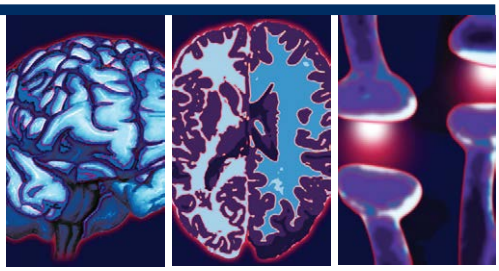
Review



Neuroactive Steroids and Related Steroids in Autism Spectrum Disorders

Hui-Fei Zheng[1,2], Wen-Qiang Wang[1†], Xin-Min Li[2], Gail Rauw[2], Jean-Michel Le Mellédo[2], Glen B Baker[2†]

Abstract

Neuroactive steroids (rapidly acting steroids that act as allosteric modulators of neurotransmitter receptors such as the γ-aminobutyric acid$_A$ ($GABA_A$) receptor and the NMDA glutamate receptor) are involved in brain development and have been proposed to be important in the etiology and/or pharmacotherapy of a number of psychiatric and neurologic disorders, but there is limited information available on their association with autism spectrum disorders (ASD). This paper reviews the possible involvement of neuroactive steroids (NASs) and some related steroids, including testosterone, androstenedione and estradiol steroids in the etiology of ASD. NASs include progesterone, pregnanolone, pregnenolone, pregnenolone sulfate, allopregnanolone, tetrahydrodeoxycorticosterone (THDOC), dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Studies on the levels of NASs and related steroids in blood, urine, saliva, and amniotic fluid in ASD are also reviewed. The results on plasma levels of NASs in ASD reported in the literature are generally inconclusive, probably because of differences among studies in terms of experimental design and factors such as age, gender, number of subjects, and medication being taken and differences in time of day at which samples are taken and storage conditions of samples. Future studies on levels of NASs in ASD should include careful consideration of the factors mentioned above, the possible advantages of saliva sampling and the use of assay procedures that provide simultaneous analysis of levels of a large number of these steroids.

Keywords:

Neuroactive steroids, Autism spectrum disorders, Neurosteroids, Testosterone, DHEA, Androstenedione, Estradiol, Pregnane steroids

Introduction

Autism spectrum disorders (ASD) are characterized by impaired social communication skills combined with restrictive/repetitive behaviors [1]. ASD occur in all ethnic groups, and there are different levels of severity. Previous studies have estimated that the prevalence of ASD is about 1%, and it is on the rise in the worldwide [2-4].

Some investigators view ASD traits as manifestations of an "extreme male brain (EMB)" [5]. The theory of EMB is the extension of the theory of empathizing-systemizing (E-S) which is a typical psychological sex difference. Evidence suggests that females have a greater drive to empathize and males have a stronger drive to systemize [5], and consistent with the theory of EMB, ASD subjects demonstrate a stronger

[1]Mental Health Research Laboratory, Xiamen Xianyue Hospital, Xiamen, Fujian Province, China

[2]Neurochemical Research Unit, Department of Psychiatry and Neuroscience & Mental Health Institute, University of Alberta, Edmonton, Canada

[†]Author for correspondence: Glen B. Baker, Neurochemical Research Unit, Department of Psychiatry and Neuroscience & Mental Health Institute, University of Alberta, Edmonton, 12-105B Clinical Sciences Building, AB T6G 2G3, Canada, Tel: +1 780 492 5994, email: glen.baker@ualberta.ca

Wen-Qiang Wang, Mental Health Research Laboratory, Xiamen Xianyue Hosptial, Xiamen, Fujan Province, 361000, China, Tel: +86 0592 5392688, email: wwq5392616@sina.com

drive to systemize but are impaired on tests of empathizing [6,7]. Schwarz et al. [8} indicated that their finding of elevated serum levels of testosterone (T) should just be T in Asperger's Syndrome adult females compared to controls supported the EMB theory. Sarachana et al. [9] showed that male and female hormones are different in regulating the expression of a novel autism candidate gene which is retinoic acid-related orphan receptor-alpha (RORA) which is a nuclear hormone receptor and a transcriptional regulator. These authors and their colleagues have found a reduction of RORA transcript and/or protein levels in lymphoblasts and postmortem profrontal cortex and cerebellum taken from ASD subjects, and that testosterone (T) and estradiol exert positive and negative feedback regulation of the RORA gene respectively [10], suggesting that RORA may influence the male bias of ASD. Further, an isoform of RORA protein in human brain, namely RORA1, appears to regulate a number of ASD-associated genes involved in neuronal function, synaptogenesis, plasticity, cognition and spatial learning.

Steroid hormones are generally synthesized from cholesterol in the gonads and adrenal glands [11] and the main metabolic enzyme is P450 side-chain cleavage enzyme (P450scc). These hormones can then pass through the blood-brain barrier to reach the brain [12], an organ in which they have important roles in the development, growth, maturity and differentiation. There has been a great deal of interest in recent years in neuroactive steroids (NASs), which have the capability of rapidly modifying neural activities [13]. NASs produce rapid, nongenomic effects in the brain, primarily through actions on ligand- or voltage-gated channels. They thus differ from classic steroid hormones which act mainly on intracellular receptors, producing long-lasting genomic effects. The NASs include dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), progesterone, tetrahydrodeoxycorticosterone (THDOC), pregnanalone, allopregnanolone, pregnenolone and pregnenolone sulfate [14-16]. Most of the research focus on NASs has been on their interaction with γ-aminobutyric acid$_A$ ($GABA_A$) receptors and N-methyl-D-aspartate (NMDA) glutamate receptors. A delicate balance between the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter GABA (E/I balance) exists in normal brain function. This balance is often distributed in several neuropsychiatric disorders and considerable recent research suggests that this is the case with ASD. Although some controvery remains, excessive glutamatergic activity and reduced GABAergic activity have been proposed in ASD [17-19]. Allopregnanolone, THDOC, pregnanolone, androstanediol and progesterone are positive modulators at the $GABA_A$ receptor, DHEA and pregnenolone are positive modulators at the NMDA receptor and DHEAS and pregnenolone sulfate are negative modulators at the $GABA_A$ receptor and positive modulators at the NMDA receptor [16,20]. Thus future comprehensive studies on NASs in ASD are warranted.

The term neurosteroids is used for NASs that are synthesized from cholesterol *de novo* in the brain [15] or formed by metabolism in the brain from precursor compounds coming from endocrine sources [11,16]. Numerous studies have shown that NASs have neurotrophic and neuroprotective properties and play a role in the development of the nervous system but also in some neurodevelopmental pathological processes [15,21,22]. Neuroactive steroids appear to play a role in the etiology and/or pharmacotherapy of several psychiatric and neurological disorders, such as depression [23-27], Alzheimer's disease [21,28-30], schizophrenia [31-36], anxiety disorders [35-38], bipolar disorder [39-42] and post-traumatic stress disorder (PTSD) [43-44].

Several studies have proposed abnormal metabolism and/or levels of steroids in ASD, particularly in adolescents with ASD. While the causes of these anomalies may be manifold, steroids which participate in the development of the brain and control many brain functions are likely to play a role in the pathogenesis and clinical/behavioral manifestations of ASD [45]. This paper is a review of the possible involvement of NASs and related steroids in ASD and also includes a review of studies that have been done on levels of NASs and related steroids in blood, urine, saliva, and amniotic fluid in ASD. PubMed was scanned using the headings Steroids in Autism and Neuroactive Steroids in Autism. The references listed were reviewed in detail and narrowed down to those related directly to the NASs indicated here in the text as well as some other steroids that are related to NASs metabolically and/or functionally.

Neuroactive Steroids and Related Steroids in ASD

■ Testosterone

Testosterone (T), a metabolite of DHEA, plays an important role in the growth and differentiation

of genital and extragenital organs. It induces masculine features, affects the early development of the central nervous system (CNS) and behavior, and induces secondary sex characteristics and sexual development [46-47]. The levels of T change with age, different development stages, medication, diseases and stress [48]. Lutchmaya et al. [49] proposed that the ratio of 2nd to 4th digit length (2D:4D) and the fetal testosterone (FT)/fetal estradiol (FE) ratio have a significant negative relationship which is independent of sex. Baron-Cohen et al. [50] followed 58 children from fetus to 4 years old and found that the FT levels in amniotic fluid are negatively related to the quality of social relationships when considering the sex differences; FT was found to be positively related to boys' restricted interests. Auyeung et al. [51] showed a significant positive association between FT levels and autistic traits in 18 to 24-month-old children. All of these studies provided evidence suggesting that T plays a role in the pathophysiology of ASD. Moreover, some studies suggested that as a male sex hormone, T can play a role in the complex etiology of aggressive behavior in prepubertal and postpubertal ASD [52,53].

Geier and Geier [54] reported that compared with the typical reported reference ranges, serum levels of T and DHEA in prepubertal ASD are significantly increased. These researchers studied a group of 70 ASD subjects (mean age=10.8 years) and found that serum levels of total T, free T, % free T and DHEA were significantly increased compared to age- and sex-matched typical laboratory reference ranges. In a large sample of males, Schmidtova et al. [55] showed that the saliva levels of T in prepubertal and pubertal ASD are significantly increased over those in healthy controls (HCs). Schwarz et al. [8] reported that the serum levels of T in ASD females are significantly increased compared to control females. Xu et al. [56] showed that compared to mothers of typical children, plasma levels of T were increased in mothers of ASD children, and suggested that there is dysregulation of T systems in mothers of ASD children which may influence the children's susceptibility to ASD. However, Croonenberghs et al. [48] found significantly decreased serum T in high-functioning ASD males compared with HCs. Other studies reported that plasma levels of T showed no difference from HCs in prepubertal and postpubertal ASD [57], and that serum levels of total and free T, DHEA-S, and estradiol showed no differences between ASD adults and HCs [58].

■ DHEA and DHEAS

DHEA is a major secretory product of the human adrenal glands and is a precursor for both androgenic and estrogenic steroids [59,60]. DHEAS, formed from DHEA by the enzyme hydroxysteroid sulfotransferase, is the normally favored storage form of DHEA [61,62] and is the most abundant steroid found in the human body [59,63,64]. DHEA and DHEAS are reported to have prominent influences on $GABA_A$ receptors [65] and have modulatory influences on neuronal excitability and synaptic plasticity [34]. Kroboth et al. [64] reviewed the effects of disease, diet, exercise and drugs on levels of DHEA and DHEAS in human plasma and indicated that the plasma levels are age- and gender-dependent. The levels decrease from the first few months of life until 5-7 years of age, then increase with age and reach peaks between the ages of 20 to 40 years, and decrease with age again after the peaks [66-68].

Studies have shown that levels of DHEA and DHEAS respond to stress and are associated with neuroprotection, mood regulation, cognitive performance and various psychiatric disorders [64,69]. There are inconsistencies in the reported levels of DHEA and DHEAS in ASD compared to HCs. Tordjman et al. [53] showed that, compared with HCs, levels of DHEA-S in plasma are increased in prepubertal ASD but found no difference in postpubertal ASD. Croonenberghs et al. [48,70] reported that a disequilibrium in peripheral serotoninergic metabolism may influence DHEAS levels and that L-5-hydroxytryptophan (5-HTP) [the precursor of 5-HT (serotonin)] induced DHEAS responses such that the DHEAS levels and the cortisol/DHEAS ratios were significantly higher in ASD subjects than in HCs. However, Ruta et al. [58] reported that the serum DHEAS levels in adult ASD are no different than those in HCs, and Strous et al. [71] reported that the plasma DHEAS levels in adult ASD are significantly decreased compared to HCs and that there was no difference in plasma levels of DHEA compared to HCs. Majewska et al. [72] compared ASD subjects with HCs and found that levels of DHEA were elevated in a 7-9 year old group but found no difference in a 3-4 year old group when measuring levels in saliva samples.

■ Androstenedione

Androstenedione, which is synthesized in both gonads and adrenal cortex, is released into the peripheral blood circulation and is then converted

into T, DHEA or estrogens via intracrine mechanisms in brain, skin, the pilosebaceous unit, and adipose tissue [73,74] As a result, these tissues contribute to the final pool of active androgens in peripheral target tissues and may also contribute to androgen-related conditions including polycystic ovary syndrome (PCOS), hirsutism, and acne [58]. Ingudomnukul et al. [75] reported that a number of androgen-related medical conditions (such as PCOS, hirsutism, acne, breast and ovarian cancers) and androgen-related characteristics (such as tomboyism, bisexualism and asexualism) are more common in adult ASD women and their mothers than in HCs, and proposed that genetics might be one of the factors involved in higher levels of androgen synthesis and/or increased local tissue sensitivity to circulating androgens in ASD.

Geier and Geier [76], in a study of ASD subjects with a mean age of 5.9 years, reported that serum androstenedione levels are increased in ASD subjects compared to HCs. Ruta et al. [58] found that serum levels of androstenedione are higher in ASD adults than HCs, with no sex difference, which is of interest since there are differences between males and females in the androstenedione metabolic pathway in normal individuals. This is further evidence of dysregulation and is referred to as the absence of the typical sexual dimorphism. Majewska et al. [72] found that saliva androstenedione levels in ASD subjects are no different in a 7-9 year old group or a 3-4 year old group and HCs. Tordjman et al.[57] did not observe a difference in plasma androstenedione levels between ASD children and HCs; but the same group reported high plasma T concentrations and hyperandrogenism in ASD children with aggressive behaviors [53].

■ Estradiol

Estradiol is one of the most active estrogens in humans; it is synthesized in the follicle and is formed from T [77-79]. Estrogens produced in the CNS are involved in regulating processes as diverse as memory, pain processing, neural plasticity regeneration, and tumor growth [80,81]. There is a paucity of studies on levels of estradiol in ASD. Ruta et al. [58] showed no difference in serum estradiol levels between ASD adults and HCs, and Xu et al. [56] found no difference in plasma estradiol levels between mothers of ASD children and HCs.

Bakker and Baum [82] suggested that estradiol may control prenatal brain and behavioral sexual differentiation by exerting defeminizing actions in male brains. In a comprehensive study on candidate genes associated with autism, Chakrabarti et al. [83] showed that the ESR2 gene, which codes for one of the two main estrogen receptors and is expressed in the brain, showed a nominally significant association with the Autism Spectrum Quotient (AQ) and the Empathy Quotient (EQ). de Bournonville et al. [84] showed that pathways of estrogens and androgens are coupled together, since androstenedione and T are precursors of androgens which can be converted into estrogens (estrone and estradiol respectively) in target tissues by the cytochrome P450 aromatase enzyme. Estradiol has been reported to closely associate with aggressive behaviors which are common in ASD subjects [85-87]. Recently, Hoffman et al. [88] showed estrogenic compounds as phenotypic suppressors in zebrafish mutants of CNTNAP2, an autism risk gene. These interesting findings suggest that the role of estrogens in the pathogenesis of ASD, and the levels of estradiol in ASD warrant further investigation.

■ Pregnane steroids

Pregnenolone is a direct metabolite of cholesterol and is very important because it is the precursor of several other NASs. Pregnenolone sulfate has been reported to stimulate the trafficking of NMDA glutamate receptors to the neuronal surface [90,91]. Allopregnanolone, a metabolite of pregnenolone (via progesterone), is produced *de novo* in both neurons and glia [92] and is also synthesized in ovaries and adrenal glands [12]. Allopregnanolone is a very strong positive allosteric modulator of the $GABA_A$ receptor [93] and has been reported to regulate that receptor through two discrete transmembrane sites [94].

Although allopregnanolone and pregnenolone have been studied extensively in psychiatric disorders such as major depressive disorder and schizophrenia [11,13,16,26,31,69,95,96], there is a paucity of studies on levels of these neuroactive steroids in ASD. Majewska et al. [72] showed that compared with HCs, salivary levels of pregnenolone and allopregnanolone were elevated in a 7-9 year old group, but they found no difference between a 3-4 year old group and HCs. However, an increasing number of investigators have become interested in the relationship between pregnenolone and ASD. Sripada et al. [97] gave oral pregnenolone to adult male HCs (mean age=22 ± 3.38) and showed enhancement of the activation of emotion

regulation and relief of anxiety symptoms (which occur frequently in ASD). Fung et al.[89] did a clinical trial using oral pregnenolone for adult ASD subjects (mean age=22.5 ± 5.8) and showed improvement of irritability symptoms and suggested using oral pregnenolone in ASD to improve social functioning and to reduce sensory abnormalities. Kazdoba et al. [98] showed that ganaxolone, a synthetic analog of allopregnanolone, can improve social and repetitive behaviors in an ASD mouse model. Braat and Kooy [99] suggested that ganaxolone is a potential candidate for treatment of fragile X syndrome which is a frequently inherited cause of syndromic autism [100]. Ligsay et al. [101] conducted a randomized, double-blind placebo-controlled trial of ganaxolone in fragile X syndrome and reported no significant improvement in the overall population investigated, but suggested that this drug might be beneficial in treatment of fragile X syndrome children displaying high anxiety or reduced cognitive abilities.

There are some studies on progesterone and ASD. Frye and Llaneza [102] showed that plasma corticosterone, progesterone and progesterone's metabolite, 5α-pregnan-3α-ol-20-one (3α,5α-THP), were significantly higher in ASD mouse models than in controls. Mamidala et al. [103] reported that maternal progesterone intervention was a significant risk factor for ASD. Whitaker-Azmitia et al. [104] suggested that low maternal progesterone may be responsible for both obstetrical complications and brain changes associated with ASD and proposed that progesterone levels should be routinely monitored in at-risk pregnancies. Baron-Cohen et al. [105] reported that amniotic fluid sample levels of progesterone, 17α-hydroxy-progesterone, androstenedione, T and cortisol are increased in ASD subjects, and these results provided the first direct evidence of elevated fetal steroidogenic activity in ASD. Deng et al. [106] found reduced serum progesterone levels in children with ASD [107]. The Cytochrome P-450scc gene (CYP11A1) and the Cytochrome P-45011beta gene (CYP11B1) are candidate genes in ASD that encode proteins that participate in the metabolism of progesterone. Increased levels of 11-deoxycorticosterone (DOC) (formed by CYP11B1-mediated conversion of deoxycortisol) result in increased formation of the $GABA_A$ receptor positive modulator neurosteroid THDOC [108].

Discussion

Some investigators in the studies mentioned in this review focused on the changes in the adolescence stage. Adolescence is a period of many physiological, psychological, and social changes in both normal and ASD individuals, and these are contributing factors to significant changes related to morphology, cognition, emotion regulation, and response to physiological stress [109-111]. Adolescence is also a period of increasing awareness and interest in both peer and romantic relationships [112] and rising prevalence of psychiatric disorders which are associated with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and an imbalance of steroids [111]. Because youngsters in adolescence begin to focus on peer relationships and developing romantic relationships, a number of serious challenges may appear, and the impairments in social skills inherent with ASD subjects become increasingly more apparent [110,113].

Studies have shown that amplified steroid release in prepubertal ASD subjects indicated the precociousness of adrenarche [114] because the amounts of synthesized androgens correlate with the size of adrenals [115]. Meanwhile, because of early maturation, the hyperplasia or hypertrophy of the adrenal reticular zone contributes to augmented steroid secretion in ASD. So, excessive steroid secretion can point to precocious adrenarche in prepubertal ASD subjects and may be a predictior of early maturation. Moreover, an excess of excitatory steroids (such as DHEA and pregnenolone) may amplify neuro-stimulant influences such as deficits in GABA neurotransmission [116] and enhance the activity of glutamate [117] in subjects with ASD, contributing to increased ASD comorbidities (anxiety, sleep disturbances and seizures) [118,119]. Due to the potential role of neuroactive steroids and related steroids in the development of ASD subjects, monitoring changes in their concentration in the adolescence stage or even earlier is important in case they may be helpful for predicting changes in mood and behavior and allow for earlier intervention in subjects with ASD.

Overall, the results on levels of NASs and related steroids in body fluids of ASD subjects reported in the literature are inconclusive. Differences in experimental design among studies and factors such as age, gender, number of subjects, time of day at which samples are taken, storage

conditions of samples and medication being taken at the time of the studies may account for varying results. Future studies should take into account these factors and analyzing levels of several steroids simultaneously. The levels of NASs may change with anxiety and stress, which may be related to strange environments and processes in collection of samples. Saliva samples are relatively convenient for caregivers to collect at home, even from infants and toddlers [120]. There should be reduced emotional changes compared to collecting blood samples and thus possibly increased accuracy of results [121]. Saliva samples have proved appropriate in the past for determining levels of several neuroactive and related steroids [72,122-126].

Because of convenient, noninvasive, relatively stress-free collection and applicability to dynamic monitoring, saliva samples are increasing in favour as a specimen choice for detecting levels of steroids [127]. Although the levels of steroids in saliva samples are several-fold lower than those in serum, they generally reflect their levels in the blood [128,129]. The collecting and analysis of saliva samples are suitable for rapid and simple application in clinical studies. In addition, it has been shown that saliva samples can be analyzed for amino acids accurately in real time [130-132]; there is considerable evidence for a GABA-glutamate imbalance in ASD [17-19,133,134] and several neuroactive steroids act as strong modulators of receptors for GABA and glutamate [91-93,135,136]. Thus, it may be useful to employ saliva samples of ASD subjects routinely to determine levels of neuroactive steroids and amino acids in clinical practice.

Conclusion

Since so many of the NASs have effects on brain development and modulatory actions on neurotransmitters such as GABA and glutamate, future studies on body fluid levels of these steroids should ideally use assay procedures that provide simultaneous analysis of as many of these NASs and related steroids as possible as well as of amino acids related metabolically and/or functionally to GABA and glutamate. Consistency among research groups with regard to study design and factors such as time of collection of samples and appropriate storage of samples would do much to advance our knowledge of possible biochemical factors involved in the detection etiology of ASD. Establishment of standardized international criteria for such studies is warranted.

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