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Estrogens and progestagens: synthesis and action in the brain

Authors: Rossetti María F.[1,2,*]; Cambiasso María J.[3,4] ; Holschbach M. Allie[5]; Cabrera Ricardo[6].

Authors Affiliations:

[1] Departamento de Bioquímica Clínica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. Santa Fe, Argentina.

[2] Instituto de Salud y Ambiente del Litoral, CONICET-Universidad Nacional del Litoral. Santa Fe, Argentina.

[3]Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC-CONICET-Universidad Nacional de Córdoba. Córdoba, Argentina.

[4]Departamento de Biología Bucal, Facultad de Odontología -Universidad Nacional de Córdoba. Córdoba, Argentina.

[5]Biomedical Sciences, Colorado State University. Fort Collins, Colorado, United States of America.

[6]Instituto de Investigaciones Biomédicas. INBIOMED-IMBECU-CONICET. Universidad de Mendoza. Mendoza, Argentina.

Email addresses:

mfrossetti@fbcb.unl.edu.ar

jcambiasso@immf.uncor.edu

maholsch@colostate.edu

ricabre@gmail.com

* Corresponding author. Departamento de Bioquímica Clínica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. Santa Fe, Argentina. E-mail: mfrossetti@fbcb.unl.edu.ar.

Declaration of interest

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Abbreviations

17β-HSD: 17β-hydroxysteroid dehydrogenase

3α-HSD: 3α-hydroxysteroid dehydrogenase

3β-HSD: 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase

5αR: steroid 5α-reductase

AD: Alzheimer's disease

Allop: Allopregnanolone

AP: activator protein

BST: Bed nucleus of the stria terminalis

DHEA: Dehydroepiandrosterone

DHEAS: Dehydroepiandrosterone sulfate

E2: Estradiol

ER: Estrogen receptor

ERE: Estrogen response element

$GABA_A$: Type-A γ-aminobutyric acid receptor

GPER1: G protein-coupled estrogen receptor 1

LH: Luteinizing hormone

MBP: Myelin basic protein

mPR: Membrane bound progesterone receptor

NF: Nuclear Factor

Ngn3: Neurogenin 3

NMDA: N-methyl-D-aspartate receptor

Oct-1: octamer-binding factor-1

OVX: Ovariectomized

P: Progesterone

P450arom: cytochrome P450arom

P450c17: 17α-hydroxylase / C17-20 lyase

P450scc: cytochrome P450 side-chain cleavage

PGMRC1: Progesterone membrane receptor component 1

PR: Progesterone receptor

PRE: Progesterone response element

Preg: Pregnenolone

PregS: Pregnenolone sulfate

Sp: selective promoter factor

SREBP-: sterol regulatory element-binding protein

StAR: Steroidogenic acute regulatory protein

T: Testosterone

TBI: Traumatic brain injury

TFs: Transcription factors

TSPO: Translocator protein

Abstract

When steroids, such as pregnenolone, progesterone and estrogen, are synthesized *de novo* in neural tissues, they are more specifically referred to as neurosteroids. These neurosteroids bind specific receptors to promote essential brain functions. Pregnenolone supports cognition and protects mouse hippocampal cells against glutamate and amyloid peptide-induced cell death. Progesterone promotes myelination, spinogenesis, synaptogenesis, neuronal survival and dendritic growth. Allopregnanolone increases hippocampal neurogenesis, neuronal survival and cognitive functions. Estrogens, such as estradiol, regulate synaptic plasticity, reproductive behavior, aggressive behavior and learning. In addition, neurosteroids are neuroprotective in animal models of Alzheimer's disease, Parkinson's disease, brain injury, and aging. Using *in situ* hybridization and/or immunohistochemistry, steroidogenic enzymes, including cytochrome P450 side-chain cleavage, 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase, cytochrome P450arom, steroid 5α-reductase and 3α-hydroxysteroid dehydrogenase, have been detected in numerous brain regions, including the hippocampus, hypothalamus and cerebral cortex. In this article, we summarize some of the studies related to synthesis and function of estrogens and progestagens in the central nervous system.

Keywords

Estrogen; progestagen; brain; neurosteroidogenic enzymes.

1. Introduction.

It is well known that steroid hormones regulate various functions associated with the brain including the development of the central and peripheral nervous system, the regulation of neurotransmitter systems, synaptic connectivity, dendritic branching, and myelination. They are also involved in cognition, emotion, mood, sexual behavior, and social behavior (1-12). Historically, the synthesis of these steroids was thought to be constrained to

steroidogenic glands such as gonads, adrenal cortex and placenta, and could then cross the blood-brain barrier to act in the brain (13). However, it is now well known that steroids are also synthesized *de novo* from cholesterol or steroidal precursors imported from peripheral sources in numerous brain regions, including the hippocampus, hypothalamus and cerebral cortex, by both neurons and glia. These locally synthesized hormones are called neurosteroids (13). Brain cholesterol is converted into pregnenolone (Preg) by the cholesterol side-chain cleavage enzyme (P450scc) in the mitochondria, as in peripheral steroidogenic organs. Subsequently, Preg is metabolized in the smooth endoplasmic reticulum into neurosteroids, such as progesterone (P), allopregnanolone (Allop), dehydroepiandrosterone (DHEA) or estradiol (E2) (14).

Neurosteroids regulate several central nervous system functions by binding their respective receptors and have key roles in neurodegenerative disease and aging. The present article focuses on two major groups of neurosteroids: estrogens (E2) and progestagens (P and Allop) and their common precursor Preg. Here, we summarize some of the studies related to the steroidogenic enzymes and classical receptors that are involved in their synthesis and action in the central nervous system.

2. Steroidogenic enzymes in the brain

The nervous system expresses all the enzymes necessary for the synthesis of neurosteroids (Fig. 1). Astrocytes express P450scc, cytochrome P450 17α-hydroxylase/c17,20-lyase (P450c17), 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase (3β-HSD), 3α- hydroxysteroid dehydrogenase (3α-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD), steroid 5α-reductase (5αR) and cytochrome P450 aromatase (P450arom), producing Preg, P, DHEA, androstenedione, testosterone (T), Allop and E2 (15-19). Oligodendrocytes express P450scc, 5αR and 3β-HSD and produce Preg, P and Allop (18; 20). The neurons express P450scc, P450c17, 3β-HSD, 5αR and P450arom and produce Preg, DHEA, androstenedione, Allop and E2 (19; 21). Distinct patterns of expression of steroidogenic enzymes in neurons, oligodendrocytes, and astrocytes, suggest that some kind of cooperation between neurons and glial cells coordinates the metabolism of various sex steroids, particularly Allop, T and P (19).

2.1. Cytochrome P450 side chain cleavage

The first enzymatic reaction of steroidogenesis is the transformation of cholesterol into Preg, catalyzed by P450scc (Fig. 1). P450scc also known as CYP11A1 (Cytochrome P450 family 11 subfamily A member 1), is located in the

inner mitochondrial membrane, where it catalyzes the conversion of cholesterol to Preg via three reactions (19). The first two steps involve hydroxylation of cholesterol side chain, generating first, 22R-hydroxycholesterol and then 20α, 22R-dihydroxycholesterol. Finally, P450scc cleaves the bond between carbons 20 and 22, resulting in the production of Preg and isocaproic aldehyde. Each step of the monooxygenase reaction requires 2 electrons (reducing equivalents), which are transferred from NADPH to P450scc through transfer proteins (22).

P450scc is always active, but its activity is limited by the availability of cholesterol in the inner membrane. Cholesterol supply to this membrane is mainly mediated by steroidogenic acute regulatory protein (StAR) and the translocator protein (TSPO) of 18 kDa (23). Increasing TSPO-mediated translocation of cholesterol from the outer to the inner mitochondrial membrane by applying TSPO agonists, stimulates steroid production (24). TSPO likely functions as a channel, accommodating a cholesterol molecule in the space delineated by five transmembrane domains. The mechanisms by which the mitochondria-targeted protein StAR drives the transfer of cholesterol and increases steroidogenesis are less well understood (24).

The expression of P450scc and the production of Preg by astrocytes, oligodendrocytes and neurons was previously described by Zwain and Yen (20). They noted that oligodendrocytes are the main source of Preg in the brain, as these cells produce Preg from cholesterol at a higher level than astrocytes or neurons, confirming previous suggestions that oligodendrocytes are primarily responsible for P450scc activity in the brain (20). Interestingly, in rat embryos , P450scc expression was mainly found in sensory structures of the peripheral nervous system, suggesting a possible role of this enzyme in the development and maturation of the brain (16). In the adult brain, P450scc enzyme was found in the cortex, amygdala, hippocampus, and midbrain (11; 19). In the human brain , the presence of mRNA of P450scc has been described in the olfactory bulb, corpus callosum , amygdala , hippocampus and cerebral cortex (19).

2.2. 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase

Preg can be converted to P by the enzyme 3β-HSD (Fig. 1). 3β-HSD is a membrane-bound protein that has two distinct enzymatic activities: 3β-dehydrogenation and isomerization of the double bond from C5,6 in the B ring (Δ5-steroids) to C4,5 in the A ring (Δ 4-steroids) (11). 3β-HSD is expressed in neurons, oligodendrocytes and astrocytes

and each of these types of cells can convert Preg to P (20). Two isoforms of 3β-HSD have been described in humans, four in rats, and six in mice. These isoforms are expressed in a tissue- and developmentally specific manner and fall into two functionally distinct groups NAD^+-dependent dehydrogenase/isomerases and NADPH-dependent 3-keto steroidreductases. In rats, 3β-HSD mRNA type I has been detected in several regions of central nervous system, including the cerebellum, hippocampus, cortex and hypothalamus (19). In the human brain, type II 3β-HSD mRNA has been detected by RT-PCR in the amygdala, hippocampus, cerebellum and spinal cord, among others. 3β-HSD protein has also been detected in the brain of both rats and humans (11). Developmental changes in 3β-HSD gene expression have been investigated in the brain of postnatal rodents. In the rat hippocampus, 3β-HSD mRNA is 2–3-fold higher on postnatal day (PND) 7 and PND 14 than PND 70 (25); and is 9-fold higher in PND 90 than in PND 450 (26). In the cerebellum, 3β-HSD is expressed transiently during PND 7 and PND 14 and disappears in the adult (25). These changes in enzyme expression could explain the decline in P levels during aging (27) that contribute to neurodegenerative diseases (9; 28-30). Gene regulation of 3β-HSD is complex and involves several different factors, such as cyclic adenosine monophosphate and protein kinase-C (31), as well as DNA methylation (32). However, most of the studies have been conducted in rat and human adrenal glands and gonads; thus, the regulation of 3β-HSD expression in the brain is still largely unknown.

2.3. Steroid 5α-reductase

P can be converted to 5α-dihydroprogesterone, by the enzyme 5αR (Fig. 1) (1). Melcangi *et al.* (18) showed that the formation of 5α-dihydroprogesterone takes place preferentially in neurons; however, type 2 astrocytes and oligodendrocytes also possess considerable 5αR activity, while activity in type 1 astrocytes is much lower. Two isoforms of 5αR, 5αR type 1 (5αR-1) and type 2 (5αR-2), have been reported in rodents and humans. 5αR-1 is the most abundant molecular form in the brain; it is present in many regions including the hypothalamus, hippocampus, cerebellum and cerebral cortex. In contrast, 5αR-2 is only expressed exclusively in the late fetal and early postnatal period in the rat brain, and it is virtually undetectable in the hypothalamus, cerebellum, pons and medulla oblongata in humans. The fact that this pattern of expression correlates with T synthesis in the fetal testis suggests that 5αR -2 could be involved in the control of brain sex differentiation (19).

The expression of 5αR-1 is relatively constant over the period PND 1 – PND 84 but then decreases at least 2-fold from PND 90 to PND 450 in the rat hippocampus. The mRNA levels of 5αR-2 also decreased between PND 1 to PND 84 (25). In addition, alterations of mRNA levels of 5αR-1 in the brain are related to neurodegenerative diseases such as Niemann-Pick Disease Type C, Parkinson's disease and multiple sclerosis (28; 33). These changes may explain the decline in Allop levels during aging and neurodegenerative disease (4; 27); and therefore, the deterioration of neuronal and cognitive functions (9; 34). Moreover, some forms of enrichment, including sensory and social stimuli change the expression of 5αR-1 in the brain. Specifically, rats housed in groups of eight animals in large cages and provided with an assortment of objects including large plastic tubes, rodent dwellings and toys of various shapes, sizes and colors during 105 days, increased the transcription of 5αR-1 in the hippocampus (26). Since this brain structure is associated with learning and memory, these results suggested that 5αR may play an important role in cognition.

Transcriptional regulation of 5αR is not well understood. Some authors suggest that changes in the DNA methylation transcription factors (TFs) could be involved. The promoter regions of 5αR-1 and 5αR-2 genes were identified and several TFs such as selective promoter factor 1 (Sp1), Sp3, activator protein 2 (AP-2) and nuclear factor 1 (NF-1) can interact with those promoters (35; 36). In addition, Blanchard *et al.* (35) described an important CpG Island of about 1000 pb in the 5αR-1 gene. CpG Islands are DNA strands of more than 200 bp where a cytosine (C) nucleotide is followed by a guanine (G) nucleotide at more than 50% above expected CG distribution. Cytosines in CG dinucleotides can be methylated to form 5-methylcytosine and thus the gene is silenced. Such methylation constitutes an important mechanism of transcriptional regulation. Accordingly, changes in the methylation patterns of specific sites located in 5αR-1 gene CpG Island were correlated with alterations in the mRNA levels in the rat hippocampus (26; 37).

2.4. 3α-hydroxysteroid dehydrogenase

The enzyme 3α-HSD is involved in the synthesis of Allop (Fig. 1). Interestingly, the expression of 3α-HSD appears to be mainly, if not exclusively, present in type 1 astrocytes. The compartmentalization of two strictly correlated enzymes (5αR and 3α-HSD) in separate central nervous system cell populations suggests the simultaneous participation of neurons and glia in the 5 alpha-reductive metabolism of hormonal steroids such as 5α-

dihydroprogesterone (18). In humans, four functional isoforms of 3α-HSD (1, 2, 3, 20a) were identified; types 2, 3 and 20a are widely expressed in the central nervous system (19). In contrast, rodents have a single 3α-HSD isozyme that is expressed in the cortex, hippocampus, olfactory bulb, basal ganglia, hypothalamus, thalamus and cerebellum (11).

3α-HSD expression is age-dependent. Higo *et al.* (25) showed that mRNA expression of this enzyme is 2.28-fold higher on PND 10 than PND 84 in the rat hippocampus. Recently, Rossetti *et al.* (26) showed that 3α-HSD expression also decreased 2.3-fold from PND 90 to PND 450. In addition, the 3α-HSD expression is reduced by the emergence of neurodegenerative diseases, such as Niemann-Pick Disease Type C (28), and increased by environmental stimuli (26), suggesting that 3α-HSD enzyme is neuroprotective. The similar expression patterns of 3α-HSD and 5αR suggests that the synthesis of Allop is paramount for learning and memory and other hippocampal-dependent mechanisms.

The involvement of certain TFs in the regulation of 3α-HSD gene expression has been examined by several authors. The 5′-flanking regions of the rat and human genes contain consensus sequences for AP-1, octamer-binding factor 1 (Oct-1) and steroid hormone response elements, which may comprise a steroid response unit (38). These Oct factors increase gene transcription whereas glucocorticoid response elements reduce transcription of this gene Penning (39). Hung and Penning (40) also suggest that NF-1 would upregulate the expression of 3α -HSD enzyme in rat liver. Recently, Rossetti *et al.* (26) proposed that the transcriptional regulation of the 3α-HSD gene in the rat brain would be mediated by differential methylation mechanisms (26; 37). Particularly, they found that the promoter was mostly methylated at a potential binding site for the sterol regulatory element-binding protein (SREBP-) and this change was correlated with alteration in the mRNA levels (26), suggesting that SREBP- also affects gene expression.

2.5. Cytochrome P450arom

P450arom catalyzes the last and obligatory step in the biosynthesis of estrogens (Fig. 1) and is necessary for sexual differentiation of the brain (41). Zwain and Yen (20) demonstrated that astrocytes and neurons, but not oligodendrocytes, express P450arom and produce E2 from T. Neurons appear to be more active than astrocytes in

aromatization of androgen to estrogen. Neurons cannot produce T, but astrocytes may provide T as a substrate for neurons to produce E2. Developmental expression of P450arom has been classified into three different groups: 1- fetal group (includes the anterior medial preoptic nucleus, the periventricular preoptic nucleus, neurons associated with the strial part of the preoptic area, and the rostral portion of the medial preoptic nucleus); 2- fetal/neonatal group (from the medial preoptic nucleus to the principal nucleus of the bed nucleus of the stria terminalis and the posterodorsal part of the medial amygdaloid nucleus) and 3- young/adult group (42). In the adult brain the pattern of P450arom distribution is restricted to interconnected nuclei, including the nucleus of the posteromedial amygdala, encapsulated region of the bed nucleus of the stria terminalis, ventrolateral portion of the ventromedial hypothalamic nucleus, and central component of the medial preoptic nucleus (43). These spatial variations of P450arom mRNA and protein provide evidence that estrogens play fundamental roles during brain development. Interestingly, P450arom is also expressed in the hippocampus, cerebral cortex, midbrain, spinal cord and cerebellum (43), suggesting that, in addition to reproductive functions, P450arom may play a role in modulation of mood, affective behaviors (e.g. depression) and/or learning and memory (44). It also plays an important role in neuroprotection after excitatory injury, experimental stroke, global ischaemia, reperfusion, and elevated intracranial pressure (45).

The regulation of P450arom in the brain is complex and not completely understood. Studies in several species have led to new perspectives on the control of this enzyme by both transcriptional and posttranscriptional mechanisms (37; 46; 47). In addition, there are regionally specific sex differences in P450arom expression during the critical period of sexual differentiation (48). However, some of the sex differences in P450arom expression could not be explained by organizational actions of gonadal hormones. Instead, genetic sex determines the expression of P450arom in specific brain areas during development, as demonstrated using the four core genotypes mouse model, in which the testis-determining gene Sry is moved from the Y chromosome onto an autosome to separate effects of gonadal sex from genetic sex (49). Individuals carrying the XY chromosome complement have higher expression levels of P450arom (mRNA and protein) in the stria terminalis and anterior amygdaloid area than individuals carrying XX chromosomes, irrespective of gonadal status (testes *vs.* ovary), indicating that brain P450arom at E16 is determined by sex chromosomes rather than gonadal hormones. The biological meaning of this effect is unknown; however, such differences *in vivo* could reflect differences in the local production of E2 by aromatization of T in these specific brain areas. According to these findings, amygdala neurons of genetic males would be exposed to

greater neuritogenic effects of E2, leading to larger dendritic trees and greater synaptic connectivity than neurons of genetic females .

3. Steroid hormones and neurotransmitter receptors in the brain

Neurosteroids exert several biological actions in the brain by both genomic actions mediated by nuclear /membrane steroid receptors and non-genomic actions mediated by neurotransmitter receptors. Thus, we focus this section on two main classical receptors; estrogen and progesterone receptors. It is important to consider the patterns of expression of these receptors within the brain, and understand how activating these receptors affects brain cell physiology and how these patterns of expression are controlled by hormones, age, sex, and experience. In addition, we briefly discuss the implication of the neurotransmitter receptors on neurosteroid actions.

3.1. Estrogen Receptors

There are two isoforms of the classical estrogen receptor (ER), ERα and ERβ, which are transcribed from unique genes (50; 51). Activating ERα or ERβ causes translocation of receptor-ligand dimers to the nucleus where they bind to estrogen response elements (EREs) on DNA (52) to control protein transcription (53) by recruiting various co-activators and co-repressors (51; 54).

In addition to this classical mode of ER expression and activation, ERs, including ERα and ERβ, are also expressed on the membrane (55-59). The mechanism of this cell membrane association is unclear but involves posttranslational lipid modification (palmitoylation) and interaction with membrane/cytoplasmic scaffolding proteins (e.g. caveolins) (55-57). In addition to membrane-associated (m) ERα and mERβ, G protein-coupled estrogen receptor 1 (GPER1) is another membrane-associated ER (58). Activating mERs alters membrane permeability (60) and activates second messenger cascades, including mitogen-activated protein kinases, extracellular-regulated kinases, and Src kinases, among others(59) and hyperpolarizes neurons in the preoptic area (61; 62). Interestingly, increasing data indicate that the potent androgen, dihydrotestosterone, can be metabolized to 3β-Diol, a steroid that binds to ERβ and may play a role in the estrogenic effects on pathological and physiological functions (63), such as anxiety (64; 65), cognition(66) and sexual differentiation of the brain (67).

ERs are found throughout the brain, but some areas with dense expression are highlighted below. Nuclear receptors are expressed in the pituitary, hypothalamus, hippocampus, amygdala, and prefrontal cortex (68; 69). Many cells with ERα are found in the bed nucleus of the stria terminalis (BST), medial amygdala, preoptic area, and various other hypothalamic nuclei (70-73). High levels are also seen in olfactory regions, the periaqueductal gray, area postrema, cerebellum, and parabrachial nucleus (72; 74-77). Like ERα, ERβ is also found in the BST, lateral septum, the medial and basolateral amygdale, the trigeminal nuclei, the preoptic region, and other hypothalamic nuclei (69; 76; 78; 79). In addition, ERβ is found in some regions with low or no ERα, such as the diagonal band of Broca, supraoptic area, and paraventricular nucleus (72; 76; 79; 80). Moderate levels are also seen in the hippocampus, substantia nigra and dorsal raphe (76; 81; 82).

ERα is expressed in cell nuclei as well as in dendrites and terminals in the hypothalamus (83). mERα is expressed on both the cytoplasmic surface and exterior surface of the cell membrane and is mostly found within presynaptic compartments of hippocampal neurons but is also seen in postsynaptic compartments and glia (84-86). mERβ is also found on the cytoplasmic surface of the cell membrane, but is primarily expressed in postsynaptic dendrites with lower expression in presynaptic axons and glia (81). GPER1, like mERα and mERβ, is found on the cytoplasmic surface of the cell membrane in pre and post-synaptic sites of hippocampal neurons (87-89) and is also found in clusters of vesicles in axon terminals (89). All three receptor types were also found in striatal neurons (mostly presynaptic sites) and glia (90). Similarly, the prefrontal cortex also has a preponderance of mERs at presynaptic sites and glia (91). GPER1 is highly expressed in the olfactory bulbs, hypothalamus, motor cortex, somatosensory piriform cortex, hippocampus, and the habenular nucleus of the epithalamus, nucleus of the solitary tract, and cerebellum (87; 89; 92-94).

Many factors influence expression of ERs. GPER1 expression is equivalent between the sexes but females in proestrus have higher GPER1 expression than estrus females, suggesting GPER expression can be modulated by even acute changes in circulating hormones (89). Estrogen replacement reduces ERα in preoptic and hypothalamic regions of female rodents (95). Extranuclear ERα levels in hippocampal neurons of mice are highest when estrogen is low, either during diestrus or after ovariectomy, but in rats they peak during proestrus when estrogens are high (84; 85; 96). Although aged and youthful monkeys have similar levels of ERα, GPER1, and P Receptor (PR)

protein, aged female rats have reduced nuclear ER expression in preoptic nuclei (97). Aged female rats also have fewer ERα-containing synapses in the hippocampus (98). Male rats retain ERα across adulthood but do have decreased ERα in response to circulating testosterone (99).

3.2. Progesterone Receptor

Like the ER, PRs are classically defined as ligand-activated transcription factors. There are two isoforms of the nuclear PR, but unlike ERα and ERβ, PRA and PRB are transcribed from the same gene. PRA is an N-terminal truncated form of the full-length isomer, PRB. Unbound PR exists as a complex with chaperone proteins that are necessary for its subsequent binding (100). Bound PR dissociates from the chaperone proteins, undergoes conformational changes, dimerizes, and interacts directly with specific P response elements (PREs) in promoter regions of targeted genes by binding to steroid receptor coactivators (101). In addition to nuclear PRs, many membrane bound (m) PRs have been identified. mPRs activate G-proteins but are not GPCRs; they are members of the progestin and adipoQ receptor family (102; 103), which have 7 transmembrane domains. In addition, the b5-like heme/steroid-binding protein family includes P membrane receptor component one (PGMRC1) (104).

PR has been seen in many brain regions, including the hippocampus, frontal cortex, hypothalamus, and cerebellum (105). It is also densely expressed in the BST and the centromedial amygdala (106). mPRs are also widely distributed throughout the brain (103), but only at very low levels throughout most of the forebrain except for dense mPRβ in the nucleus of the oculomotor cranial nerve (107). PGMRC1, but not mPRs, are abundant in forebrain structures that regulate neuroendocrine function (102). PGRMC1 is also in the hippocampus, cortex, and cerebellum (105).

Unlike ER, estrogens increase PR expression (108). Estrogen treatment increases PRA expression in male but not rat female cerebellum (105), but there is no sex difference in PR expression within the BST or centromedial amygdala (106). Estrogen also increases PRA expression in the hippocampus and olfactory bulb, whereas P has no effect (105). The effects of aging on PR expression are less clear. Whereas one group has found less cytosolic PR binding in the preoptic area of middle-aged ovariectomized (OVX) rats after 2 days of estrogen exposure compared to young OVX rats (109), other groups have reported no age differences on mRNA levels or PR binding (97; 110). It does

seem clear that whereas neonatal rodents have nuclear PR, expression of PR is largely extra nuclear in adult mice and rats (85).

3.3. Neurotransmitter Receptors

One of the best-documented examples of a non-genomic action of a steroid is the ability of several progesterone derivatives to activate type-A γ-aminobutyric acid ($GABA_A$) receptors, which are members of the ligand gated ion channel family and contain many distinct binding sites for GABA, benzodiazepines, barbiturates and convulsants. The $GABA_A$ receptor is the principal inhibitory neurotransmitter receptor in the brain and can be made up of different subunits of the α, β, γ and δ subtypes, and their composition is region and developmental stage specific (10). Although there is no absolute specificity for neurosteroid modulation of $GABA_A$ receptors, the α- and γ-subunits also affect $GABA_A$ neuromodulation by either positive modulators, such as Preg and Allop, or negative modulators, such as Preg sulfate (PregS), DHEA and DHEA sulfate (DHEAS) (111). $GABA_A$ receptors containing the δ-subunit can be less sensitive to neurosteroid modulation (10). Fluctuations in the concentration of neurosteroids and changes in GABAergic signaling have been implicated in a variety of physiological and pathophysiological conditions including stress, pregnancy, reproductive/sexual behaviors, depression, anxiety, seizure and epilepsy (7; 112-114), suggesting that $GABA_A$ receptors are important mediators of the action of these compounds.

N-methyl-D-aspartate (NMDA) receptors are tetrameric ion channels containing two of four possible GluN2 subunits. These receptors have been implicated for decades in neurological diseases such as stroke, traumatic brain injury, dementia and schizophrenia. The GluN2 subunits substantially contribute to functional diversity of NMDA receptors and are distinctly expressed during development and among brain regions (115). Some neurosteroids such as E2 act as a negative modulator, whereas DHEA, Preg and their sulfate esters are thought to be positive allosteric modulators of NMDA receptors. Unlike $GABA_A$ receptor interactions, the interaction of neurosteroids with the NMDA receptor is not well documented, and no specific interactions have been described (7; 10).

Although $GABA_A$ and NMDA receptors appear to be primarily responsible for the action of neurosteroids; other kind of receptors have also been studied in the literature, such as sigma receptors, AMPA receptors and kainate receptors (10; 116). These receptors are also regulated allosterically by several neurosteroids such as PregS, DHEAS and P and have been implicated in different nervous system functions and pathologies.

4. Neurosteroids and their effects on nervous system functions.

4.1. Pregnenolone and sulfated neurosteroids

Preg and DHEA are precursors of estrogens, progestins and androgens, but they also influence neuronal functions and are likely to play particularly important roles in the aging nervous system (13). Preg is essential in maintaining cognitive functions and protects mouse hippocampal cells against glutamate and amyloid peptide-induced cell death (13). Preg also regulates neurotransmission, acting at both pre and postsynaptic sites to control synaptic release of neurotransmitters such as GABA, glutamate, noradrenaline, dopamine and serotonin (10).

In addition to these neurosteroids, their sulfated counterparts have distinct effects in the nervous system. DHEAS and PregS are the most abundant sulfated neurosteroids in the brain. Whereas Preg is a positive allosteric modulator of GABA receptors, PregS is a negative modulator of the same receptor. Sulfated neurosteroids are involved in a large number of biological functions in humans and other mammals (117-120). For example, PregS increases luteinizing hormone (LH) secretion by modulating GABA and glutamate receptors. This LH surge is inhibited by COUMATE (an irreversible inhibitor of the steroid sulfatase (STS) enzyme), showing that imbalances in neurosulfation can indirectly affect reproductive function (121-124). Furthermore, we observed that this hypothalamic sulfation prevents variations in steroid hormones by reducing STS gene expression and reduces receptive behaviors such as lordosis. This indicates that neurosulfation can fine-tune reproductive physiology and behavior by controlling expression and activity of enzymes involved (125). Thus, neurosteroid sulfation in the hypothalamus plays a key role in the reproductive function of the female rat.

4.2. Progestagens

4.2.1. Progesterone

In the central nervous system, P increases the number of oligodendrocytes expressing the myelin basic protein (MBP). MBP has many splice variants, which are developmentally regulated. In adult myelin, the role of the predominant 18.5 kDa isoform is to maintain the structural integrity and compaction of the myelin sheaths (126). This suggested that P promotes myelination by increasing the transcription of certain myelin genes (126). In the brain, P regulates spinogenesis, synaptogenesis, neuronal survival and dendritic growth (29; 30; 127; 128) and plays a neuroprotective role in numerous animal models of neurodegenerative diseases (24; 34). For example, after traumatic brain injury (TBI) P decreases cell death, gliosis, and cognitive deficits (9). Gonzalez Deniselle *et al.* (129) indicated that P restores motoneuron morphology and the expression of α3 subunit Na,K-ATPase mRNA, a neuronal enzyme controlling ion fluxes, neurotransmission, membrane potential, and nutrient uptake, and increased both muscle strength and survival time of Wobbler mice, a genetic model of spinal cord motor neuron disease. P prevents depression-like behavior in a model of Parkinson's disease induced by 6-hydroxydopamine in male rats (130). P also exerts marked neuroprotective effects after spinal cord injury, cerebral ischemia and stroke (34). Moreover, Liu *et al.* (131) showed that β-amyloid peptide ($A\beta_{25-35}$), a main etiological factor of Alzheimer's disease (AD), is exacerbated by low levels of P *in vivo* in the rat prefrontal cortex and hippocampus, suggesting that P is also essentially for learning and memory. In addition to its role in organization and protection of neural structures, P activity in the brain is a necessary component in female reproduction (132).

4.2.2. Allopregnanolone

P also acts on the nervous system through one of its most important metabolites, Allop. Allop increases neurogenesis and neuronal survival and reduces apoptosis in the hippocampus (7; 26). Allop also increases the density of dendritic spines as well as drebrin clusters in cultured hippocampal neurons, indicating that it increases excitatory synapse density (133). Allop also seems to be involved in learning and memory. For example, Frye (134) showed that young rats in proestrus and late pregnancy (reproductive states associated with higher cortical Allop levels) exhibit better performance on the object recognition task than diestrous rats or rats in early pregnancy. The infusion of E2 benzoate alone or with P into the hippocampus of ovariectomized rats has amnesic effects and Allop

can reverse this effect, suggesting that these effects are not mediated through the P receptor and beneficial effects may include the promotion of the cognitive performance of the hippocampus (135).

Interestingly, Allop has a key role in neurodegenerative disease. Reduced Allop levels were observed in the prefrontal cortex and in temporal cortex of patients with AD (26). Similarly, Allop content in the white matter (33) and in cerebrospinal fluid (136) is reduced in patients with multiple sclerosis and Parkinson's disease, respectively. In the triple transgenic mouse model of AD (3xTgAD), Allop prevents neurogenic and cognitive deficits (26). In addition, Allop restores hippocampal-dependent learning and memory and neural progenitor survival in aging wild type mice (26). Allop also increases Purkinje and granule cell survival in a mouse model of the human neurodegenerative disease Niemann–Pick disease type C (26). Allop is also neuroprotective in other experimental models, such as TBI, ischemia and spinal cord injury (34).

4.3. Estrogens

E2 has multiple important physiologic effects on several tissues and cellular phenotypes. E2 acts permanently on the developing brain to establish sex differences by regulating the growth, differentiation and survival of neurons and glia (137; 138). In the adult brain, E2 acts as an autocrine-paracrine factor that regulates synaptic plasticity, adult neurogenesis, reproductive behavior, aggressive behavior, pain processing and cognition (5; 45; 138-141). E2 also impacts cellular physiology by modulating calcium handling, immediate-early-gene expression (c-Fos) and kinase activity (cAMP response element-binding and extracellular-signal-regulated kinases) (142-144). The specific mechanisms of E2 action in the brain are region- and sex-specific and often involve neuronal/glial cross-talk (145). For instance, in primary cultures of dissociated neurons, the neuritogenic effect of E2 is differentially exerted depending on the genetic sex of the neurons (146), the region of the brain (2; 146-148), the maturity of the neurons (146; 149; 150) and the glial environment in which they develop (151-154). Moreover, at 14 days of embryonic age (E14), E2 only stimulates axogenesis in hypothalamic neurons from males, and this effect is contingent on the upregulation of neurogenin 3 (Ngn3), a proneural gene that is involved in neuritogenesis (142). The silencing of Ngn3 abolished sex differences in neuritogenesis, decreasing the differentiation of female neurons. Moreover, the sex difference in Ngn3 expression is determined by sex chromosomes, as demonstrated using the four core genotypes mouse model, in which the testis-determining gene Sry is moved from the Y chromosome onto an

autosome to separate effects of gonadal sex from genetic sex (49). These findings indicate that the developmental actions of estrogen on neurite growth and differentiation can result from modulatory interactions with endogenous proneural factors (Ngn3) and the genetic sex rather than directly from estrogenic action alone (146).

5. Conclusions and future perspectives

Neurosteroids are synthesized within the nervous system. Steroidogenic enzymes and receptors are widely distributed in specific populations of neurons, neuronal precursors, and glia. Neurosteroids exert several neurotrophic and neuroprotective actions and therefore provide tremendous opportunities for developing therapeutic approaches. Although hormone replacement therapies have been studied in numerous laboratories, the results are somewhat contradictory. Hopefully, future studies will elucidate the mechanisms that regulate steroid synthesis and action in the brain and explore more alternatives to estrogen and progestagens (P and Allop) replacement.

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Figure Legends

Figure 1. Pathway of neurosteroid synthesis. Steroidogenic acute regulatory protein (StAR); cytochrome P450 side chain cleavage (P450scc); 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase (3β-HSD); cytochrome P450 17α-hydroxylase/c17,20-lyase (P450c17); steroid 5α-reductase (5αR); 3α-hydroxysteroid dehydrogenase (3α-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD) and cytochrome P450arom (P450arom).Astrocytes express P450scc, P450c17, 3β-HSD, 3α-HSD, 17β-HSD, 5αR and P450arom, producing pregnenolone (Preg), progesterone (P), dehydroepiandrosterone (DHEA), androstenedione, testosterone, allopregnanolone (Allop) and estradiol (E2) (15-19). Oligodendrocytes express P450scc, 5α- reductase and 3β-HSD and produce Preg, P and Allop (18; 20). The neurons express P450scc, P450c17, 3β-HSD, 5α-reductase and P450arom and produce Preg, DHEA, androstenedione, AlloP and E2 (19).