Oestrogens and Progestagens: Synthesis and Action in the Brain

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When steroids, such as pregnenolone, progesterone and oestrogen, are synthesised 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. Oestrogens, such as oestradiol, regulate synaptic plasticity, reproductive behaviour, aggressive behaviour and learning. In addition, neurosteroids are neuroprotective in animal models of Alzheimer’s disease, Parkinson’s disease, brain injury and ageing. Using in situ hybridisation and/or immunohistochemistry, steroidogenic enzymes, including cytochrome P450 side-chain cleavage, 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase, cytochrome P450 arom, steroid 5α-reductase and 3α-hydroxysteroid dehydrogenase, have been detected in numerous brain regions, including the hippocampus, hypothalamus and cerebral cortex. In the present review, we summarise some of the studies related to the synthesis and function of oestrogens and progesterogens in the central nervous system.

Key words: oestrogen, progestagen, brain, neurosteroidogenic enzymes

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Cholesterol

\[ \text{STAR} \rightarrow \text{P450scc} \]

\[ \text{Pregnenolone} \rightarrow \text{Dehydroepiandrosterone} \]

\[ 3\beta\text{-HSD} \rightarrow \text{P450c17} \rightarrow \text{Progesterone} \rightarrow \text{Androstenedione} \rightarrow \text{Oestrone} \]

\[ 5\alpha\text{-dihydroprogesterone} \rightarrow 3\alpha\text{-HSD} \rightarrow \text{Testosterone} \rightarrow 17\beta\text{-Oestradiol} \rightarrow \text{P450arom} \]

\[ \text{Allopregnanolone} \]

**Fig. 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, dehydroepiandrosterone (DHEA), androstenedione, testosterone, allopregnanolone (Allop) and oestriadiol (E2) (15–19). Oligodendrocytes express P450scc, 5α-reductase and 3β-HSD and produce Preg, progesterone and Allop (18,20). The neurons express P450scc, P450c17, 3β-HSD, 5α-reductase and P450arom and produce Preg, DHEA, androstenedione, Allop and E2 (19).

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, progesterone, DHEA, androstenedione, testosterone, allopregnanolone (Allop) and oestradiol (E2) (15–19). Oligodendrocytes express P450scc, 5α-reductase and 3β-HSD and produce Preg, progesterone 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 neurones, oligodendrocytes and astrocytes suggest that some kind of cooperation between neurones and glial cells coordinates the metabolism of various sex steroids, particularly Allop, testosterone and progesterone (19).

**Cytochrome P450 side chain cleavage**

The first enzymatic reaction of steroidogenesis is the transformation of cholesterol into Preg, catalysed 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 catalyses the conversion of cholesterol to Preg via three reactions (19). The first two steps involve hydroxylation of cholesterol side chain, first generating 22R-hydroxycholesterol and then 20α, 22R-dihydroxycholesterol. Finally, P450scc cleaves the bond between carbons 20 and 22, resulting in the production of Preg and iso-capric aldehyde. Each step of the monoxygenase reaction requires two electrons (reducing equivalents), which are transferred from NADPH to P450scc via transfer proteins (22).

P450scc is always active, although 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 neurones was previously described by Zwaan and Yen (20). They noted that oligodendrocytes are the main source of Preg in the brain because these cells produce Preg from cholesterol at a higher level than astrocytes or neurones, 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).

3β-hydroxysteroid dehydrogenase/Δ5–Δ4-isomerase

Preg can be converted to progesterone by the enzyme 3β-HSD (Fig. 1). 3β-HSD is a membrane-bound protein that has two distinct enzymatic activities: 3β-dehydrogenation and isomerisation of the double bond from C5,6 in the B ring (Δ5-steroids) to C4,5 in the A ring (Δ4-steroids) (11). 3β-HSD is expressed in neurones, oligodendrocytes and astrocytes and each of these types of cells can convert Preg to progesterone (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/isoformases 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, amongst 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 two- to three-fold higher on postnatal day (PND)7 and PND14 than PND70 (25); and is nine-fold higher on PND90 than on PND450 (26). In the cerebellum, 3β-HSD is expressed transiently during PND7 and PND14 and disappears in the...
Steroid 5α-reductase

Progesterone 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 neurones; however, type 2 astrocytes and oligodendrocytes also possess considerable 5αR activity, whereas 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. By contrast, 5αR-2 is only expressed exclusively in the late foetal and early postnatal period in the rat brain, and it is almost undetectable in the hypothalamus, cerebellum,pons and medulla oblongata in humans. The fact that this pattern of expression correlates with testosterone synthesis in the foetal testis suggests that 5αR-2 could be involved in the control of brain sex differentiation (19).

The expression of 5αR-1 is relatively constant from PND1 to PND84 but then decreases at least two-fold from PND90 to PND450 in the rat hippocampus. The mRNA levels of 5αR-2 also decreased between PND1 to PND84 (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 ageing 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). Because this brain structure is associated with learning and memory, these results suggest that 5αR may play an important role in cognition.

Transcriptional regulation of 5αR is not well understood. Some studies 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, selective promoter factor 3, activator protein 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 approximately 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 the 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).

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 compartmentalisation of two strictly correlated enzymes (5αR and 3α-HSD) into separate central nervous system cell populations suggests the simultaneous participation of neurones and glia in the 5α-reductive metabolism of hormonal steroids such as 5α-dihydroprogesterone (18). In humans, four functional isozymes of 3α-HSD (1, 2, 3, 20a) were identified; types 2, 3 and 20a are widely expressed in the central nervous system (19). By 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 PND10 than PND84 in the rat hippocampus. Recently, Rossetti et al. (26) showed that 3α-HSD expression also decreased 2.3-fold from PND90 to PND450. 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 (26,27), 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 in several studies. The 5’-flanking regions of the rat and human genes contain consensus sequences for AP-1, octamer-binding factor 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 the transcription of this gene Penning (39). Hung and Penning (40) also suggest that NF-1 would up-regulate 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-1) and this change was correlated with alteration in the mRNA levels (26), suggesting that SREBP– also affects gene expression.

Cytochrome P450 aromatase

P450arom catalyses the last and obligatory step in the biosynthesis of oestrogens (Fig. 1) and is necessary for sexual differentiation of the brain (41). Zwain and Yen (20) demonstrated that astrocytes and neurones, but not oligodendrocytes, express P450arom and produce E2 from testosterone. Neurones appear to be more active than astrocytes.
in the aromatisation of androgen to oestrogen. Neurones cannot pro-
duce testosterone but astrocytes may provide testosterone as a sub-
strate for neurones to produce E2. Developmental expression of
P450arom has been classified into three different groups: (i) a foetal
group (includes the anterior medial preoptic nucleus, the periventricu-
lar preoptic neurones, neurones associated with the strial part of the
preoptic area, and the rostral portion of the medial preoptic nucleus); (ii) a foetal/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 (iii) a
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 ventromed-
dial hypothalamic nucleus, and central component of the medial pre-
optic nucleus (43). These spatial variations of P450arom mRNA and
protein provide evidence that oestrogens play fundamental roles dur-
ing 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 behaviours (e.g.
depression) and/or learning and memory (44). It also plays an impor-
tant 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 dur-
ing the critical period of sexual differentiation (48). However, some of the
sex differences in P450arom expression could not be explained by organisational actions of gonadal hormones. Instead, genetic sex determines the expression of P450arom in specific brain
areas during development, as demonstrated using the four core
zymes mouse model, in which the tests-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, irre-
spective of gonadal status (testes versus 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 differ-
ences in the local production of E2 by aromatisation of testosterone in
these specific brain areas. According to these findings, amygdala
neurones of genetic males would be exposed to greater neurot-
genic effects of E2, leading to larger dendritic trees and greater
synaptic connectivity than neurones of genetic females.

Steroid hormones and neurotransmitter receptors in the
brain

Neurosteroids exert several biological actions in the brain as a
result of both genomic actions mediated by nuclear/membrane
steroid receptors and nongenomic actions mediated by neurotrans-
mitten receptors. Thus, we focus on two main classical receptors:
oestrogen and progesterone receptors (PRs). It is important to con-
sider the patterns of expression of these receptors within the brain,
as well as to understand how activating these receptors affects
brain cell physiology and how these patterns of expression are con-
trolled by hormones, age, sex and experience. In addition, we briefly
discuss the implication of the neurotransmitter receptors on neu-
rosteroid actions.

Oestrogen receptors

There are two isomorphs of the classical oestrogen receptors (ERs),
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 oestrogen response ele-
ments on DNA (52) to control protein transcription (53) by recruit-
ing various co-activators and co-repressors (51,54).

In addition to this classical mode of ER expression and activa-
tion, ERs, including ERα and ERβ, are also expressed on the mem-
brane (55–59). The mechanism of this cell membrane association is
unclear but involves post-translational lipid modification (palmito-
lation) and interaction with membrane/cyttoplasmic scaffolding pro-
teins (e.g. caveolins) (55–57). In addition to membrane-associated
mERα and mERβ, G protein-coupled oestrogen receptor 1 (GPER1)
is another membrane-associated ER (58). Activating mERs alters
membrane permeability (60) and activates second messenger cas-
cades, including mitogen-activated protein kinases, extracellular-
regulated kinases and Src kinases, amongst others (59), and hyper-
polarises neurones in the preoptic area (61,62). Interestingly,
increasing data indicate that the potent androgen, dihydrotestos-
terone, can be metabolised to 3β-diol, a steroid that binds to ERβ
and may play a role in the oestrogenic 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, although 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 grey, area post-
rema, cerebellum and parabrachial nucleus (72,74–77). Similar to
ERα, ERβ is also found in the BST, lateral septum, the medial and
basolateral amygdala, 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 termi-
nals in the hypothalamus (83). mERα is expressed on both the
cyttoplasmic surface and exterior surface of the cell membrane and
is not only mostly found within presynaptic compartments of hip-
 pocampal neurones, but also is seen in postsynaptic compartments.
and glia (84–86). mERβ is also found on the cytoplasmic surface of the cell membrane, although it is primarily expressed in postsynaptic dendrites with lower expression in presynaptic axons and glia (81). GPER1, similar to mERα and mERβ, is found on the cytoplasmic surface of the cell membrane in pre- and postsynaptic sites of hippocampal neurones (87–89) and is also found in clusters of vesicles in axon terminals (88). All three receptor types were also found in striatal neurones (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, habenular nucleus of the epithalamus, nucleus of the solitary tract and cerebellum (87,89,92–94).

Many factors influence the expression of ERs. GPER1 expression is equivalent between the sexes, although females in pro-oestrus have higher GPER1 expression than oestrus females, suggesting that GPER expression can be modulated by even acute changes in circulating hormones (89). Oestrogen replacement reduces ERα in preoptic and hypothalamic regions of female rodents (95). Extracellular ERα levels in hippocampal neurones of mice are highest when oestrogen is low, either during di-oestrus or after ovariectomy but, in rats, they peak during pro-oestrus when oestrogens are high (84,85,96). Although aged and youthful monkeys have similar levels of ERα, GPER1 and 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).

**Progesterone receptors**

Similar to 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, dimerises and interacts directly with specific progesterone 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 adipokine receptor family (102,103), which have seven transmembrane domains. In addition, the b5-like heme/steroid-binding protein family includes progesterone membrane receptor component 1 (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), although only at very low levels 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 found in the hippocampus, cortex and cerebellum (105).

Unlike ER, oestrogens increase PR expression (108). Oestrogen treatment increases PRA expression in the male but not rat female cerebellum (105), although there is no sex difference in PR expression within the BST or centromedial amygdala (106). Oestrogen also increases PRA expression in the hippocampus and olfactory bulb, whereas progesterone has no effect (105). The effects of ageing on PR expression are less clear. Although one study found less cystolic PR binding in the preoptic area of middle-aged ovariectomised (OVX) rats after 2 days of oestrogen exposure compared to young OVX rats (109), other studies have reported no age differences for mRNA levels or PR binding (97,110). It does appear to be clear that neonatal rodents have nuclear PR, whereas the expression of PR is largely extra nuclear in adult mice and rats (85).

**Neurotransmitter receptors**

One of the best-documented examples of a nongenomic action of a steroid is the ability of several progesterone derivatives to activate GABA<sub>A</sub> 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<sub>A</sub> receptor is the principal inhibitory neurotransmitter receptor in the brain and can be made up of different subunits of 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<sub>A</sub> receptors, the α- and γ-subunits also affect GABA<sub>A</sub> neuromodulation by either positive modulators, such as Preg and Allop, or negative modulators, such as Preg sulphate (PregS), DHEA and DHEA sulphate (DHEAS) (111). GABA<sub>A</sub> receptors containing the δ-subunit can be less sensitive to neurosteroid modulation (10). Fluctuations in the concentration of neurosteroids and changes in GABAergic signalling have been implicated in a variety of physiological and pathophysiological conditions, including stress, pregnancy, reproductive/sexual behaviours, depression, anxiety, seizure and epilepsy (7,112–114), suggesting that GABA<sub>A</sub> receptors are important mediators of the action of these compounds.

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 E<sub>2</sub> act as a negative modulator, whereas DHEA, Preg and their sulphate esters are considered to be positive allosteric modulators of NMDA receptors. Unlike GABA<sub>A</sub> 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<sub>A</sub> and NMDA receptors appear to be primarily responsible for the action of neurosteroids, other kinds 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 progesterone, and have been implicated in different nervous system functions and pathologies.
Neurosteroids and their effects on nervous system functions

Pregnenolone and sulphated neurosteroids

Preg and DHEA are not only precursors of oestrogens, progestins and androgens, but also influence neuronal functions and are likely to play particularly important roles in the ageing nervous system (13). Preg is essential for 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 the synaptic release of neurotransmitters such as GABA, glutamate, noradrenaline, dopamine and serotonin (10).

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

Progestagens

Progesterone

In the central nervous system, progesterone 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 progesterone promotes myelination by increasing the transcription of certain myelin genes (126). In the brain, progesterone 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, progesterone decreases cell death, gliosis and cognitive deficits (9). Gonzalez Deniselle et al. (129) indicated that progesterone restores motoneurone morphology and the expression of α3 subunit Na, K-ATPase mRNA, a neuronal enzyme controlling ion fluxes, neurotransmission, membrane potential and nutrient uptake, and also increased both muscle strength and survival time of Wobbler mice, a genetic model of spinal cord motor neurone disease. Progesterone prevents depression-like behaviour in a model of Parkinson’s disease induced by 6-hydroxydopamine in male rats (130). Progesterone also exerts marked neuroprotective effects after spinal cord injury, cerebral ischaemia and stroke (34). Moreover, Liu et al. (131) showed that β-amyloid peptide (Aβ25–35), a main aetiological factor of Alzheimer’s disease (AD), is exacerbated by low levels of progesterone in vivo in the rat prefrontal cortex and hippocampus, suggesting that progesterone is also essentially for learning and memory. In addition to its role in organisation and protection of neural structures, progesterone activity in the brain is a necessary component in female reproduction (132).

Allopregnanolone

Progesterone 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 dendrin clusters in cultured hippocampal neurones, indicating that it increases excitatory synapse density (133). Allop also appears to be involved in learning and memory. For example, Frye (134) showed that young rats in pro-oestrus and late pregnancy (i.e. reproductive states associated with higher cortical Allop levels) exhibit better performance on the object recognition task than dio-oestrous rats or rats in early pregnancy. The infusion of E2 benzoate alone or with progesterone into the hippocampus of ovariecotomised rats has amnesic effects and Allop can reverse this effect, suggesting that these effects are not mediated through the PR 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 ageing 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 traumatic brain injury, ischaemia and spinal cord injury (34).

Oestrogens

E2 has multiple important physiological 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 neurones and glia (137,138). In the adult brain, E2 acts as an autocrine-paracrine factor that regulates synaptic plasticity, adult neurogenesis, reproductive behaviour, aggressive behaviour, pain processing and cognition (5,45,138–141). E2 also impacts cellular physiology by modulating calcium handling,
Conclusions and future perspectives

Neurosteroids are synthesised within the nervous system. Steroidogenic enzymes and receptors are widely distributed in specific populations of neurones, 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 clarify the mechanisms that regulate steroid synthesis and action in the brain and explore more alternatives to oestrogen and progesteragens (progesterone and Allop) replacement.

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