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REVIEW

Neurosteroid metabolism in the human brain

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Abstract

This review summarizes the current knowledge of the biosynthesis of neurosteroids in the human brain, the enzymes mediating these reactions, their localization and the putative effects of neurosteroids. Molecular biological and biochemical studies have now firmly established the presence of the steroidogenic enzymes cytochrome P450 cholesterol side-chain cleavage ($P450_{SCC}$), aromatase, 5α-reductase, 3α-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase in human brain. The functions attributed to specific neurosteroids include modulation of γ-aminobutyric acid A ($GABA_A$), N-methyl-D-aspartate (NMDA), nicotinic, muscarinic, serotonin ($5\text{-}HT_3$), kainate, glycine and sigma receptors, neuroprotection and induction of neurite outgrowth, dendritic spines and synaptogenesis. The first clinical investigations in humans produced evidence for an involvement of neuroactive steroids in conditions such as fatigue during pregnancy, premenstrual syndrome, post partum depression, catamenial epilepsy, depressive disorders and dementia disorders. Better knowledge of the biochemical pathways of neurosteroidogenesis and their actions on the brain seems to open new perspectives in the understanding of the physiology of the human brain as well as in the pharmacological treatment of its disturbances.

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Introduction

Steroid hormones are mainly synthesized in the adrenal glands, the gonads and the feto-placental unit. They can easily cross the blood–brain barrier due to their high lipid solubility. The brain is an important target organ of steroid hormones. Moreover, an extensive steroid metabolism occurs in the brain and several brain regions are well equipped with enzymes necessary for steroid hormone biosynthesis (1–4). Steroid hormones play an important role in the development, growth, maturation and differentiation of the brain.

Although, as in the case of aromatase, the activity of steroidogenic enzymes had already been identified in human fetal brain tissue 25 years ago (5), the majority of biochemical, physiological and behavioral studies on aromatase in brain tissue had been carried out in rodents or other vertebrate species. The difficulty in obtaining fresh human brain tissue, coupled with presumably low expression or activity of the respective enzymes, has so far precluded studies in humans. This is also the case with other steroidogenic enzymes. Steroidogenesis requires numerous sequential enzymatic reactions to convert cholesterol to glucocorticoids, mineralocorticoids or sex hormones. Since the steroids produced within a tissue depend upon the enzymes present in this tissue, only systematic studies on the expression of all relevant steroidogenic enzymes would allow insight into the steroidogenic pathways and the capacity within the respective tissue, i.e. the human brain. Although reports on the expression and activity of the most important steroidogenic enzymes in the human brain have been published in recent years, a comprehensive review is still missing. This article reviews the current knowledge of steroid hormone metabolism within the human brain and the evidence we have for its importance.

Animal studies have shown that steroids synthesized *de novo* in the central nervous system can affect multiple brain functions (i.e. neuroendocrine and behavioral functions) via intracellular receptors that regulate transcriptionally directed changes in protein synthesis. These physiological actions generally occur within hours or days. In addition to the classic sites of steroid synthesis, neurosteroids can rapidly alter excitability of the central nervous system through binding to neurotransmitter-gated ion channels, thus modulating γ-aminobutyric acid A ($GABA_A$) and N-methyl-D-aspartate (NMDA) receptors (6–8). These actions occur within seconds or milliseconds via ligand or voltage-gated ion channels. Compounds that have direct nongenomic effects on neuron excitability are referred to as neuroactive steroids (9). Neurosteroids,

by definition, are steroids that still accumulate in the brain in the absence of steroidogenic glands and are synthesized in the brain from endogenous precursors by enzymes that are present *in situ* (6). Neurosteroids can be further classified into two groups: neuroactive and neuroinactive steroids. The term 'neuroactive' steroids refers to steroids that are active on neural tissue. They may be synthesized in the brain or in endocrine glands. The term 'neuroinactive' steroids refers to steroids synthesized in the brain that are inactive on neural tissues. In 1941, Selye first described the anesthetic and sedative properties of progesterone and some of its metabolites (10). The potencies of neuroactive steroids in biochemical and electrophysiological investigations correlate with their sedative, anticonvulsant, and anxiolytic effects *in vivo*. There are a number of reviews concerning the biosynthesis and metabolism of neurosteroids and neuroactive steroids in animals (7–9, 11–13).

Steroid conversions in the human brain

Cytochrome P450 cholesterol side-chain cleavage ($P450_{SCC}$)

The first and rate-limiting step in the synthesis of steroid hormones is the conversion of cholesterol to pregnenolone, catalysed by the enzyme cytochrome $P450_{SCC}$. Human $P450_{SCC}$ is encoded by a single gene on chromosome 15, the CYP11A1 gene (14). In addition to the major sources of steroid production, the adrenal glands and gonads, $P450_{SCC}$ mRNA and enzyme are also present in the placenta, the primitive gut and in the brain (15–17). However, the major role of $P450_{SCC}$ in the brain is probably the regulation of brain neurosteroid levels (18). Recently, we investigated the expression of CYP11A1 mRNA in tissue specimens from temporal and frontal neocortex, subcortical white matter from the temporal lobe and in the hippocampus from children and adults (19, 20). In these brain areas, CYP11A1 mRNA was expressed in significant amounts in all tissue samples investigated, at a rate, however, $\approx$200 times lower than in adrenal tissue, which is known for the highest CYP11A1 expression. Thus, CYP11A1 mRNA expression in the human brain is within the range previously estimated for rat brain in qualitative RT-PCR experiments (17, 18, 21). In humans, CYP11A1 mRNA concentrations in the temporal lobe increase markedly during childhood and reach adult levels at puberty (19). CYP11A1 mRNA concentrations were significantly higher in the temporal and frontal neocortex as well as in the hippocampus of women compared with those of men (19, 20). These data demonstrated for the first time an age- and sex-dependent expression of CYP11A1 mRNA in the human brain. Few data are available on the relative amount of CYP11A1 mRNA in the brain of male and female animals, but qualitative studies report no obvious sex differences in rats (17, 22). Due to the insensitivity of qualitative RT-PCR in detecting differences in mRNA expression at high cycle numbers, a careful quantitative re-examination of results obtained in rat brain with respect to sex differences of CYP11A1 mRNA expression seems to be necessary. Whereas *in situ* hybridization and cell culture experiments in rat brain demonstrated predominant CYP11A1 expression in the subcortical white matter (21, 23), no such differences could be detected between neocortex and subcortical white matter tissue in the human brain (19). The presence of CYP11A1 mRNA in human brain tissue provides evidence that pregnenolone can be produced in the central nervous system.

Aromatase

Cytochrome P450 aromatase, which catalyses the conversion of androgens to estrogens in specific brain areas (3), is the product of the CYP19 gene, which has been cloned and sequenced (24, 25).

In the brain, androgens may be metabolized following two different major pathways. First, the aromatase pathway (transformation of testosterone into estradiol and of androstenedione into estrone), and secondly, similar to the one present in the majority of the peripheral androgen dependent structures, e.g. prostate, the 5α-reductase pathway (transfomation of testosterone into dihydrotestosterone).

Aromatase activity itself has been detected only in a few fetal brain specimens (26–28). Previously published data demonstrated aromatase activity in human temporal and in frontal brain areas (29). The authors studied biopsy materials removed at autopsy from normal adult control subjects and from patients with Alzheimer's disease. Temporal aromatase activity was always significantly higher than frontal aromatase activity regardless of sex and/or disease state. This difference was also confirmed by our own studies on the expression of temporal and frontal CYP19 mRNA in fresh brain tissue specimens from adult patients with epilepsy undergoing neurosurgery (30). CYP19 mRNA was not only expressed in temporal and frontal neocortex, but also in the human hippocampus and in subcortical white matter of the temporal lobe (30, 31). Sex specific differences in CYP19 mRNA expression could be observed in none of these brain areas. In our laboratory, aromatase activity in the temporal lobe was recently characterized in a similar cohort of patients with epilepsy (32). Aromatase activity was present in all tissue specimens under investigation. We demonstrated a specific, dose-responsive and competitive inhibition of its activity by atamestane, which is a known specific and competitive inhibitor of placental aromatase activity (33). Aromatase activity in the human brain was low compared with its high activity in the placenta. However, rates of aromatase activity in the brain were in the same order of magnitude as in

human adipose and testicular tissue (34, 35). Subsequent experiments with cerebral neocortex and subcortical white matter specimens of children and adults revealed significantly higher aromatase activity in the cerebral neocortex than in the subcortical white matter (32). This difference could not be found for CYP19 mRNA expression in the human temporal lobe (31). However, in the human temporal neocortex, CYP19 mRNA concentrations were significantly lower in children than in adults (31), a finding which could not be confirmed by measurement of aromatase activity (31). These contradictory findings indicate that aromatase might be regulated at the post-translational level.

Expression of the CYP19 gene, involving alternative splicing of multiple forms of exon 1, has been demonstrated to contribute to tissue-specific expression of aromatase (36–40). Honda and co-workers found that the aromatase gene in human brain is expressed with a brain-specific exon 1 and promotor, using poly (A)+ RNAs in human amygdala and hippocampus (40). However, a recent study (41) demonstrated that the expression of aromatase in human brain is not necessarily under the control of the brain specific exon 1 or exon 1f as proposed by Honda *et al.* (40). Instead, Sasano and co-workers found different patterns of utilization of exon 1 in different areas of the human brain tissue derived from autopsies (41). Hypothalamic and/or limbic structures utilized exon 1f and/or 1d (gonadal type) while other areas (pons, frontal lobe) utilized 1b (fibroblast type). There were no differences of utilization of exon 1 and mRNA expression of aromatase between female and male brains. Total amounts of aromatase mRNA varied among the brain regions and individuals under investigation. Due to the limited number of patients (4 men, 2 women), a statistical comparison could not be carried out. The authors suggest that the variability may possibly be caused by the effects of terminal and/or postmortem changes on the preservation of mRNA in tissue specimens.

5α-Reductase

Numerous animal studies have shown that in the central nervous system progesterone is rapidly metabolized to 5α-dihydroprogesterone (5α-DHP), which is then further reduced to the potent neurosteroid 3α,5α-tetrahydroprogesterone (3α,5αTHP) (8). These reductive conversions are catalysed by 5α-reductase and 3α-hydroxysteroid dehydrogenase (3α-HSD). In humans, two isozymes of 5α-reductase, which differ in tissue distribution and biochemical characteristics as well as in their responsiveness to specific inhibitors of their enzymatic activity, have been identified (42, 43).

The majority of physiological and biochemical studies on the expression of 5α-reductase in the brain were carried out in rodents and other vertebrate species (1, 2, 44, 45). However, some investigators documented 5α-reductase activity in human fetal brain (46–48), but 5α-reductase activity in the brain of adults was only demonstrated in a few frontal lobe and temporal lobe tissue specimens (49, 50).

Recently, we have demonstrated the predominant expression of 5α-reductase type 1 mRNA in a large series of human temporal neocortex and subcortical white matter as well as hippocampal tissue specimens obtained from patients with chronic temporal lobe epilepsy (51, 52). The expression levels were about 100 times lower than in human liver tissue. 5α-Reductase type 2 mRNA was not expressed. Another study reported on 5α-reductase type 1 mRNA expression in a few human cerebellum, hypothalamus and pons tissue specimens which were collected postmortem (53). Also, a predominant expression of 5α-reductase type 1 mRNA was found in rat brain (2, 54).

Previously, it was reported that 5α-reductase type 1 mRNA is expressed in rat brain at all stages of brain development and in adulthood, with a small increase around the time of birth, whereas 5α-reductase type 2 mRNA is only transiently expressed during the late fetal and early postnatal life (55). The expression patterns of this isoform overlapped the secretory profile of testosterone. The authors hypothesize that increased levels of circulating androgens occurring in male rats around the time of birth could modulate 5α-reductase type 2 expression. Hence, transient androgen-regulated expression of 5α-reductase type 2 may be important for sexual differentiation of the brain and for the formation of anxiolytic/anesthetic steroids originating from 3α-hydroxylation of 5α-reduced derivates of progesterone involved in the stress responses associated with parturition. However, we still do not know whether 5α-reductase type 2 mRNA might be expressed transiently during fetal or early postnatal life within the human brain.

In our laboratory, we also measured 5α-reductase activity in human temporal neocortex and subcortical white matter tissue specimens (51, 56). We used androstendione as the substrate; in a former study on human fetal brain tissue almost the same enzyme activity rates were found with either androstenedione or progesterone as the substrate (46). While enzyme activity was present in all tissue specimens under investigation, the apparent K_m values and the pH profile substantiated the predominant expression of the type 1 isoform. Furthermore, we investigated the inhibitory effects of MK386, a specific inhibitor of the 5α-reductase type 1 isoform, and of finasteride, a specific inhibitor of the 5α-reductase type 2 isoform on 5α-reductase activity (56). MK386 was a strong inhibitor of human brain tissue 5α-reductase activity, with an IC_{50} value of 2.0 nmol/l, whereas finasteride turned out to be a poor inhibitor of the reaction, with an IC_{50} value of 142.8 nmol/l (56). Moreover, we observed a potent inhibition of the pH-dependent

reaction by MK386 but not by finasteride; this further substantiates an, at least predominant, activity of the 5α-reductase type 1 isozyme in the human brain (56). There were no sex-specific differences in the expression levels of 5α-reductase type 1 mRNA in human temporal lobe and hippocampal tissue or in the activity of 5α-reductase (51, 52, 56). These findings are consistent with previous animal studies, where no significant sex specific differences concerning 5α-reductase activity were found in neural tissue of rhesus macaques during fetal development (57) or in rats during postnatal development (58, 59).

3α-Hydroxysteroid dehydrogenase

Multiple cDNAs encode proteins related to 3α-HSD in humans (60). However, three functional 3α-HSD isozymes (type 1, 2 and 3) have been characterized on the basis of their affinity for 5α-dihydrotestosterone (61–64).

Recently, we could only demonstrate the expression of the mRNA of the type 2 isozyme of 3α-HSD in the hippocampus and the temporal lobe of patients with temporal lobe epilepsy, whereas the mRNA of the type 1 isozyme of 3α-HSD was not expressed (52, 56). 3α-HSD type 2 is thought to be responsible for the production of neurosteroids (61), whereas the type 1 isozyme is expressed exclusively in the liver. In liver metabolism, 3α-HSD type 1 plays an essential role leading to physiologically inactive metabolites of steroid hormones. The mRNA expression levels of 3α-HSD type 2 in the human hippocampus were approximately one fifth of that in human liver tissue. For the quantification of 3α-HSD type 2 mRNA there was no need for a nested competitive RT-PCR assay as required for 5α-reductase type 1 mRNA. This emphasizes the fact that the mRNA expression levels of 3α-HSD are much higher than those of 5α-reductase type 1 mRNA. This is also consistent with the suggestion that 5α-reduction is the rate-limiting step in the production of 3α,5α-reduced metabolites of progesterone (65).

In accordance with data on 3α-HSD activity in the rat brain, expression of 3α-HSD type 2 mRNA in the human hippocampus did not differ between the sexes (52, 66).

Penning and co-workers demonstrated that, apart from 3α-HSD type 2 being expressed in human brain, 3α-HSD type 3 and 20α(3α)-HSD (EC 1.1.1.149) are also expressed to a larger extent in the human brain (64). Moreover, they showed that all human 3α-HSD isoforms and the human 20α-HSD act as 3-, 17- and 20-ketosteroid reductases as well as 3-, 17- and 20-hydroxysteroid oxidases. Based on spectrophotometric and radiometric data, the authors concluded that all isoforms were capable of producing the neuroactive tetrahydrosteroids that modulate the $GABA_A$ receptor. Thus, the meaning of the differential expression of the single isoforms is less established than ever.

17β-Hydroxysteroid dehydrogenase

The seven human isozymes of 17β-hydroxysteroid dehydrogenase (17β-HSD), which have so far been cloned, play a major role in the regulation of the biological activity of sex hormones. They are essential for the biosynthesis of the strong androgens and estrogens, testosterone and estradiol from their weaker precursors androstenedione and estrone (67, 68). These conversions are reversible and thus can lead to a deactivation of the respective sex hormones (69). The different isozymes show an individual cell-specific expression and substrate specificity. The importance of the 17β-HSD activity in the maintenance of physiological levels of estradiol and testosterone is reflected by the ubiquitous distribution of 17β-HSD in peripheral tissues (70).

17β-HSD activity in the human brain was reported about 30 years ago (49, 71). However, studies on the expression of the enzyme in the human brain are still rare. Western immunoblot analysis revealed the presence of 17β-HSD 1 in human fetal brain (72). Recently, we demonstrated the expression of 17β-HSD 1, 3, 4 and 5 mRNA in the human temporal lobe and hippocampus, whereas an in tandem pseudogene of 17β-HSD 1 and 17β-HSD 2 mRNA was not expressed (73, 74). Moreover, we characterized androgenic and estrogenic 17β-HSD activity in the human temporal lobe and found the NADPH-dependent reduction of androstenedione and estrone as well as the NAD-dependent oxidation of testosterone and estradiol (75). Substrate specificity, cofactor requirement patterns, pH optima and kinetic properties suggest the activity of at least two isozymes, namely the activating 17β-HSD 3 and the deactivating 17β-HSD 4, in the human brain. There was no sexual dimorphism in the expression or activity of 17β-HSDs. However, the expression levels of 17β-HSD 3, 4 and 5 mRNAs as well as the conversion of androstenedione, testosterone, estrone and estradiol were signifiantly higher in the subcortical white matter than in the cerebral neocortex (73–75). The predominant expression of 17β-HSD in the subcortical white matter suggests that glial cells could play a role in the biosynthesis and deactivation of sex steroids in the brain. Among a host of potential functions of glia, glial cells are involved in the formation of myelin, suggesting a possible correlation between sex steroids, these enzymatic activities and the formation or functions of myelin.

In a recent study on the human 17β-HSD 7 gene (*HSD17B7*), its promotor revealed binding sites for brain-specific transcription factors corresponding to expression domains in the developing brain as identified by in silico Northern blot (68). 17β-HSD 8 expression has not yet been investigated in the human brain.

Other steroidogenic enzymes

Other important steroidogenic enzymes are 3β-hydroxysteroid dehydrogenase (3β-HSD), cytochrome $P450c_{17}$,

21-hydroxylase (cytochrome P450c21), 11β-hydroxylase (cytochrome P45011β) and cytochrome P450 aldosterone synthetase (P-450aldo).

3β-Hydroxysteroid dehydrogenase catalyzes the conversion of Δ^5-3β-hydroxysteroids into Δ^4-3-ketosteroids (i.e. the conversion of pregnenolone into progesterone). Cytochrome P450c_{17}, possessing both 17α-hydroxylase and 17,20 lyase activity, is responsible for the conversion of C_{21} steroids (pregnenolone, progesterone) into C_{19} steroids (dehydroepiandrosterone (DHEA) and androstenedione).

21-Hydroxylase converts progesterone to 11-deoxycorticosterone and 17-hydroxyprogesterone to 11-deoxycortisol, the substrates required for the production of the main adrenal steroids, corticosterone, aldosterone and cortisol.

11β-Hydroxylase (cytochrome P45011β) catalyzes the formation of glucocorticoids (cortisol and corticosterone). Cytochrome P450 aldosterone synthetase (P-450aldo), which exerts three enzyme activities (11β-hydroxylation, 18-hydroxylation, and 18-oxidoreduction), catalyzes the formation of mineralocorticoids (aldosterone).

Studies on the expression of 21-hydroxylase in the brain are scarce. In rodents, 21-hydroxylase was detected in the brain stem using the reverse transcription-polymerase chain reaction assay and immunohistochemical methods (76, 77), whereas other investigators could not find 21-hydroxylase mRNA in any extra-adrenal tissue (78). As this may be due to the limited sensitivity of the mRNA quantification assay, we investigated the expression of 21-hydroxylase mRNA in the human hippocampus using a highly sensitive nested RT-PCR assay (79). This study was the first to demonstrate that 21-hydroxylase mRNA is expressed in the human hippocampus. The expression levels in the hippocampus are approximately 10 000 times lower than in the adrenal gland which is known for high 21-hydroxylase expression (79). However, we could not measure the enzyme activity of 21-hydroxylase since only small amounts of tissue specimens were available. Thus, although our results clearly demonstrate that 21-hydroxylase mRNA is expressed in small amounts in the human hippocampus, it remains debatable whether hippocampal tissue contains sufficient 21-hydroxylase to produce neuroactive steroid concentrations of physiological or pathophysiological relevance.

The mRNAs of 3β-HSD 1 and 2 as well as cytochrome P45011β and cytochrome P450 aldosterone synthetase were not expressed in the human temporal lobe nor in the hippocampus (own unpublished data). A sensitive, nested competitive RT-PCR assay was used for these investigations. However, several studies demonstrated the expression of 3β-HSD mRNA (21, 80, 81) and 3β-HSD protein (81) in the rat brain. Data concerning the expression of cytochrome P45011β in rodent brain are conflicting: while some authors report the expression throughout the rat brain (77, 82), others found only low expression levels in rat brain (17, 83) or no expression in mouse brain (77). Cytochrome P450 aldosterone synthetase expression and activity has been demonstrated in various regions of rat brain including hypothalamus, hippocampus, amygdala and cerebellum (82, 84).

Cytochrome P450c_{17} mRNA was not expressed in the human temporal lobe or hippocampus (own unpublished data). Former studies failed to demonstrate 17α-hydroxylase activity or P450c17 mRNA in the adult rat brain (6, 17). However, using ribonuclease protection assays and immunocytochemistry, P450c17 mRNA as well as P450c17 protein were detected in the brain of rat embryos (85). Conflicting data have been reported in adults: Compagnone and co-workers (85) reported expression of P450c17 mRNA only in the peripheral nervous system of rats and mice, whereas others demonstrated the presence of P450c17 mRNA in various brain regions of adult rodents (77).

Clinical implications

Molecular biological and biochemical studies have now firmly established the presence of the above mentioned steroidogenic enzymes cytochrome P450$_{SCC}$, aromatase, 5α-reductase, 3α-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase in human brain and provide evidence that neurosteroids and neuroactive steroids can be produced within the human brain. However, the (patho)physiological significance of these findings remains to be elucidated. Fig. 1 and Table 1 present a summary of current knowledge and open questions on biochemical pathways of steroid metabolism in the human brain.

Steroid hormone effects on the brain have typically been associated with gene regulation via intracellular steroid receptors. In contrast to reproductive and neuroendocrine actions of steroids via these intracellular receptors, which regulate transcriptionally directed changes in protein synthesis, modulatory actions on the GABA receptor system can rapidly alter the excitability of neurons (7, 8). GABA, a major inhibitory neurotransmitter, mediates fast synaptic inhibition by activating ligand-gated chloride channels. Binding of 3α-reduced neurosteroids to $GABA_A$ receptors leads to either inhibition or potentiation of the inhibitory effects of GABA. Hence, anticonvulsive, anesthetic and anxiolytic effects of neuroactive steroids are mediated by their capacity to positively modulate $GABA_A$ receptor function, i.e. these substances act to increase GABA-ergic effects by increasing the frequency and duration of chloride channel openings (7, 8). On the other hand, inhibition of $GABA_A$ receptor function which is mostly documented for the neurosteroids pregenenolone sulfate and DHEA sulfate (DHEAS) produces effects ranging from anxiety and excitability to seizure susceptibility (9, 11, 12). However, there is strong evidence for a putative specific

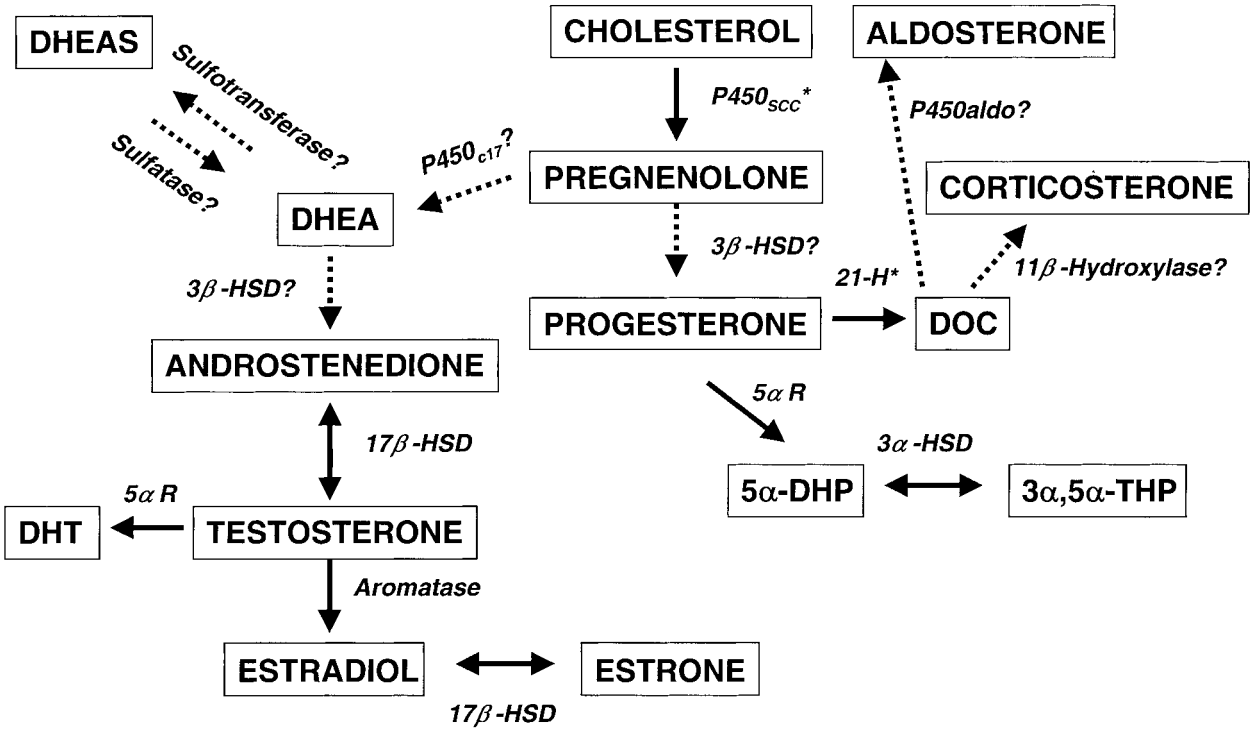

Figure 1 Current knowledge and open questions concerning the biochemical pathways of neurosteroidogenesis in the human brain. Solid arrows indicate that the activity of the respective enzyme as well as the expression of its mRNA has been documented with the exception of $P450_{SCC}$ and 21-hydroxylase (marked by an asterisk) as here only the expression of its mRNA has been shown. Dashed arrows indicate that the occurrence of the enzyme has not yet been found in the nervous system. DOC, deoxycorticosterone; DHT, dihydrotestosterone; 5α-DHP, 5α-dihydroprogesterone; 3α,5α-THP, 3α,5α-tetrahydroprogesterone (allopregnanolone); 5αR, 5α-reductase; 3α-HSD, 3α-hydroxysteroid oxidoreductase; 3β-HSD, 3β-hydroxysteroid oxidoreductase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 21-H, 21-hydroxylase.

steroid binding site at the $GABA_A$ receptor (86). In addition, other neurosteroid actions have been described in the brain including the inhibition of voltage-gated Ca^{2+} currents and NMDA receptor function as well as the modulation of other receptors, such as nicotinic, muscarinic, serotonin ($5\text{-}HT_3$), kainate, glycine and sigma receptors (13, 86–90). Moreover, it has been postulated that neurosteroids act on nerve cells through membrane receptors coupled to G proteins (91) and may also interact with various neuropeptide receptors (92). There is also evidence that neurosteroids may regulate gene expression by activating progesterone receptors (86, 93). In addition, other steroid effects apart from the modulation of neurotransmitter receptors have emerged: for example, estrogen might serve as a neuroprotective antioxidant (94). In summary, neurosteroids exert both genomic and nongenomic effects, and regulate neuronal function via their concurrent influence on gene expression and transmitter-gated ion channels. These actions

Table 1 Expression of mRNA and activity of steroidogenic enzymes in the human brain.

Enzyme	mRNA expression	Enzyme activity	Reference
Cytochrome $P450_{SCC}$	+	?	19, 20
21-Hydroxylase	+	?	79
Cytochrome P450 aromatase	+	+	26–33, 41
5α-Reductase type 1	+	+	46–53; 56
3α-Hydroxysteroid dehydrogenase type 2	+	+	52, 56
3α-Hydroxysteroid dehydrogenase type 3	+	?	64
17β-Hydroxysteroid dehydrogenase type 1	+	?	73
17β-Hydroxysteroid dehydrogenase type 3	+	+	73, 75
17β-Hydroxysteroid dehydrogenase type 4	+	+	73, 75
17β-Hydroxysteroid dehydrogenase type 5	+	?	74

+, Expression or activity has been demonstrated.

suggest that neurosteroids play a crucial role in mediating many brain functions. Moreover, the systemic effects of neurosteroids may be beneficial for a variety of neuropsychiatric disorders.

To date, the majority of physiological and behavioral studies have been carried out in rodents or other animal species. In recent years, evidence for an intensive neurosteroid metabolism within the human brain has emerged and now the first clinical investigations exist to support the results obtained in preclinical animal studies.

The potential anesthetic properties of neurosteroids had already been suggested in 1941 (10), and have led to the development of steroid anesthetics, e.g. alphaxalone (95). However, side effects have hindered the development of steroid anesthetics for routine clinical use (9).

The observation that epileptic seizures in cyclic women are less frequent in the luteal phase, when circulating levels of progesterone are high, appears to be associated with cyclical variations in the metabolism of progesterone to allopregnanolone in the brain (8, 96, 97). Progesterone and 3α-reduced neuroactive steroids have potent anticonvulsant effects (98, 99). Synthetic derivates of neuroactive steroids are under investigation for treatment of epilepsy disorders. Some preliminary investigations in healthy volunteers and in patients with medically intractable epilepsies have already been undertaken. Ganaxolone, for example, showed a promising pharmaco-kinetic profile and was well tolerated in a trial with healthy volunteers (100, 101). It was also effective in clinical studies with patients with epilepsy (102). Although promising, caution is necessary considering the potential side effects. For example, progesterone and 3α,5α-THP have benzodiazepine-like effects (98, 99), and progesterone withdrawal may lead to an increase in seizure susceptibility.

The development of sensitive assays to measure cerebral fluid or blood neurosteroid concentrations enabled researchers to document alterations in neurosteroidogenesis in human diseases. Recently, Ströhle and co-workers demonstrated decreased 3α,5α-tetrahydroprogesterone plasma concentrations in patients with major depression compared with healthy control subjects, and clinically effective antidepressant treatment was accompanied by an increase of 3α,5α-tetrahydroprogesterone in the plasma of these patients (103).

Substances which activate the $GABA_A$ receptor exert anxiolytic effects. Benzodiazepines and barbiturates are the most widely used anxiolytics in medicine. The GABA agonistic 3α-reduced neuroactive steroids may have a potential advantage as anxiolytics in comparison to benzodiazepines because of their more favorable tolerance and abuse liability profile in various drug discrimination paradigms (104). However, whether the anxiolytic properties of 3α-reduced neuroactive steroids suggested by animal studies can be confirmed in clinical trials with human beings is still a matter of speculation.

Neuroactive steroids may also be involved in physiological conditions where fluctuations of the hormonal balance occur. For example, increased fatigue during pregnancy may be a consequence of higher concentrations of progesterone and GABA agonistic 3α-reduced neuroactive steroids like 3α,5α-THP (105), whereas a rapid decline in these substances may lead to the premenstrual syndrome or post partum depression (106, 107). In rats, others have related endogenous 3α,5α-THP synthesis/metabolism rate during pregnancy with changes in $GABA_A$ receptor subunits (108), suggesting that the pharmacological changes in $GABA_A$ receptor subunits and their associated behavior are regulated by local production of 3α,5α-THP. Using a pseudopregnant rat model, Smith and co-workers (109) showed that withdrawal of 3α,5α-THP caused a relative insensitivity of $GABA_A$ to neurosteroid neuromodulation, and induced increased anxiety. Moreover, fluctuations in neuroactive steroid concentrations may, in part, contribute to the increased risk of developing psychiatric diseases in women at the perimenstrual phase, during pregnancy and the post partum period, and around menopause.

In alcoholic patients, reduced plasma concentrations of GABA agonistic 3α-reduced neuroactive steroids have been found during ethanol withdrawal (110). This decline in 3α-reduced neuroactive steroid concentrations may contribute to the increased seizure liability during ethanol withdrawal.

In humans, DHEA and DHEAS are the most abundant circulating steroid hormones and their concentrations decrease with age (111). Stress also decreases DHEA and DHEAS concentrations (112). As both age and stress are associated with neuronal vulnerability to degeneration, it was hypothesized that DHEA and DHEAS may be neuroprotective agents. Indeed, neuroprotection by DHEA and DHEAS was observed *in vivo* in hippocampal structures (113). The mechanisms by which DHEA and DHEAS act are still unknown. Decreased DHEAS concentrations have also been reported in patients with Alzheimer's disease and multi-infarct dementia (114–116). To date, trials in which DHEA was administered for a short period of two weeks have failed to demonstrate any benefit of DHEA therapy in cognitive performance (117–119). However, high-quality trials need to be undertaken in which the duration of DHEA treatment is in excess of a few weeks with a large enough number of participants to detect possible effects and where the outcome measures include objective tests of cognitive function.

In recent years, a great deal of research has focused on the effects of sex hormones on cognitive functions in humans. Perhaps the most prominent examples are the effects of estrogens and androgens on verbal fluency, the performance of spatial tasks, verbal memory tests

and fine-motor skills (120–122). The hormonal influences on memory processes appear to involve actions in brain structures such as the hippocampus and basal forebrain. There is now considerable documentation of estrogenic influences on hippocampal morphology and neurochemistry including the enhancement of the cholinergic system, which is involved in learning and memory (for reviews see: 123–127). Estrogens exert neurotropic and neuroprotective effects such as induction of neurite outgrowth, dendritic spines, and synaptogenesis. They have an influence on long-term potentiation and excitability, enhance gene expression and exhibit intrinsic antioxidant activity. Verbal fluency, memory, fine motor skills and the performance of spatial tasks are all subject to sex-specific differences. Thus, estrogen effects differ quantitatively or qualitatively between the sexes. Sex-specific differences also have an effect on the incidence of psychopathologies such as anxiety disorders, depression and migraine, which are more common in females, whereas substance abuse, antisocial behavior as well as pain sensitivity are more common in males. Estrogen replacement therapy has beneficial effects on verbal memory tests in surgically postmenopausal women (121). Alzheimer's disease occurs more frequently in elderly women than in elderly men (128), and there seems to be a possible link between estrogen deficiency and dementia. One study revealed a significant reduction in Alzheimer's disease as a cause of death in women who had taken estrogens postmenopausally, with an apparently greater protective effect resulting from higher dosage and longer exposure (128). Nevertheless, the authors have pointed out the need for prospective randomized treatment trials to substantiate the beneficial effects of estrogen replacement therapy. Although testosterone levels in elderly men decrease with age, production never ceases completely so that the prohormone for the production of estradiol exists in men throughout their life time. As the human brain contains aromatase, which is necessary for the conversion of testosterone to estradiol, estradiol is available to the brains of men throughout their life, whereas it is not available to the brains of untreated postmenopausal women.

Conclusions

Molecular biological and biochemical studies have now firmly established that several key enzymes of steroidogenesis, namely cytochrome $P450_{SCC}$, aromatase, 5α-reductase, 3α-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase, are present in human brain (Fig. 1, Table 1). Their presence in the cerebral cortex and in the subcortical white matter indicates that various cell types, either neurons or glial cells, are involved in the biosynthesis of neurosteroids and neuroactive steroids in the brain. We still do not know whether and how the steroidogenic enzymes are involved in the pathophysiology of the nervous system. However, clinical investigations in humans are now producing evidence for an involvement of neuroactive steroids in conditions such as fatigue during pregnancy, premenstrual syndrome, post partum depression, catamenial epilepsy, depressive disorders and dementia disorders. Results from preclinical and clinical studies strongly support the hypothesis that neuroactive steroids could be useful for therapeutic management of such disorders in the future.

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