes responsible for the biosynthesis of neurosteroids in the brain of vertebrates; they review the neuronal mechanisms that control the activity of these enzymes.

PREG and its metabolites such as pregnenolone sulfate (PREGS) (both PREG and PREGS [PREG(S)]), DHEA and DHEA sulfate (DHEAS) (both DHEA and DHEAS [DHEA(S)]) are neurosteroids. Figure 1 presents biosynthesis of PREG(S), DHEA(S), and other steroids [6]. Briefly, trophic hormones activate a chain of reactions that lead to the hydrolysis of cholesterol esters into free cholesterol, and the transport of cholesterol into mitochondria where it is converted to PREG by cytochrome P450 side-chain cleavage (P450 scc; now referred to as CYP11A1). This is the first step in steroidogenesis [7].

PREG can be converted to 17-OH-PREG by the enzyme 17α-hydroxylase. The following step is conversion of 17-OH-PREG to DHEA (3β-hydroxy-5-androsten-17-one) by the microsomal cytochrome P450c17 enzyme (17a-hydroxylase/17,20-desmolase) in the brain and the adrenals [4,8]. Lastly, hydroxysteroid sulfotransferases convert PREG to PREGS, and DHEA to DHEAS, whereas steroid sulfatases convert PREGS to PREG, and DHEAS to DHEA [9,10]. DHEA(S) concentrations in the human brain were found to be much higher than

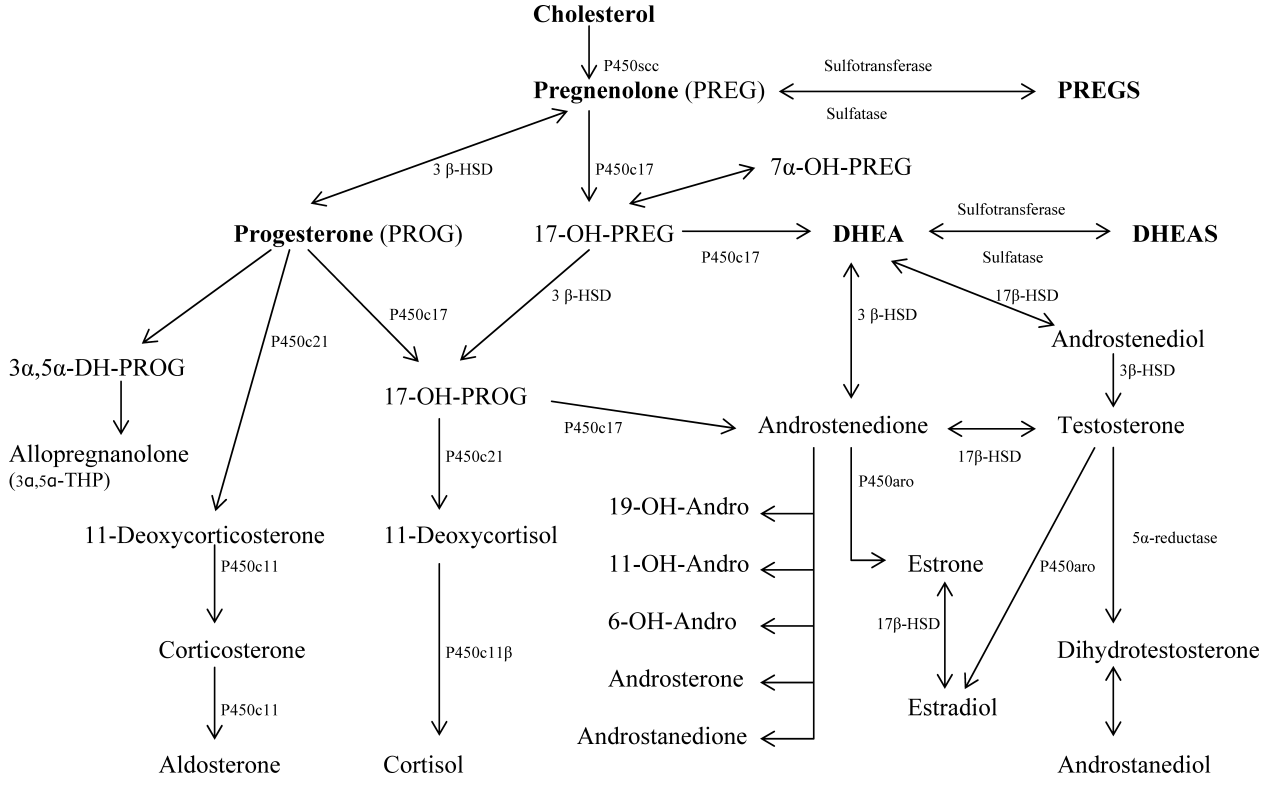

Figure 1 Biosynthesis of pregnenolone, and metabolic pathway that demonstrates the synthesis of PREG, DHEA, and other steroids. Abbreviations: Andro, androstenedione; PREG, pregnenolone; PREGS, pregnenolone sulfate; 17-OH-PREG, 17-dihydropregnenolone; 7α-OH-PREG, 7α-dihydropregnenolone; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; PROG, progesterone; 17-OH-PROG, 17-dihydroprogesterone; THP, tetrahydroprogesterone (allopregnanolone); 3α,5α-THP = 3α,5α-tetrahydroprogesterone; 3α,5α-DH-PROG, 3α,5α-dihydroprogesterone; P450scc, P450 side-chain cleavage enzyme (now referred to as CYP11A1); P450c17, 17α-hydroxylase/17,20-desmolase (CYP17A1); P450c21, 21α-hydroxylase; P450c11, 11α-hydroxylase; P450c11β, 17β-hydroxylase; HSD, hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; P450aro = P450 aromatase; 5α-reductase, 5α-hydroxysteroid reductase.

in peripheral circulation and also exceeded their very low cerebrospinal fluid (CSF) levels, ranging from about 1% to 5% of the corresponding plasma concentrations. DHEA(S) levels decrease markedly with age in humans, and levels in elderly populations are reduced to 20 to 30% of peak levels in young adulthood (reviewed in [6]).

Neuroprotective Effects

Investigation of the long-term course of schizophrenia with progression to different residual syndromes has suggested that schizophrenia may be a neuroregressive illness. Although the molecular mechanisms of neurodegeneration and pathogenesis of schizophrenia remain largely unknown, a significant body of literature indicates that the main mechanisms implicated in the disease process may include apoptosis, excitotoxicity, oxidative stress, stress and others [11]. A role for apoptosis in schizophrenia has long been hypothesized, but studies investigating this hypothesis have only recently begun. Apoptosis appears to be downregulated in the cortex of patients with chronic schizophrenia that could reflect either a pathophysiological failure to mount an effective response to an apoptotic insult or an appropriate compensatory response to an earlier insult [12].

Biological actions of PREG(S) and DHEA(S) include neuroprotection against apoptosis, *N*-methyl-D-aspartate (NMDA) or oxidative damage, promotion of neurite growth, opposition of glucocorticoids, neurite growth, and antagonistic effects on oxidants and glucocorticoids, a modulatory effect on neuronal excitability and synaptic plasticity; they have many functions associated with response to stress, mood regulation, and cognitive performance [6,13,14].

In particular, DHEA is produced at high concentrations in the human embryo enhancing neuronal development, but its production rate and levels in serum, brain and adrenals decrease gradually with advancing age [15,16]. This decline was associated to age-related neuronal dysfunction and degeneration, suggesting a neuroprotective effect of endogenous DHEA against noxious agents [17]. This hypothesis is substantiated by experimental findings showing that DHEA protect neural crest derived cells against serum deprivation-induced apoptosis with EC50 1.8 nM. This potent antiapoptotic effect of DHEA is mediated by G-protein coupled specific membrane binding sites, the subsequent activation of prosurvival kinases Src and PKC and transcription factors CREB and NF-κB, upstream effectors of the antiapoptotic Bcl-2 proteins [18].

In addition, DHEA and DHEAS inhibit *apoptosis* in human peripheral blood lymphocytes through a mechanism independent of either androgen receptors or estrogen receptors [19].

DHEA(S) exhibit reduction of risk of age-related neurodegenerative disorders [20,21]. Animal and *in vitro* studies have shown that DHEA and DHEAS stimulate neuronal outgrowth and development [22,23] and improve glial survival, learning, and memory [15,22]. Recent studies described a protective effect of DHEA on neuronal survival after oxidative, ischemic or traumatic damage [24,25]. This specifically extends to a protective effect of DHEA on the hippocampus [21], which supports the proposed antiglucocorticoid effect of DHEA, as glucocorticoids are known to affect hippocampal structure and function. DHEA(S) protect chromaffin cells and the sympathoadrenal PC12 cells (an established model for the study of neuronal cell apoptosis and survival) against serum deprivation-induced apoptosis [25].

PREG(S) and DHEA(S) stimulate hypothalamic-pituitary-adrenal (HPA) axis activity and cerebral brain-derived neurotrophic factor (BDNF) protein levels in adult male rats [16,26]. In detail, they induce corticotropin-releasing hormone and/or arginine vasopressin synthesis and release at the hypothalamic level, thus enhancing plasma adrenocorticotropin hormone and corticosterone concentrations. This stimulation of the HPA axis occurs concomitantly with BDNF modifications at the hippocampus, amygdala and hypothalamus levels. These results strongly suggest that part of the HPA axis and antidepressant effects of neuroactive steroids could be mediated by BDNF, particularly at the amygdala level. They also suggest that neurosteroid effects on central BDNF could partially explain the trophic properties of these molecules.

DHEA(S) demonstrates neuroprotective effects on *NMDA-induced neurotoxicity* in primary cultured rat hippocampal neurons [27]. In addition, DHEA(S) block the neurotoxic effects of cortisol on hippocampal cells [28] and protect neurons against glutamate and amyloid ß-protein toxicity [29], and glucocorticoid toxicity [30]. The participation of aromatase in the neuroprotective effect of these neurosteroids was assessed in a study that suggested that estradiol formation by aromatase mediates neuroprotective effects of PREG and DHEA against excitotoxic-induced neuronal death in the hippocampus [31]. Recently, Akan et al. [32] investigated the effects of PREG and PREGS on cell viability and amyloid beta peptide peptide toxicity in a concentration- and exposure time-dependent manner in rat PC-12 cells. PREG showed a dose-dependent protective effect against amyloid ß-peptide in PC-12 cells. But its sulfate ester did not have the same effect on amyloid beta peptide toxicity. PREG has neuroprotective effects against both glutamate and amyloid beta protein neuropathology and

glutamate neurotoxicity. Leskiewicz et al. [33] examined the effect of PREG, DHEA(S), and allopregnanolone on staurosporine-, glutamate-, and NMDA-induced damage in primary cortical neuronal culture. It was shown that glutamate-induced cell damage was attenuated by PREG, DHEA, and DHEAS, but not by allopregnanolone. The results of the present *in vitro* studies suggest that excitatory neurosteroids PREG and DHEA(S) at physiological concentrations participate in the inhibition of cortical neuronal degeneration elicited by staurosporine and glutamate. DHEA(S) supplementation greatly increases neuronal survival and differentiation and reduces astroglial proliferation rates in mouse brain cells in cultures [34]. Treating adult male rats with subcutaneous pellets of DHEA increased the number of newly formed cells in the dentate gyrus of the hippocampus, and also antagonizes the suppression of corticosterone. In other words, DHEA regulates neurogenesis in the hippocampus and modulates the inhibitory effect of increases corticoids on both the formation of new neurons and their survival [35].

In addition to aforementioned neuroprotective effects, DHEA(S) have been shown to display antioxidant [36] and antistress properties [37,38]. In particular, DHEA protects hippocampal cells from oxidative stress-induced damage [36], while DHEA and DHEAS influence on the HPA axis adaptation to stress and other psychiatric symptoms [39].

Thus, these findings suggest that PREG(S), and DHEA(S) may act as endogenous neuroprotective factors. The decline of neurosteroid levels during aging and schizophrenia may leave the brain unprotected against neurotoxic challenges. Therefore, PREG and DHEA may be suitable candidates for the treatment of schizophrenia and schizoaffective disorder patients.

Central Nervous System Receptors

At the cellular level, in addition to the effect on postsynaptic receptors, PREG(S) and DHEA(S) have modulatory effects on the release of multiple neurotransmitters like glutamate, GABA, acetylcholine, norepinephrine, dopamine and 5-HT (reviewed in [40]). Neurosteroids directly affect major Central Nervous System (CNS) receptors, especially the gamma-aminobutyric acid ($GABA_A$), NMDA, and sigma receptors [19,41]. More specifically, certain naturally occurring PREG can enhance $GABA_A$ receptor function in a direct manner, and consequently have anxiolytic, analgesic, anticonvulsant, sedative, hypnotic and anaesthetic properties [42]. In particular, PREG and DHEA may act as potential signaling molecules for neocortical organization during brain development, regulate the neuronal function by affecting neuronal excitability through prominent modulatory effects on the $GABA_A$, sigma-1, NMDA [19,43,44], cholinergic [45], and dopaminergic [46] systems. These modulations may lead to important changes for neuronal *excitability*.

There is evidence supporting a receptor-dependent basis for the direct physiological effects of DHEA(S). The data supporting an intracellular receptor for DHEA(S) are relatively weak and do not allow us to determine whether DHEA(S) directly, or a metabolite of DHEA(S), acts as a direct receptor ligand [47].

Many of the modulatory effects of PREG(S) and DHEA(S) occur in brain regions involved in learning and memory, emotion, motivation, motor, and cognition. Neurosteroids demonstrate cognitive-enhancing effects and have been reported to improve memory [48]. In preclinical studies, memory-enhancing effects of PREGS and DHEAS have been attributed to their NMDA-agonistic properties [49].

While several neurotransmitter systems have been linked to neurocognitive abnormalities in schizophrenia, including prefrontal glutamatergic, cortical dopaminergic, and cholinergic neurotransmission function, the association between neurosteroids and neurocognitive function is not yet fully understood and has been sparsely investigated. DHEA(S) studies that investigated neurocognitive functions in schizophrenia patients noted that neurocognitive impairment was associated with low DHEAS levels [50,51], high DHEAS levels [52] or high DHEA levels [50] in plasma, or no association was found altogether [53].

Davis et al. [54] investigated whether circulating levels of DHEAS independently contribute to aspects of cognitive function in women in a community-based, cross-sectional study of 290 women, aged 21–77 years. In the multiple linear regression analysis, the authors found that higher endogenous DHEAS levels are independently and favorably associated with executive function, concentration, and working memory. A limitation of this study was that DHEAS was measured on only one occasion.

Neurosteroids are also involved in the control of a number of behavioral, neuroendocrine and metabolic processes, such as regulation of food intake, locomotor activity, sexual activity, aggressiveness, anxiety, depression, body temperature, and blood pressure [5].

Because biological actions of PREG and DHEA include neuroprotective and antistress effects, a modulatory effect on neuronal excitability and synaptic plasticity, it is important to examine the alterations of these neurosteroids in schizophrenia and schizoaffective disorders.

Alterations

There is accumulating evidence that alterations in PREG(S) and DHEA(S) may be involved in the

pathophysiology of schizophrenia, mood and cognitive disorders [6,53,55–57]. Previous clinical studies demonstrated low circulating levels of PREG in the elderly, including those with dementia [58], in individuals with schizophrenia [59], in male patients with generalized anxiety disorder [60], and in non-medicated male patients with generalized social phobia [61].

Blood DHEA and DHEAS levels of schizophrenia patients and healthy subjects were found to differ across studies, ranging from normal to low, and to high levels. Overall, serum DHEA and DHEAS concentrations range from 15.7 to 90.9 nmol/L, and from 4,928 to 12,777 nmol/L, respectively, among schizophrenic patients, as well as, from 24.0 to 68.8 nmol/L, and from 5,375 to 13,477 nmol/L among healthy subjects, respectively. Meta-analysis of differences in mean concentrations of serum DHEA(S) between schizophrenic patients and control subjects show a significant nonzero effect ($p < 0.001$), and significant heterogeneity of data ($p < 0.001$; see more details and references in [6].

Contradictory and confusing reports on serum DHEA levels in schizophrenia led us to compare the serum a concentration of PREG, and DHEA between 15 medicated schizophrenic patients and 12 healthy subjects at four time points: at the start of the study and after 2, 4, and 8 weeks [6]. Controlling for age, serum concentrations of PREG were lower, while the DHEA level and the molar ratio values of DHEA to PREG were higher in schizophrenic patients compared to healthy controls. PREG and DHEA levels and their molar ratio did not change significantly during the study period either among schizophrenic patients or healthy controls. The blood levels of PREG appear to be associated with trait-anxiety scores in schizophrenic patients, while associations of clinical symptoms with the two neurosteroids did not reach a significant level when the confounding effect of emotional distress and anxiety scores was controlled. Thus, low serum PREG concentrations in schizophrenia appear to be associated with trait-anxiety scores independent of symptoms. PREG and the DHEA to PREG molar ratio may represent trait-like markers of impaired hormonal response to stress in schizophrenia. Although the role of PREG and DHEA in non-specific response to distress and anxiety or in the pharmacotherapy of schizophrenia is as yet unclear, this report adds evidence to the assumption that, for example, PREG and DHEA concentrations, are not related to specific diagnoses, but to more general psychological states such as anxiety, that can occur in various disorders. Conflicting reports underscore the fact that the neurobiology underlying the plasma steroid levels in schizophrenia is multifaceted. A further longitudinal large-scale case-control comparison of PREG and DHEA levels in treated and untreated, as well as in subjects who have undergone a washout of antipsychotics schizophrenic patients is warranted.

Thus, the involvement of PREG(S) and DHEA(S) in schizophrenia and schizoaffective disorders is not fully established. However, because PREG and DHEA are multifunctional and exhibit a variety of properties in the CNS, their potential therapeutic benefits may be useful for the treatment of patients with schizophrenia and schizoaffective disorders.

Treatment

Two treatment models have been applied in the DHEA administration studies:

1. *a 'replacement model'* with physiological (25–50 mg) or near-physiological (100 mg) daily doses of DHEA [62–66];
2. *a 'pharmacological model'* of treatment using more than 100 mg/day DHEA. Few reports are available regarding the use of higher DHEA doses (up to 1,600 mg/day [67]), and supraphysiologic doses (1,600 mg–2.25 g [68]).

Clinical trials with the use of oral DHEA have been extensively studied in healthy elderly, postmenopausal women, in various somatic and neuropsychiatric disorders (see for review [55]). Several randomized, double-blind, placebo-controlled clinical trials have been conducted with these neurosteroids for treatment of schizophrenia patients. DHEA augmentations (50–200 mg/day) for a period of 1–12 weeks were examined in cross-sectional [69–71] and crossover [72–74] designs. Comparative analyses of the obtained findings are presented in the review [75]. Table 1 presents the main parameters and results from clinical trials with DHEA and PREG augmentations.

The first randomized, double-blind, placebo-controlled study compared patients receiving DHEA (n = 15) and placebo (n = 12) and indicated a significant efficacy of DHEA augmentation (100 mg/day) after 6 weeks in the management of *negative, depressive, and anxiety symptoms* of schizophrenia [69]. Subjects receiving DHEA demonstrated a significant increase in DHEA(S) plasma levels that were correlated with improvement in negative symptoms, but not with improvement in depressive and anxiety symptoms. Limitations of the small sample size and lack of cognitive, side effects and quality of life assessments are noted.

A second randomized, double-blind, placebo-controlled study investigated the effect of DHEA

Table 1 Dehydroepiandrosterone (DHEA) and pregnenolone (PREG) as an adjunctive treatment in schizophrenia and schizoaffective disorders: the main parameters and results from clinical trials

Study	Participants (daily dose)			Length of trial	Antipsychotics	Significant effect of DHEA or PREG augmentation compared to placebo			
	DHEA	PREG	Placebo	(weeks)		Symptoms	Cognition	Side effects	Quality of life
[69]	15 (100 mg)	-	12	6	FGAs, SGAs	Negative, depressive, and anxiety symptoms	No data	No data	No data
[70]	15 (100 mg)	-	15	1	FGAs, SGAs	No effect	No data	Extrapyramidal symptoms	No data
[71]	16 (150 mg)	-	15	12	Olanzapine	No effect	No effect	No effect	No data
[72]	55 (200 mg)	-	55	6	FGAs, SGAs	No effect	Visual sustained attention, and motor skills	No effect	No effect
[73]	16 (400 mg)	16 (30 mg), 10 (200 mg)	16	8	FGAs, SGAs	Positive symptoms**	Attention and working memory performance**	Extrapyramidal symptoms***	No data
[81]	-	8* (500 mg)	9	8	SGAs	No effect	No effect	No effect	No effect
Total	117 (100–400 mg)	34 (30–500 mg)	122	1–12	FGAs, SGAs	The clinically significant benefits remain unclear.			

Design: a randomized, double-blind, placebo-controlled study [69–71,73,81]; a randomized, double-blind, placebo-controlled crossover study [72].

*Dosing: 100 mg/day for 2 weeks, 300 mg/day for 2 weeks, and 500 mg/day for 4 weeks.

**Significant for PREG augmentation, 30 mg/day.

***Significant for PREG and DHEA augmentations.

FGAs, first-generation antipsychotics; SGAs, second-generation antipsychotics.

administration for 7 days on medication-induced extrapyramidal symptoms (EPS) among inpatients with schizophrenia or schizoaffective disorder that were randomized in a double-blind fashion to receive either 100 mg DHEA or placebo in addition to a constant dosage of antipsychotic medication [70]. Parkinson-like side effects showed a favorable effect of DHEA with a significant time effect, as well as a significant group by time interaction and with no change noted on akathisia. An increase in DHEA blood levels was negatively associated with change of Parkinson-like side effects as well as with change of total EPS ratings. The authors concluded that DHEA appears to demonstrate a significant effect on EPS, with improvement observed particularly in Parkinsonian symptoms. The Brief Psychiatric Rating Scale scores showed a significant decrease during the 7 days of the study, *which was identical for DHEA and placebo groups.*

A third randomized, double-blind, placebo-controlled study performed by the same research group included 40 patients with chronic schizophrenia stabilized on olanzapine. The subjects were randomized in a double-blind fashion to receive either DHEA (150 mg/day) or placebo augmentation for a period of 12-weeks [71]. Sixteen patients who received DHEA and 15 patients who received placebo completed the study. The analysis *of negative symptoms* measured with the Scale for the Assessment of Negative Symptoms (SANS) revealed a significant treatment by time interaction, *but not between DHEA and placebo arms*. At the same time, DHEA augmentation was not superior to placebo in improving the scores of the Positive and Negative Symptom Scale (PANSS), measures of side effects (the Simpson–Angus Extrapyramidal Symptom Scale [SAS], Barnes Akathisia Scale [BAS], and Abnormal Involuntary Movements Scale [AIMS]), in cognitive performance (Mindstreams battery), and aggressive behavior (the Life History of Aggression scale).

Several methodological concerns should be mentioned regarding this trial.

1. Scales data were analyzed by using 2 × 7 repeated measures ANOVA models with main factor of treatment (DHEA vs. placebo) and a repeated measurements factor of time (weeks 0–12). Unfortunately, findings concerning significance of the main treatment factor (DHEA vs. placebo) were not reported.
2. Furthermore, the ANOVA for repeated measures and correlation coefficients are presented without the Bonferroni correction for multiple testing.
3. In this study effect size as the partial eta squared (η^2) was calculated based on the ANCOVA models. The η^2 means the proportion of total variability attributable to a factor (the group) by time interaction. The effect size in this study was very small (<0.20), in particular, the η^2 was only 0.172, which means that "the group by time interaction on SANS factor" accounted for 17.2% of the overall variance. Likewise, the η^2 on the SAS, and the BAS scores were 0.063, and 0.093, respectively. *This means that three months of DHEA administration failed to detect any efficacy in treating symptoms of schizophrenia, and side effects (SAS, BAS).*
4. Finally, since blood DHEA(S) levels among DHEA treated patients compared to the placebo group failed to reach significance, the relationship of DHEA therapy with reduction of negative symptom ratings remains doubtful.

Thus, these cross-sectional DHEA trials [69–71] did not replicate one another in terms of the depressive or anxiety symptoms, and in the medication-induced adverse side effects. They did not show a consistent and unequivocal significant favorable effect of DHEA administration on negative symptoms compared to placebo.

In order to resolve some of the concerns that have risen in the cross-sectional trials, a randomized, double-blind, placebo-controlled crossover study was conducted in two mental health centers [72]. During this trial 55 patients received either DHEA (200 mg per day) or placebo in identical capsules for 6 weeks following which they were switched to either placebo or DHEA for a further 6 weeks. Patients continued to receive their regular treatment with daily doses of antipsychotic medication kept constant for at least 2 weeks prior to entering the study and throughout the study period. The crossover analysis revealed no statistically significant treatment effect of DHEA on severity of illness symptoms (PANSS), side effects, or on quality of life measures compared with placebo treatment. However, this investigation, while preliminary, supports prior findings of some improvement noted in visual sustained attention, and motor skills due to DHEA administration. DHEA treatment was well tolerated without any serious adverse effects.

Findings from this trial [72,73] were used for multiple regression analysis for predicting sustained attention, memory, and executive function scores across three examinations from circulating levels of DHEA, DHEAS, androstenedione, and cortisol through DHEA administration in schizophrenia [74]. The findings indicated that circulating DHEAS and androstenedione levels were positive predictors of cognitive functioning, and DHEA levels was a negative predictor. Overall, blood neurosteroid levels and their molar ratios accounted for 16.5% of the total variance in sustained attention, 8–13% in visual memory tasks, and about 12% in executive functions. In addition, effects of symptoms, illness duration, daily doses of antipsychotic agents, side effects, education, and age of onset accounted for variability in

cognitive functioning in schizophrenia. Thus, this study suggests that alterations in circulating levels of neurosteroids and their molar ratios may reflect pathophysiological processes, which, at least in part, underlie cognitive dysfunction in schizophrenia.

Previous trials with PREG conducted on healthy volunteers under stressful conditions demonstrated significant improvements in mood, general well-being, psychomotor performance, and learning [76–78]. Low doses of oral PREG (30 mg/day) were generally well tolerated in 17 healthy volunteers who received pregnenolone for 4 weeks [79].

Findings from two pilot clinical trials with PREG augmentation in schizophrenia were recently reported. The first of them, an 8-week, controlled, double-blind, randomized, parallel-group trial with "low dose" and "high dose" of PREG (30 mg/day and 200 mg/day, respectively), and 400 mg/day DHEA augmentation of on-going antipsychotics in the treatment of chronic schizophrenia and schizoaffective disorders patients was conducted in two large state referral institutions [80]. It was hypothesized that PREG augmentation of ongoing and unchanged antipsychotic therapy may be able to improve psychotic symptoms and cognitive performance in chronic schizophrenia and schizoaffective disorder patients compared to DHEA and placebo administration. Data were collected from February 2005 until June 2007 (70 patients). A total of 58 patients were randomized, 44 patients (12/13 women and 32/45 men) completed the trial. Ten patients met criteria for schizoaffective disorders; all other subjects met criteria for schizophrenia. After an 8-week period, compared with placebo, PREG administration of 30 mg/day was associated with significant reduction in positive symptom scores, side effects (EPS), and improvement in attention and working memory performance, whereas subjects treated with 200 mg/day of PREG did not differ on outcome variable scores for the study period. The condition of patients receiving placebo and 30 mg/day of PREG improved more than those subjects treated with DHEA with regard to general psychopathology severity, and general functioning, while DHEA was superior to placebo in improving EPS. No significant main effect of the type of antipsychotics or type of antipsychotics time interaction on the Clinical Global Impression-Severity scale (CGI-S), PANSS subscale, the Global Assessment of Functioning Scale, Extrapyramidal Symptom Rating Scale (ESRS) or Barnes Akathisia Rating Scale (BARS) ratings was observed for patients receiving PREG (30 or 200 mg/day), DHEA or placebo. Interestingly, a significant efficacy of DHEA augmentation was observed with 50–150 mg/day [69,71], but not with 200 mg/day [72] or 400 mg/day [80]. Moreover, the augmentation of 400 mg/day of DHEA resulted in significantly less improvement of CGI-S, and PANSS general psychopathology subscale scores compared to placebo. Therefore, we suggest an inverted-U clinical response on a daily dose of PREG and DHEA augmentations (although there could be other reasons for the inconsistent results: methodological issues, different sample characteristics, different baseline severity of illness, and varying durations of combination treatment). *Negative symptoms and akathisia did not significantly benefit from any treatment*. The administration of PREG and DHEA was well tolerated. Circulatory pregnenolone was found significantly higher among the patients treated by both neurosteroids compared to the placebo group; however, it was significantly higher among those receiving 200 mg/day PREG compared to 30 mg/day PREG and the DHEA groups. This study demonstrates no effects of PREG administration on the other hormones measured in this trial, while treatment with DHEA significantly elevated blood levels of PREG (but to a lesser extent than PREG 200 mg/day), as well as DHEA, DHEAS, androstenedione, 3a-androstane-3a-17b-diol-glucoronide, testosterone, and estradiol compared to PREG-30, PREG-200 and placebo. No between-group differences in the levels of progesterone, 17-OH-progesterone, and cortisol were demonstrated. The patients receiving PREG do not have a risk for elevation of androgenic metabolites, like DHEA, which may in turn potentially predispose them to various problems such as prostatic hypertrophy in men and hirsutism in women. PREG's treatment effects cannot be explained by an impact of their neuroactive metabolites like DHEA, DHEAS, androstenedione, 3a-androstane-3a-17b-diol-glucoronide, testosterone, and estradiol. Considering the lack of any significant effect of PREG on the measured hormonal profile in this study, it may be suggested that PREGs therapeutic effects as noted are mediated by other mechanisms, including further potential hormonal influences not investigated in this study. In addition, direct neuromodulatory effects on the $GABA_A$, NMDA, sigma-1, dopaminergic, cholinergic or neurotrophic systems may mediate PREG's effect. Thus, although based on a relatively small sample size, this study suggests that low-dose PREG treatment for 8 weeks, used as an adjunct to antipsychotics, has a valuable ameliorating effect on positive symptoms, attention and memory impairments and antipsychotic-induced extrapyramidal side effects in chronic schizophrenia and schizoaffective patients. These initial results will clearly require replication in a larger cohort.

A second "proof-of-concept" randomized, placebo-controlled, double-blind trial was started in June 2005, and aimed at investigating adjunctive PREG for cognitive and negative symptoms in patients with

schizophrenia or schizoaffective disorder receiving stable doses of second-generation antipsychotics [81]. Following a 2-week single-blind placebo lead-in, patients were randomized to PREG (100 mg/day for 2 weeks, 300 mg/day for 2 weeks, and 500 mg/day for 4 weeks) or placebo, for 8 weeks. Of 21 patients randomized, 17 patients completed the entire 8-week study post-randomization; one patient randomized to the placebo group completed only 4 weeks of the study ($n = 9$/group). Authors reported that 8 patients receiving PREG demonstrated significantly greater improvements in SANS scores (mean change = 10.38) compared with 9 patients receiving placebo (mean change = 2.33, $p = 0.048$, *uncorrected for multiple comparisons*). However, application of the Bonferroni correction to the 8 psychiatric rating scales used in this study (PANSS, SANS, CGI-S, Calgary Scale for Depression in Schizophrenia [CSDS], SAS, BARS, AIMS, Quality of Life Scale for rating the schizophrenic deficit syndrome [QLS]) corresponds to setting the p value *a priori* at $p < 0.006$ ($p = 0.05/8$). Furthermore, eight patients randomized to PREG augmentation demonstrated improvement on "affect" subscale scores of SANS (mean change, 4.25 ± 1.68 SD) compared with nine patients receiving placebo (mean change 0.22 ± 1.04 SD; $p = 0.035$, *uncorrected for multiple comparisons*). Again, application of the Bonferroni correction to the 5 SANS subscales corresponds to setting the p value *a priori* at $p < 0.010$. In addition, changes in other four SANS subscales (alogia, avolition/apathy, anhedonia/asociality, and attention), PANSS (negative, positive, and general psychopathology subscales), CGI-S, CSDS, SAS, BARS, AIMS, QLS scores, and in mean composite changes in two neurocognitive measures were not significantly different in patients randomized to PREG compared with placebo. Thus, when Bonferroni correction was applied, improvement on any outcome measure did not reach a significant level (negative trial). Over the last few decades, statistical analysis has increasingly been used in medical studies. Nevertheless, the hypothesis test has often been misused and misinterpreted. In adition, the number of subjects required is calculated (power analysis) because without this calculation, reliable conclusions cannot be drawn from the p-values. For instance, a conclusion in this study (Pregnenolone may be a promising therapeutic agent for negative symptoms and merits further investigation for cognitive symptoms in schizophrenia [81]), based on a small sample and nonsignificant difference after Bonferroni correction between PGEG and placebo groups, can lead to an unsound clinical judgement.

Overall, the results of six clinical trials with two neurosteroids are based on 117 patients who received DHEA and 34 patients treated with PREG. The clinically significant benefits of both DHEA and PREG augmentations remain unclear. It is crucial to replicate these trials with larger samples of schizophrenia or schizoaffective patients, and for a longer duration of treatment. A gap between *animal studies* and *clinical trials* of neurosteroids may be explaned, at least partly, by important differences between rodents and primates in terms of their circulating DHEA and DHEAS concentrations, and suggests that age-related changes within the human DHEA metabolic pathway may contribute to the relative inefficacy of DHEA replacement therapies in humans [82].

Antipsychotic Agents

Since the most schizophrenia patients were medicated, it is still possible that the antipsychotic treatment could affect DHEA(S) circulatory levels in some schizophrenia patients. Animal studies have shown that olanzapine and clozapine administration are associated with an increase in the brain GABA-positive neurosteroid allopregnanolone and decreases in the GABA-negative modulators DHEA(S) levels, respectively [83].

It is possible that olanzapine- and fluoxetine-induced PREG elevations may contribute to the antidepressant actions of these agents [84].

Ritsner and associates repetedly examined the possible effect of the antipsychotic treatment on DHEA(S) cirulatory levels in schizophrenia patients [56,59,85–87]. For instance, patients who received first-generation (FGAs), second-generation (SGAs) and both types of antipsychotic agents had no significant differences in the cortisol to DHEA, and cortisol to DHEAS molar ratios. Futhermore, no significant correlations were found between these molar ratios with daily doses of antipsychotic agents [56]. Likewise, DHEA to PREG molar ratio, serum concentrations of PREG, and DHEA did not reach a significant level between schizophrenia patients who received FGAs, SGAs and both types of antipsychotis [85,86]. Patients who received FGAs and SGAs had comparable serum DHEA(S) and their metabilites' levels [87]. However, it should be noted that antipsychotic treatment and elevated prolactin might stimulate the adrenal cortex to secrete DHEA. Indeed, plasma levels of prolactin and DHEA were found elevated during sulpiride treatment in four out of five healthy men compared with those of the controls [88]. Thus, the possible contribution of antipsychotic treatment to alterations in DHEA metabolism in schizophrenic patients is unclear, and the alterations in both prolactin and neurosteroids may be related to independent effects of antipsychotic treatment. In addition, no significant main effect of the type of antipsychotics on the CGI-S, PANSS, GAF, ESRS, or BARS ratings was observed for patients receiving PREG, or DHEA [69,80].

Conclusions

Studies examining PREG and DHEA effects in the CNS and the role they play in the modulation of many neurotransmitter systems and brain protection are essential for understanding the pathology of the illness. These insights underscore the need for developing novel treatment strategies such as neuroprotective strategies using neurosteroids and other compounds, which should help overcome the limitations of current antipsychotic drugs and improve the cognitive deficits and negative symptoms, as well as functioning and quality of life outcomes of people affected by schizophrenia. There is limited investigation of PREG and DHEA in schizophrenia. Although there are some contradictory findings regarding blood levels of PREG and DHEA in schizophrenia patients, they may possess intrinsic antipsychotic properties. Based on the accumulated evidence from animal and human studies, it is also possible to conclude that PREG/DHEA play a significant role in the expressions of stress response, anxiety, and cognitive functioning. Indeed, beyond the use of antipsychotic agents, it will be of interest to examine whether PREG and DHEA that demonstrate neuroprotective and other properties in preclinical studies may prove to have ameliorative effects on psychopathological symptoms, neurocognitive and quality of life deficits, side effects and functioning in schizophrenia. Pilot clinical trials indicate that PREG and DHEA augmentation may improve some clinical symptoms and neurocognitive response in schizophrenia. Clinical trials for the evaluation of these neurosteroids pose a few challenges in terms of experimental design, dosing strategy, data analysis and interpretation, and merit further investigation in schizophrenia and related disorders.

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Conflict of Interest

The author has no conflict of interest pertaining to this manuscript.

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