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Corticosteroid and Neurosteroid Dysregulation in an Animal Model of Autism, BTBR Mice

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Abstract

Autism spectrum disorders (ASD) are a constellation of neurodevelopmental disorders associated with disruptions in social, cognitive, and/or motor behaviors. ASD are more prevalent among males than females and characterized by aberrant social and language development, and a dysregulation in stress responding. Levels of progesterone (P₄) and its metabolite 3 α ,5 α -THP are higher and more variable in females compared to males. 3 α ,5 α -THP is also a neurosteroid, which can be rapidly produced *de novo* in the brain, independent of peripheral gland secretion, and can exert homeostatic effects to modulate stress responding. An inbred mouse strain that has demonstrated an ASD-like behavioral and neuroendocrine phenotype is BTBR T⁺/J (BTBR). BTBR mice have deficits in cognitive and social behavior and have high circulating levels of the stress hormone, corticosterone. We hypothesized that central 3 α ,5 α -THP levels would be different among BTBR mice compared to mice on a similar background C57BL/6J (C57/J) and 129S1/SvImJ (129S1). Tissues were collected from BTBR, C57/J and 129S1 male mice and levels of corticosterone, P₄, and 3 α ,5 α -THP in plasma and hypothalamus, midbrain, hippocampus, and cerebellum were measured by radioimmunoassay. Circulating levels of corticosterone, P₄, and 3 α ,5 α -THP were significantly higher among BTBR, than C57/J and 129S1, mice. Levels of P₄ in the cerebellum were significantly higher than other brain regions among all mouse strains. Levels of 3 α ,5 α -THP in the hypothalamus of BTBR mice were significantly higher compared to C57/J and 129S1 mice. These findings suggest that neuroendocrine dysregulation among BTBR mice extends to 3 α ,5 α -THP.

Keywords

affect; learning; memory; stress; neurosteroid; autism spectrum disorder; allopregnanolone; corticosterone

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1. Introduction

Autism Spectrum Disorder (ASD) affects approximately 1 in 150 children. It is characterized by aberrant social (social interaction, communication), cognitive (perseveration), and/or motor (repetitive stereotyped behaviors) behavior [1] and dysregulation in stress-responding [2–3]. Along with difficulty tolerating novelty and environmental stressors [4], those with ASD often suffer from co-morbid neurological disorders, such as seizures [5]. Thus, ASD is a pervasive developmental disorder that is associated with dysregulation in several behaviors.

There are gender differences in the incidence and/or symptomology of ASD, such that males are four times more likely to be diagnosed compared to females. As well, boys with ASD performed better on eye-hand integration and perception skills, and have higher nonverbal IQ social quotients compared to girls. When nonverbal IQ was controlled for, gender differences on integration and perception disappeared. However, boys demonstrated more unusual visual responses and more stereotypic play compared to girls [6]. Given the prominence of gender differences in ASD incidence and/or symptomology, hormones or other neuroendocrine factors may be involved in ASD and need to be further elucidated.

Hypothalamic pituitary adrenal axis (HPA) stress-responding is modified among people with ASD [7], such that circulating levels of the steroid, cortisol, are elevated [2,8]. However, behavior may be influenced by external or internal factors, such as time of day or hormonal state [9], and some studies have not reported HPA differences [10–13]. Thus, there may be neuroendocrine differences in ASD that are related to the expression and/or development of ASD.

One neuroendocrine factor that may be involved in ASD is a progesterone (P_4) metabolite, 5α -pregnan- 3α -ol-20-one ($3\alpha,5\alpha$ -THP). Sources of $3\alpha,5\alpha$ -THP are the ovaries, adrenals, and *de novo* formation in the brain. $3\alpha,5\alpha$ -THP levels vary across the life span, such that females experience variable and higher levels, while males have lower and more stable levels [14]. $3\alpha,5\alpha$ -THP can feedback on HPA responding to reinstate parasympathetic tone [15–19]. Furthermore, female rodents demonstrate enhanced social, cognitive and affective behaviors when $3\alpha,5\alpha$ -THP levels are elevated [20–21]. Among male rodents, disruption in $3\alpha,5\alpha$ -THP formation can enhance aggression and decrease cognitive function [22]. As well, $3\alpha,5\alpha$ -THP administration or enhancing its formation provide neuroprotection [23–24]. Given sex differences in $3\alpha,5\alpha$ -THP and its potential effects on behaviors associated with ASD, we began to examine the role of $3\alpha,5\alpha$ -THP in an animal model of ASD. Our foray into this was restricted to male mice, to control for inherent sex differences in progestogens.

An inbred mouse strain that has demonstrated reliable decrements in social behavior and learning is BTBR T +tf/J (BTBR) mice compared to other mouse strains, including C57BL/6J (C57/J), DBA/2J, FVB/NJ, and BALB/cByJ [25–27]. BTBR mice also display repetitive behaviors, do not prefer novel appetitive reward scents, demonstrate altered fear and object memory, and show heightened stress-responding and corticosteroid levels [28–31]. The BTBR phenotype is neither due to differences in basal levels of anxiety, nor motor capability [26], and is not altered by cross-fostering to C57/J mice [29], which have demonstrated normative performance in roto-rod learning and react less in the contextual and cued fear tasks [32]. In the following experiment, we investigated whether corticosterone and $3\alpha,5\alpha$ -THP levels, were different among BTBR, and C57/J and 129S1/SvImJ (129S1) mice. We hypothesized that if $3\alpha,5\alpha$ -THP has a role in ASD-like phenotype, that BTBR mice would be expected to have atypical corticosterone and/or $3\alpha,5\alpha$ -THP levels, compared to C57/J and 129S1 mice.

2. Materials and Methods

Methods were pre-approved by the IACUC at the University at Albany-SUNY and carried out using adequate measures to minimize pain or discomfort, as outlined in NIH Guide for the Care and Use of Laboratory Animals (#80-23, 1996).

2.1. Animals and Housing

Adult, age-matched (50–55 days of age) male mice from the BTBR (n=7), C57/J (n=6) and 129S1 (n=6) mouse strains were generated at the University at Albany-SUNY from stocks originally acquired from The Jackson Laboratory. All mice were group-housed, had *ad libitum* access to rodent chow and tap water in their home cages, and handled daily for health checks until removed from the housing room for tissue collection.

2.2. Tissue Collection and Measurement

Mice were removed directly from their respective home cages one at a time in a randomized order just before being sacrificed. Mice were sacrificed one strain at a time. The area used for sacrifice was cleaned between subjects and strains. Trunk blood was obtained following cervical dislocation and rapid decapitation. Blood was kept on ice and coagulated until centrifugation at $3,000 \times g$. Plasma was stored at -20°C . Whole brains were extracted, flash frozen on dry ice, within 2 min, and stored at -80°C until radioimmunoassay.

2.3. Radioimmunoassay for steroid hormones

Immediately prior to radioimmunoassay for P_4 , $3\alpha,5\alpha\text{-THP}$, and corticosterone, conducted per previous methods [33–36], the hypothalamus, midbrain, hippocampus, and cerebellum were dissected out.

2.4. Statistical Analyses

One-way analyses of variance (ANOVAs) were used to examine if there were differences between C57/J, 129S1 and BTBR mouse strains in plasma hormone levels. Two way ANOVAs with strain as a between and brain region a within factor were used to examine central and P_4 and $3\alpha,5\alpha\text{-THP}$. Repeated measures ANOVA was used to determine interactions between brain region and strain on P_4 and $3\alpha,5\alpha\text{-THP}$ levels. The alpha level for statistical significance was $p < 0.05$.

3. Results

3.1. BTBR, vs C57/J and 129S1, mice have higher corticosterone, P_4 , & $3\alpha,5\alpha\text{-THP}$ plasma levels

There were significant effects of strain on plasma levels of corticosterone [$F(2,16)=8.92$, $p < 0.05$], P_4 [$F(2,16)=15.47$, $p < 0.05$] and $3\alpha,5\alpha\text{-THP}$ [$F(2,16)=13.71$, $p < 0.05$]. As Figure 1 shows, BTBR mice had significantly higher levels of corticosterone P_4 , and $3\alpha,5\alpha\text{-THP}$, than did C57/J and 129S1 mice.

3.2. BTBR mice may have higher $3\alpha,5\alpha\text{-THP}$ levels in hypothalamus compared to C57/J and 129S1 mice

There were significant effects of brain region [$F(3,48)=102.29$, $p < 0.05$], but not strain on P_4 levels. Progesterone levels were greater in the cerebellum than any other brain region.

There were significant effects of brain region [$F(3,48)=23.58$, $p < 0.05$], and an interaction between brain region and strain [$F(6,48)=3.92$, $p < 0.05$] on $3\alpha,5\alpha\text{-THP}$ levels. $3\alpha,5\alpha\text{-THP}$

levels were significantly higher in the hypothalamus of BTBR compared to C57/J and 129S1 mice.

4. Discussion

These findings support our hypothesis that stress hormone and $3\alpha,5\alpha$ -THP levels are altered among BTBR mice, an animal model of ASD. Among C57/J and 129S1 mice, corticosterone, P_4 and $3\alpha,5\alpha$ -THP levels in plasma were basal, whereas BTBR mice demonstrate a marked elevation of these steroids, comparatively. In the brain, P_4 levels were higher in the cerebellum of C57/J, 129S1 and BTBR mice. However, among BTBR mice $3\alpha,5\alpha$ -THP levels are higher in hypothalamus, compared to C57/J and 129S1 mice. These data support the notion that $3\alpha,5\alpha$ -THP synthesis may be deficient among BTBR mice, which may underlie aspects of the ASD phenotype associated with this mouse strain.

The present findings confirm previous results that show BTBR mice have elevated glucocorticoid levels. Indeed, BTBR mice have demonstrated higher baseline corticosteroid levels compared to C57/J mice, as well as enhanced anxiety responding in the elevated plus maze following tail suspension [31]. These findings confirm our results showing BTBR mice have elevated corticosterone levels compared to other mouse strains. However, possibly owing to the nature of stressors, previously reported levels were higher than ours, perhaps in part due to animals being directly taken out of their home cages without prior handling, or perhaps differences associated with stress of termination. These findings are congruent with clinical investigations that report perturbed HPA [2,3,37–38] or exacerbated cortisol [39] responses among people with ASD. Thus, BTBR mice show altered stress-responding which may be related to their expression of an ASD-like behavioral phenotype.

In addition to stress-responding, BTBR mice have demonstrated altered cerebellar, cognitive and affective behaviors. In particular, BTBR mice display repetitive behaviors, altered fear and object memory, have a low preference for social novelty, and have altered affective behavior following an acute stressor [26,28–29,30–31]. Indeed, $3\alpha,5\alpha$ -THP can mediate each of these processes, such that when $3\alpha,5\alpha$ -THP levels are elevated, female rodents demonstrate increased sociability, cognitive performance and/or affective behaviors, as well as improved stress-responding, compared to when levels are lower [40]. We have expanded upon the BTBR phenotype by demonstrating BTBR mice have altered $3\alpha,5\alpha$ -THP levels, in addition to altered corticosterone levels, compared to other mouse strains. The behavioral tasks BTBR mice have been examined in, as well as altered stress-responding and neuroendocrine factors that have been demonstrated, provide face validity for the BTBR mouse strain as an animal model of ASD and for neuroendocrine factors to potentially play a role in ASD expression.

The potential involvement of neurosteroids in ASD has been investigated heretofore but largely with other neurosteroids. Dehydroepiandrosterone sulfate (DHEAS), produced by the adrenals, has been examined in children and adolescents with ASD, but no differences were found among males compared to controls [41]. However, DHEA and DHEA-S levels were investigated in male adults with ASD, demonstrating those with ASD have lower DHEA-S levels compared to controls [42]. Low levels of DHEA-S may impact N-methyl-D-aspartate (NMDA) receptors [43–44], and given the role of NMDARs in the behavioral phenotype and treatment of psychiatric disorders [45,46], neurosteroids, such as DHEA-S, may impact ASD. Furthermore, the most commonly prescribed psychotropic treatment for ASD is selective serotonin reuptake inhibitors (SSRIs) [47–49], although antipsychotics and antiepileptic drugs are also prescribed [50,51]. A common feature of some SSRIs, antipsychotics, and AEDs is their effects to enhance $3\alpha,5\alpha$ -THP formation [52–56]. Thus, the role of neuroendocrine factors, such as $3\alpha,5\alpha$ -THP, in ASD phenotypes need to be

further elucidated to determine their role in gender differences in incidence and/or expression of ASD.

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Figure 1.

Compared to C57/J and 129S1 mice, BTBR mice had significantly higher plasma corticosterone, progesterone, and 3α,5α-THP levels. * indicates $p < 0.05$.

Figure 2.

C57/J, 129S1 and BTBR mice have higher progesterone levels in cerebellum compared to other brain regions. BTBR mice may have higher 3α,5α-THP levels in hypothalamus than do C57/J and 129S1 mice, resulting in an interaction between brain region and strain in 3α,5α-THP levels, but not progesterone levels. * indicates $p < 0.05$.