Androgens Contribute to the Process of Neuronal Development: Implications in Explanation of Autism Pathogenesis

ORIGINAL ARTICLE

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Abstract

Fetal testosterone significantly influences the brain development. It affects number of neurons and conformation of dendritic spines within the sexual dimorphic preoptic area in the hypothalamus. Excessive testosterone levels in utero possibly contribute to the masculinization of the brain. Evidences of these facts are plausible in the anatomic field as well as behavioral effects both in rat models and in humans. Rats exposed to excessive testosterone doses in utero show masculinized brain anatomy and behavior, such as better spatial visualization performance typical for males. In humans, congenital adrenal hyperplasia that causes elevated androgen level possibly results in masculinized behavior observed in these individuals. There are reasons for the theory of the connection existence between testosterone influence on the brain functions and the pathogenesis of neurodevelopmental disorders. In this review, pathogenesis of autism, the most genetic neurodevelopmental disease is discussed. Autism is a disease with broad genetic heterogeneity and polygenic inheritance. Autism associated genes are localized throughout the genome, with the chromosome 7q most frequently involved. One of these genes encodes reelin protein that is crucial for neuronal migration in the developing brain. The connection between androgens, neuronal migration and neurodevelopmental disorder pathophysiology is also discussed.

INTRODUCTION

It is generally known that endocrine and neuronal regulations are interconnected in the area of hypothalamus and pituitary gland. Axons of hypothalamic neurons producing hormones such as anti-diuretic hormone (ADH) and oxytocin (OCT) release these hormones via exocytosis in the posterior pituitary to the circulation. Liberins and statins produced in hypothalamic neurons transport these hormones to the eminentia mediana, and to the anterioir pituitary via local vascular net. In anterior pituitary statins and liberins positively or negatively regulate the secretion of the trophic hormones that affect the regulation of other endocrine glands. Another type of hormones, produced in the brain and performing its function at the same place, are the neurotransmitters such as histamine, serotonin or melatonin that work as mediators of the action potentials among the neurons. These hormones are produced in special part of the nervous system and regulate the functions within the whole organism. On the other hand, there are hormones produced in extraneuronal areas that affect the functions and the anatomy of the central nervous system. Such hormones are steroid hormones, mainly produced in gonads or adrenal gland. Sex hormones are transported by the circulation to the target tissues where they directly or indirectly via their metabolites regulate transcription of the target genes. Here we review the effect of the sex steroids on the central nervous system functions, brain development and the origin of the neurodevelopmental disorders such as autism.

Testosterone effect of the brain

Testosterone as a sex steroid hormone plays a crucial role in neurodevelopment. It affects special brain regions and provides sexual differentiation also at the level of central nervous system. Testosterone is present postnataly in cortical regions important for cognition. Mechanism of its action is well understood. Testosterone, after its synthesis from cholesterol in testes or adrenal gland, can pass through the cell membrane into all cells. But only cells containing androgen receptor can perform its biological effect. Testosterone binding to its receptor results in receptor complex formation that serves in the nucleus as a transcription factor (Moilanen et al., 1998). It controls the expression of target genes involved in processes such as neurotransmitter production and release, synapse conformation changes, controlling of the neuronal apoptosis and altering neurochemical profiles (for review see Alonso – Solis et al., 1996). Thus the presence of androgen receptors in the cytoplasm of the cells decides whether the testosterone presence will be transformed into the biological effect. Androgen receptors are present in the brain regions crucial for memory and learning such as hippocampus, amygdala and prefrontal cortex, but are not present in other cortical regions of the brain (Beyenburg et al., 2000, Finley et al., 1999). The evidence that testosterone modulates spatial cognition is given by the fact that these functions are improved after supplementation therapy in hypogonadal states in older men (Cherrier et al., 2001). Testosterone could be metabolized in the target tissue by enzyme aromatase that converts it to the estradiol, the main female sex hormone. Hypotheses that testosterone converted to estradiol improves verbal memory are confirmed by the findings that verbal memory in older men after testosterone supplementation therapy improves only if estradiol is being produced (Cherrier et al., 2005). On the other hand, spatial memory improves independently on estradiol production (Cherrier et al., 2005). In addition, saliva testosterone levels are positively correlated with spatial abilities in women and negative correlation was found in men, suggesting the existence of optimal free testosterone levels contributing to the optimal spatial performance (Ostatnikova et al., 2002). In rat experiments comparing castrated animals, animals with testosterone or estradiol supplementation and healthy individuals, estradiol was shown to improve the spatial memory but testosterone had no significant effect (Gibbs, 2005). Other animal experiments showed an increase in spatial abilities following testosterone treatment. Decrease, although not significant, in spatial abilities was observed when animals were treated with both, testosterone and anastrozol, an aromatase inhibitor. These results suggest that testosterone aromatization to estradiol plays some role in the influence of spatial performance (Okkelova et al., 2003). Spatial performance is one of the psychological attributes of the masculine behavior. One of the brain regions that reflect the sex differences is the sexually dimorphic nucleus of the preoptic area (SDN-POA) in the anterior hypothalamus (Gorski et al., 1980). It controls the hormone secretion resulting in male or female behavior (Hsu et al., 2000). Lesions in the entire POA causes reduced acquisition of the copulatory behaviors (Dejonge et al., 1989). The volume of the SDN-POA is crucial for masculine behavior. Males have many fold larger SDN-POA than females (Houtsmuller et al., 1994). The enzyme aromatase is abundant in the hypothalamus (Shinoda et al., 1994). Aromatisation of testosterone to estradiol causes the estradiol binding to its receptors resulting in masculine SDN-POA. This binding inhibits

the cell death and thus causes the larger SDN-POA. Treating newborn female rats with testosterone results in the cell death inhibition leading to larger SDN-POA (Davis et al., 1996). Treating newborn female rats with estradiol has the same effect (Gorski et al., 1980). It seems that estrogen, main feminine hormone, serves to masculinize the brain of males. In addition, testosterone induces the formation of prostaglandin E2 (PGE2) formation that positively regulates dendritic spines within the POA, another aspect of masculinization important in the development of the copulatory behavior (Amateau et al., 2004). Some authors suggest that PGE2 acts downstream from estrogen pathway, because treating newborn females with estrogen or PGE2 results in the same effect on dendritic spine formation and perinatally blocking PGE2 demasculinizes the brain of males (Amateau et al., 2004).

Effect of fetal testosterone

Differences in fetal testosterone concentrations were shown to result in differences in brain anatomy and in behavioral tasks in rat models. Brain regions affected by the fetal testosterone such as hypothalamus, limbic system and neocortex show differences in anatomical parameters (Arnold et al., 1984). In humans, exposure to high levels of prenatal testosterone results in masculinized social behavior in the areas of spatial performance and cognitive abilities (Knickmeyer et al., 2005). This study shows a negative correlation between fetal testosterone levels and social relationship. In addition, the body of evidence exists that fetal testosterone influences the levels of another hormone produced by neurons, serotonin, and its pathway. Perinatal testosterone intake results in masculinization of the serotonin nerve fiber distribution (Simerly et al., 1985).

Testosterone influences serotonin level

Serotonin plays a role in the development of sexual dimorphic POA in the hypothalamus (Handa et al., 1986). Special role of testosterone in serotonin pathway was demonstrated by gonadectomy rat experiments, where serotonin receptor expression was altered in various brain regions (Zhang et al., 1999) and differences in serotonin hypothalamic levels were observed (Martinez-Conde et al., 1985). In addition, testosterone modulates serotonin effect on migration of LHRH neurons (Adamskaia et al., 23). The influence of testosterone on sex differentiation is evident in many biological pathways, although it is not exactly and fully understood.

Unbalanced testosterone levels contribute to the development of the autistic disorder

Geschwind and Galaburda postulated a theory suggesting that prenatal testosterone levels are responsible for differences in the development of the central nervous system (Galaburda et al., 1978). Testosterone seems to slow down the growth of the left hemisphere areas resulting in excessive development of the homological areas in the right hemisphere. Right cerebral hemisphere seems to be dominant for specialized cognitive abilities such as mathematical-logical skills and spatial abilities. Thus excessive prenatal testosterone seems to modulate attributes, in which male population outperform the female one.

Moreover, high prenatal testosterone levels result in masculinized brain anatomy and behavior patterns. Baron Cohen et al. (2005) postulated the empathizing-systemizing theory proposing that males perform better in systemization and females perform better in empathization and social behavior. Systemizing is the capacity to predict and to respond to the behavior of inferring the rules that govern the system. Empathizing is the capacity to predict and to respond to the behavior of people by inferring their mental states and responding to these with an appropriate emotion (Baron-Cohen et al., 2005). Extreme male behavior patterns and special neuroanatomical differences were observed in the behavior and neuroanatomy of people suffering from autism, a neurodevelopmental disease. Autistic people are strong systemizers with deficits in empathy.

In animal models, it has been shown that fetal testosterone plays a critical role in brain development and social behavior. In humans, exposure to high levels of prenatal testosterone results in masculine behavior. Performance of human experiments is of course limited, only the observation of ill patients is possible. For instance, congenital adrenal hyperplasia (CAH) causes abnormally high levels of fetal testosterone measured in amniotic fluid. Evidence that autistic traits are based on hypermasculinized brains resulting in hypermasculine way of thinking and behavior is that autistic traits are increased in CAH patients (Knickmeyer et al., 2006). In addition, fetal testosterone mediates serotonin levels that are increased in autistic patients (Belmonte et al., 2004).

Pathogenesis of autistic disorder

Autism is a neurodevelopmental disorder characterized by social deficits, impaired language and communication, stereotyped, repetitive behaviors (Leo Kanner's first definition postulated in 1943). The disease is manifested in the first three years of life and persists into adulthood.

Its etiopathology is poorly understood. It is a multifactorial disorder. The prevalence of autism is about 7/10,000. The importance of genetic factors has been highlighted by epidemiological studies showing that autistic disorder is one of the most genetic neuropsychiatric diseases (heritability more than 90%) (Folstein and Rosen-Sheidley, 2002, Veenstra-VanderWeele and Cook, 2004). The relative risk of the first relatives is about 100-fold higher than the risk in the normal population and the concordance in monozygotic twin is about 82-92%.

Autistic disorder can be syndromic, that means associated with a known monogenic disorder such as tuberous sclerosis and fragile X syndrome (Folstein and Rosen-Sheidley, 2002), or nonsyndromic, Concrete evidences for the proposal that increased cumulative mercury exposures may be the causes of increased number of autistic cases in the last decades are reviewed by Mutter et al. (2007).

Idiopathic autism.

Plenty of epidemiological studies attempted to assess the main etiologic factor as a primary cause of the disorder, but it seems to be more mazed than cleared out. More of the possible factors have been identified but none of them occurs in all of the studied cases of autistic disorder. Vaccinations with live virus and thimerosal (toxic mercury component) were found to be associated with autistic phenotype (Hviid et al., 2003). In some cases, prenatal toxic teratogenic exposures are linked with autistic features. Frequent abnormalities of sulfoxidation and sulfation that cause impaired liver detoxification resulting in high level of xenobiotics were frequently found in autistic children (reviewed by McFadden 1996). In addition, different types of pesticides, such as DDT belong to a group of epigenetic factors disrupting sexual brain organization during neurodevelopment (Dorner et al., 2007). In some autistic cases immune system impairments were also found, such as impaired antibody production and aberrant cytokine profiles (reviewed by Van Gent et al., 1997). Intestinal problems, food intolerance and intoxication from casein and gluten often occur in autism as well as the sulfur metabolism impairments and copper-zinc imbalance (reviewed by Millward et al., 2004).

Phenotypic profile shows altered neurodevelopment, unbalanced local/long distance connectivity, inhibitory/excitatory connectivity (Courchense and Pierce, 2005, Levitt et al., 2004) reduced apoptosis/increased cell proliferation, altered cell migration with disrupted cortical and subcortical cytoarchitectonics, abnormal cell differentiation with reduced neuronal size, altered synaptogenesis (Bauman and Kemper, 2005, Picket and London, 2005). Autistic spectrum disorder is expected to be genetically heterogenic with polygenic/oligogenic inheritance and possibly presence of gene-gene, gene-environment interactions. Many autism susceptibility loci have been identified, but one region emerges more frequently: the long arm of chromosome 7 (Folstein and Mankoski, 2000). Most of the recent studies dealing with genes associated with autism are reviewed by Persico et al. (Persico and Bourgeron, 2006). The described autism associated genes are involved in many molecular processes such as cell adhesion, migration, neurotransmition, apoptosis, cellular signaling, cytoskeleton dynamics and chromatin remodeling. Some of these genes are involved in neuroendocrine regulations such as genes encoding neurohormones oxytocin and vasopressin. Oxytocin and vasopressin are neuropeptides with sexually dimorphic effects on brain and behavior especially during in utero development. Effect of these neuropeptides on the brain is provided via their binding to the receptors. Receptors for oxytocin and vasopressin are localized especially in the brainstem regions crucial for regulation of social and reproductive behavior. Single nucleotide polymorphisms of gene encoding oxytocin receptor are associated with autism spectrum disorders (Lerer et al., 2007, Jacob et al., 2007, Wu et al., 2005). Oxytocin levels are crucial for influencing the social and emotional bonds between mother and child, as well as in long lasting relationships. Oxytocin ensures trust, loyalty and devotion. Autistic patients have low blood levels of oxytocin (Carter et al., 2007). Furthermore, specific patterns and densities of oxytocin binding sites may also be influenced by steroid hormones (Esch and Stefano, 2007).

Importance of reelin pathway in the manifestation of autistic disorder

Since Chromosome 7q was described to be associated with autism, the importance is focused on reelin, the protein that is produced by Cajal-Retzius cells in hippocampal cortex and secreted into circulation. Reelin protein is encoded by RELN gene located on 7q22. Reelin protein has a major role in neuronal migration and in prenatal development of neuronal connections (D´Arcangelo et al., 1995). Mice lacking RELN (Reeler mice) show altered neuronal migration and altered cytoarchitectonics (D´Arcangelo et al., 1995). Low reelin expression was found to be associated with autism (Fatemi et al., 2002, Fatemi et al., 2005). Many of RELN gene polymorphisms have been screened (Serajee et al., 2006). Polymorphic GGC repeats in 5'UTR were significantly correlated with autism, particularly the long variants

(more than 12). This genotype causes decreased reelin protein and mRNA level in serum and brain that is associated with vulnerability to autism spectrum disorder (Persico et al., 2006, Lugli et al., 2003). Reelin is a glycoprotein (388 kDa, 3461 amino acids) produced by Cajal-Retzius cells in hippocampal cortex, in external granular layer (EGL) in cerebellum, before the migration of granule cells to the internal granule cell layer (IGL). In the adult brain reelin is expressed by GABA-ergic interneurons (cortex) and glutamatergic neurons (cerebellum). Regions in which the reelin expression occurs are characterized by laminated structures. It has been shown that reelin controls neuronal migration and positioning (brain lamination) (D´Arcangelo et al., 1995). Reelin postnatally regulates development of neuro-muscular junctions (NMJ) via its protease activity (Quattrocchi et al., 2003). Examination of human experiments concerning post mortem autistic brains showed that reelin protein and mRNA levels are significantly reduced in frontal and cerebellar cortices in post mortem autistic brains (Fatemi et al., 2005). In addition, reelin protein and mRNA levels are reduced in sera of autistic patients (Fatemi et al., 2002).

Reelin pathway involves reelin receptors: VLDLR receptor, APOE-R2, a3b1 integrins (Hiesberger et al., 1999). These receptors upon binding reelin communicate with the adaptor protein Dab1 (Disabled-1) that triggers the machinery of kinases phosphorylating and activating other kinases (and phosphatases network) leading to MAP1B phosphorylation and Tau dephosphorylation (Hiesberger et al., 1999, Takei et al., 2000). Map1b and Tau are the main members of neuronal microtubule associated proteins (MAPs). MAPs are abundantly localized in axons. Activated MAPs provide axonal extension, dynamic stability of microtubules (MTs) and cross talk between MTs and actin filaments. MAP1B is active while phosphorylated and Tau while dephosphorylated (Figure 1.). As described above, reelin pathway keep these proteins in their active form. Reeler mice experiments showed that mice lacking reelin pathway show following abnormalities. Neurons failed to reach their correct locations in developing brain (impaired migration). These mice show disrupted organisation of cerebellar and frontal cerebral cortices, they show impaired brain lamination, which is normally controlled by reelin. In addition, reeler mice and VLDLR -/- ApoER2 -/- mice show hyperphosphorylation of Tau protein resulting in impaired axonal growth and synaptic plasticity. MAP1B -/- mice show abnormal brain lamination as a result of the failure in neuronal migration, disorganised neuronal layering, reduction in area of axons and MTs number per axon, inhibited axonal outgrowth, extensive axonal growth cones and impaired neuronal migration (Takei et al., 2000, Gonzalez-Billault et al., 2004). In addition, reelin protein is necessary in neuronal migration also because of its proteolytic activity (Quattrocchi et al.,2002).

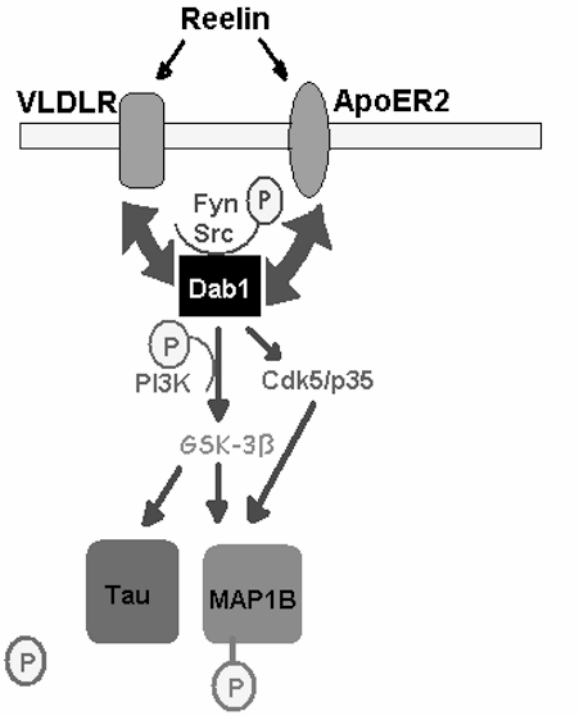

Fig. 1 Reelin signalling pathway. In th target tissue reelin binds to its receptors VLDLR and ApoER2 resulting in Dab1 phosphorylation via Fyn/Src kinase. Signalling cascade leads to the phosphorylation of the MAP1B and hypophosphorylation of the Tau protein, microtubule associated proteins that provide proper axonal outgrowth and neuronal migration process.

Testosterone and neuronal migration. Possible relationship?

Testosterone was described to enhance the quality and the frequency of birdsongs (Ball et al., 2003). Song behavior is controlled in brain by testosterone and its metabolite estradiol via its receptors that are expressed in many specialized forebrain song control nuclei. Furthermore, testosterone was found to influence the expression of reelin in the brain of male European starlings (Absil et al., 2003). Levels of testosterone change seasonally in the lifecycle of these birds. Reelin protein contributes to the incorporation of new neurons to the song control nucleus of the songbird brain. Thus it is possible that the linkage

between testosterone levels and reelin mediated neuronal development exists also in mammals including humans, mediating the manifestation of autistic phenotype.

SUMMARY AND PERSPECTIVES

Hormonal influence on the central nervous system is crucial in the prenatal and perinatal period of life. It seems that steroid hormones have the influence on the central nervous system during postnatal period. Sex steroids influence the sexual differentiation and the neuronal development in utero. Postnatal effects of the sex steroids apparently contribute to spatial abilities and cognitive performance. Testosterone via binding androgen receptors or more evidently via conversion to estradiol upon binding estrogen receptors provide sexual differentiation of special sexual dimorphic regions of the brain such as preorbital area of the hypothalamus. Increase of the cell number and dendritic spines in this region leads to the masculinized pattern. Excessive in utero testosterone contributes to the greater development of the right hemisphere responsible for special cognitive and spatial abilities characteristic for male phenotype. Thus testosterone seems to contribute to the systemizing pattern in men. The importance of the role of sex steroids in the development of the nervous system is given by the fact that differences in steroid hormonal status were described in the neurodevelopmetal disorders. Autistic phenotype is suggested to develop as a result of hypermasculinized brain consequently to hyperandrogenic environment. However, the difference in hormonal status is only one of the theories suggesting possible causes of autism. The pathogenesis of the disorder is not well understood yet. Other studies are necessary to elucidate whether the excessive sex steroids are crucial for neuronal changes in developing brain and to clear up the mechanism of this process.

Abbreviations: ADHD: antidiuretic hormone; OCT: oxytocine; SDN-POA: sexually dimorphic nucleus of the preoptic area; PGE2: prostaglandin E2; LHRH: Luteinising hormone releasing hormone; Dab1: disabled1; VLDLR: very low density lipoprotein receptor; APOE-R2: Apolipoprotein E- receptor2; MAP1B: microtubule associated protein 1B; MT: microtubules

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