

# Neurosteroid Biosynthesis in the Human Brain and Its Clinical Implications

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**ABSTRACT:** This paper summarizes the current knowledge concerning the biosynthesis of neurosteroids in the human brain, the enzymes mediating these reactions, their localization, and the putative effects of neurosteroids. The presence of the steroidogenic enzymes cytochrome P450<sub>SCC</sub>, aromatase, 5 $\alpha$ -reductase, 3 $\alpha$ -hydroxysteroid dehydrogenase, and 17 $\beta$ -hydroxysteroid dehydrogenase in the human brain has now been firmly established by molecular biological and biochemical studies. 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 neuroactive steroids in the brain. The following functions are attributed to specific neurosteroids: modulation of GABA<sub>A</sub>, N-methyl-D-aspartate (NMDA), nicotinic, muscarinic, serotonin (5-HT<sub>3</sub>), kainate, glycine and sigma receptors, neuroprotection and induction of neurite outgrowth, dendritic spines, and synaptogenesis. We still do not know whether and how the steroidogenic enzymes are involved in the pathophysiology of the nervous system. The first clinical investigations in humans produced evidence for an involvement of neuroactive steroids in conditions such as fatigue during pregnancy, premenstrual syndrome, postpartum depression, catamenial epilepsy, and depressive disorders. Further and improved knowledge of the biochemical pathways of neurosteroidogenesis and their actions on the brain may enable new perspectives in the understanding of the physiology of the human brain as well as in the pharmacological treatment of its disturbances.

**KEYWORDS:** neurosteroid; human brain; 5 $\alpha$ -reductase; 3 $\alpha$ -hydroxysteroid dehydrogenase; aromatase; 17 $\beta$ -dehydrogenase

## INTRODUCTION

Steroid hormones are mainly synthesized in the gonads, the adrenal glands, and the fetoplacental unit. The brain is an important target organ of steroid hormones. In the brain, an extensive steroid metabolism occurs. Also, several brain regions are well equipped with enzymes necessary for steroid hormone biosynthesis.<sup>1-4</sup> Development, growth, maturation, and differentiation of the brain are strongly influenced by steroid hormones. As shown in animal studies, steroids synthesized *de novo* in the

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central nervous system (i.e., neurosteroids) can affect multiple brain functions (i.e., neuroendocrine and behavioral functions) via intracellular receptors which regulate transcriptionally directed changes in protein synthesis. In addition to the classical genomic actions of steroids, neurosteroids are able to rapidly alter excitability of the central nervous system through binding to neurotransmitter-gated ion channels, thus modulating  $\gamma$ -aminobutyric acid A (GABA<sub>A</sub>) and *N*-methyl-D-aspartate receptors.<sup>5,6</sup>

In the case of aromatase, the activity of steroidogenic enzymes was identified in human fetal brain tissue 25 years ago.<sup>7</sup> However, the majority of biochemical, physiological, and behavioral studies on aromatase in brain tissue were carried out in rodents or other animal species. For a long time, studies in humans have been precluded owing to the difficulty in obtaining fresh human brain tissue, coupled with presumably low expression or activity of the respective enzymes. This also applies to other steroidogenic enzymes. Steroidogenesis requires a number of sequential enzymatic reactions to convert cholesterol to sex hormones, glucocorticoids, or mineralocorticoids. As 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, that is, the human brain. Reports on the expression and activity of the most important steroidogenic enzymes in the human brain have been published in recent years. The present paper reviews the current knowledge of steroid hormone metabolism within the human brain and the evidence we have for its importance.

## SYNTHESIS AND METABOLISM OF STEROIDS IN THE HUMAN BRAIN

### *Cytochrome P450<sub>scc</sub>*

Expression of P450<sub>scc</sub> (cytochrome P450<sub>scc</sub>, CYP11A1), induces *de novo* synthesis of neurosteroids, since it is the single enzyme mediating the conversion of the steroid precursor, cholesterol, to pregnenolone. Human P450<sub>scc</sub> is encoded by a single gene on chromosome 15, the CYP11A1 gene.<sup>8</sup> Not only is P450<sub>scc</sub> present in the adrenal glands and gonads, the major sources of steroid hormone production, but it is also present in the placenta, primitive gut, and brain.<sup>9-11</sup> Once pregnenolone is produced from cholesterol, it may be converted to progesterone and other neuroactive steroids. However, the major role of P450<sub>scc</sub> in the brain is probably the regulation of brain neurosteroid levels.<sup>12</sup> We recently 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 patients with medically intractable chronic temporal lobe epilepsy.<sup>13,14</sup> In these brain areas, CYP11A1 mRNA was expressed in significant amounts in all tissue samples investigated, but at a rate  $\approx$  200 times lower than in adrenal tissue, which is known for 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.<sup>11,12,15</sup> In humans, CYP11A1 mRNA concentrations in the temporal lobe increase markedly during childhood and reach adult levels at puberty.<sup>13</sup> In the temporal and frontal neocortex as well as in the hippocampus of women CYP11A1

mRNA concentrations were significantly higher when compared to those of men.<sup>13,14</sup> An age- and sex-dependent expression of CYP11A1 mRNA in the human brain could be demonstrated with these data for the first time. 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.<sup>11,16</sup> On account of 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 called for. Whereas *in situ* hybridization and cell culture experiments in rat brain demonstrated predominant CYP11A1 expression in the subcortical white matter,<sup>15,17</sup> no such differences could be detected between neocortex and subcortical white matter tissue in the human brain.<sup>13</sup> Evidence that pregnenolone can be produced in the central nervous system is provided by the presence of CYP11A1 mRNA in human brain tissue.

#### *Aromatase*

Cytochrome P450 aromatase catalyses the conversion of androgens into estrogens in specific brain areas.<sup>3</sup> It is the product of the CYP19 gene, which has been cloned and sequenced.<sup>18,19</sup>

Only in a few fetal brain specimens has aromatase activity itself been determined.<sup>20-22</sup> Previously published data demonstrated aromatase activity in human temporal and in frontal brain areas.<sup>23</sup> The authors studied biopsy materials removed at autopsy from normal adult control subjects and from patients with Alzheimer's disease. Regardless of sex and/or disease state, temporal aromatase activity was always significantly higher than frontal aromatase activity. 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 medically intractable chronic epilepsy undergoing neurosurgery.<sup>24</sup> 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.<sup>24,25</sup> No sex-specific differences in CYP19 mRNA expression could be observed in any of these brain areas. In our laboratory, we were able to characterize aromatase activity in the temporal lobe in brain tissue specimens of a similar cohort of patients with epilepsy.<sup>26</sup> 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.<sup>27</sup> Compared to its high activity in the placenta, aromatase activity in the human brain was low. However, rates of aromatase activity in the brain were in the same order of magnitude as in human adipose and testicular tissue.<sup>28,29</sup> Through subsequent experiments with cerebral neocortex and subcortical white matter specimens of children and adults a significantly higher aromatase activity in the cerebral neocortex than in the subcortical white matter was revealed.<sup>26</sup> For CYP19 mRNA expression in the human temporal lobe this difference could not be found.<sup>25</sup> However, in the human temporal neocortex, CYP19 mRNA concentrations were significantly lower in children than in adults.<sup>25</sup> This finding could not be confirmed by measurement of aromatase activity.<sup>26</sup> These contradictory findings indicate that aromatase might be regulated on the post-translational level.

### *5 $\alpha$ -Reductase*

Numerous animal studies have shown that, in the brain, progesterone is rapidly metabolized to 5 $\alpha$ -dihydroprogesterone (5 $\alpha$ -DHP). This is then further reduced to the potent neurosteroid 3 $\alpha$ ,5 $\alpha$ -tetrahydroprogesterone (3 $\alpha$ ,5 $\alpha$ -THP).<sup>6</sup> These conversions are catalyzed by 5 $\alpha$ -reductase and 3 $\alpha$ -hydroxysteroid dehydrogenase (3 $\alpha$ -HSD). In humans, two isozymes of 5 $\alpha$ -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.<sup>30,3</sup>

The majority of physiological and biochemical studies on the expression of 5 $\alpha$ -reductase in the brain were carried out in rodents and other animal species.<sup>1,2,32,33</sup> However, some investigators documented 5 $\alpha$ -reductase activity in human fetal brain.<sup>34-36</sup> Only in a few frontal lobe and temporal lobe tissue specimens was 5 $\alpha$ -reductase activity demonstrated in the brain of adults.<sup>37,38</sup>

The predominant expression of 5 $\alpha$ -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 medically intractable chronic temporal lobe epilepsy was recently demonstrated by us.<sup>39,40</sup> The expression levels were about 100 times lower than in human liver tissue. 5 $\alpha$ -reductase type 2 mRNA was not expressed. Another study reported on 5 $\alpha$ -reductase type 1 mRNA expression in a few human cerebellum, hypothalamus, and pons tissue specimens that were collected post mortem.<sup>41</sup> Also, in rat brain, a predominant expression of 5 $\alpha$ -reductase type 1 mRNA was found.<sup>2,42</sup>

We also measured 5 $\alpha$ -reductase activity in human temporal neocortex and subcortical white matter tissue specimens.<sup>39,43</sup> 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. We also investigated the inhibitory effects of MK386, a specific inhibitor of the 5 $\alpha$ -reductase type 1 isoform, and of finasteride, a specific inhibitor of the 5 $\alpha$ -reductase type 2 isoform on 5 $\alpha$ -reductase activity.<sup>43</sup> MK386 was a strong inhibitor of human brain tissue 5 $\alpha$ -reductase activity, with an IC<sub>50</sub> value of 2.0 nmol/l, whereas finasteride turned out to be a poor inhibitor of the reaction, with an IC<sub>50</sub> value of 142.8 nmol/l.<sup>43</sup> Moreover, we observed a potent inhibition of the pH-dependent reaction by MK386, but not by finasteride. An at least predominant activity of the 5 $\alpha$ -reductase type 1 isozyme in the human brain is further substantiated by these findings.<sup>43</sup> There were no sex-specific differences in the expression levels of 5 $\alpha$ -reductase type 1 mRNA in human brain tissue or in the activity of 5 $\alpha$ -reductase.<sup>39,40,43</sup> These findings are consistent with previous animal studies, where no significant sex-specific differences concerning 5 $\alpha$ -reductase activity were found in rat brain.<sup>44,45</sup>

### *3 $\alpha$ -Hydroxysteroid Dehydrogenase*

Multiple cDNAs encode proteins related to 3 $\alpha$ -HSD in humans.<sup>46</sup> However, at least four 3 $\alpha$ -HSD isozymes exist which share at least 84% of its amino acid sequence identity.<sup>47-50</sup> These are known as type 1 3 $\alpha$ -HSD (AKR1C4), type 2 3 $\alpha$ -HSD (AKR1C3), type 3 3 $\alpha$ -HSD (AKR1C2), and 20 $\alpha$ (3 $\alpha$ )-HSD (AKR1C1). This isoform is predominantly a 20 $\alpha$ -HSD, and this change in positional specificity implies that it may play an important role in regulating progesterone action.<sup>50</sup>

Penning and co-workers demonstrated that all human  $3\alpha$ -HSD isoforms and the human  $20\alpha$ -HSD act as 3-, 17- and 20-ketosteroid reductases as well as 3-, 17- and 20-hydroxysteroid oxidases.<sup>50</sup>

In a recent study, we could only demonstrate the expression of the mRNA of type 2 and 3 isozyme of  $3\alpha$ -HSD as well as  $20\alpha$ -HSD in the hippocampus and the temporal lobe of patients with temporal lobe epilepsy, whereas the mRNA of the type 1 isozyme of  $3\alpha$ -HSD was not expressed.<sup>40,43</sup> The expression levels of  $3\alpha$ -HSD 2 were about one-fifth of that in liver tissue, those of  $3\alpha$ -HSD 3 about one-tenth of that in liver tissue, and those of  $20\alpha$ -HSD were about 2% percent ( $\approx 1/40$ ) of that in liver tissue (own unpublished data). The expression levels of  $3\alpha$ -HSD 2 and 3 as well as  $20\alpha$ -HSD mRNAs did not differ significantly between hippocampal tissue from epileptic men and hippocampal tissue of women. This is in accordance with data on  $3\alpha$ -HSD activity in the rat brain.<sup>40</sup>

All of these three isoforms,  $3\alpha$ -HSD 2 and 3 and  $20\alpha$ -HSD, are capable of producing the neuroactive tetrahydrosteroids that modulate the GABA<sub>A</sub> receptor.<sup>50</sup> Consequently, the meaning of the differential expression of the single isoforms is less established than ever.

### *17 $\beta$ -Hydroxysteroid Dehydrogenase*

Seven human isozymes of 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD) have been cloned so far. They all play a major role in the regulation of the biological activity of sex hormones and they are essential for the biosynthesis of the strong androgens and estrogens testosterone and estradiol from their weaker precursors androstenedione and estrone.<sup>51,52</sup> These conversions are reversible and thus can lead to a deactivation of the respective sex hormones.<sup>53</sup> The different isozymes show an individual cell-specific expression and substrate specificity. The ubiquitous distribution of 17 $\beta$ -HSD in peripheral tissues reflects the importance of the 17 $\beta$ -HSD activity in the maintenance of physiological levels of estradiol and testosterone.<sup>54</sup>

17 $\beta$ -HSD activity in the human brain has been reported about 30 years ago.<sup>37,55</sup> However, only very few studies on the expression of the enzyme in the human brain exist to date. Western immunoblot analysis revealed the presence of 17 $\beta$ -HSD 1 in human fetal brain.<sup>56</sup> Recently, we demonstrated the expression of 17 $\beta$ -HSD 1, 3, 4 and 5 mRNA in the human temporal lobe and hippocampus.<sup>57,58</sup> An in-tandem pseudogene of 17 $\beta$ -HSD 1 and 17 $\beta$ -HSD 2 mRNA were not expressed.<sup>57,58</sup> We also characterized androgenic and estrogenic 17 $\beta$ -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.<sup>59</sup> Substrate specificity, pH optima, cofactor requirement patterns, and kinetic properties suggest the activity of at least two isozymes, namely, the activating 17 $\beta$ -HSD 3 and the deactivating 17 $\beta$ -HSD 4, in the human brain. The activity of 17 $\beta$ -HSDs and the expression levels of the mRNAs did not differ significantly between the sexes. However, the expression levels of 17 $\beta$ -HSD 3, 4 and 5 mRNAs as well as the conversion of androstenedione, testosterone, estrone, and estradiol were significantly higher in the subcortical white matter than in the cerebral neocortex.<sup>57-59</sup> The predominant expression of 17 $\beta$ -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 my-

elin. This suggests a possible correlation between sex steroids, these enzymatic activities, and the formation or functions of myelin.

In a recent study on the human 17 $\beta$ -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.<sup>52</sup> To date, 17 $\beta$ -HSD 8 expression has not been investigated in the human brain.

### *Other Steroidogenic Enzymes*

Other important steroidogenic enzymes are 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD), cytochrome P450<sub>c17</sub>, 21-hydroxylase (cytochrome P450c21), 11 $\beta$ -hydroxylase (cytochrome P45011 $\beta$ ) and cytochrome P450 aldosterone synthetase (P-450aldo).

3 $\beta$ -HSD catalyzes the conversion of  $\Delta^5$ -3 $\beta$ -hydroxysteroids into  $\Delta^4$ -3-ketosteroids (i.e., the conversion of pregnenolone into progesterone). Cytochrome P450<sub>c17</sub>, which possesses both 17 $\alpha$ -hydroxylase and 17,20 lyase activity, is responsible for the conversion of C<sub>21</sub> steroids (pregnenolone, progesterone) into C<sub>19</sub> steroids (DHEA and androstenedione).

21-hydroxylase converts progesterone to 11-deoxycorticosterone and 17-hydroxyprogesterone to 11-deoxycortisol. These are the substrates required for the production of the main adrenal steroids, corticosterone, aldosterone and cortisol. 11 $\beta$ -hydroxylase (cytochrome P45011 $\beta$ ) catalyzes the formation of glucocorticoids (cortisol and corticosterone). Cytochrome P450 aldosterone synthetase (P-450aldo), which exerts three enzyme activities (11 $\beta$ -hydroxylation, 18-hydroxylation, and 18-oxidoreduction), catalyzes the formation of mineralocorticoids (aldosterone).

Only a small number of studies on the expression of 21-hydroxylase in the brain exist to date. In rodents, 21-hydroxylase was detected in the brain stem using the reverse transcription polymerase chain reaction assay and immunohistochemical methods.<sup>60,61</sup> Other investigators could not find 21-hydroxylase mRNA in any extra-adrenal tissue.<sup>62</sup> Since 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.<sup>63</sup> Our study demonstrated for the first time the expression of 21-hydroxylase mRNA in the human hippocampus. In the hippocampus, the expression levels are approximately 10,000 times lower than in the adrenal gland, which is known for high 21-hydroxylase expression.<sup>63</sup> However, we were unable to measure the enzyme activity of 21-hydroxylase as only small amounts of tissue specimens were available. 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 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD) 1 and 2 as well as cytochrome P45011 $\beta$  and cytochrome P450 aldosterone synthetase were neither expressed in the human temporal lobe nor in hippocampus (our own unpublished data). For these investigations a sensitive, nested competitive RT-PCR assay was used. However, several studies demonstrated the expression of 3 $\beta$ -HSD mRNA<sup>15,64,65</sup> and 3 $\beta$ -HSD protein<sup>65</sup> in the rat brain. Data concerning the expression of cytochrome P45011 $\beta$  in rodent brain are conflicting: some authors report the expression through-

out the rat brain,<sup>61,66</sup> while others found only low expression levels in rat brain<sup>11,67</sup> or no expression in mouse brain.<sup>61</sup> In various regions of rat brain, including hypothalamus, hippocampus, amygdala and cerebellum, cytochrome P450 aldosterone synthetase expression and activity have been demonstrated.<sup>66,68</sup>

Cytochrome P450c<sub>17</sub> mRNA was not expressed in the human temporal lobe or hippocampus (our own unpublished data). Previous studies failed to demonstrate 17 $\alpha$ -hydroxylase activity or P450c17 mRNA in the adult rat brain.<sup>11,69</sup> However, P450c17 mRNA as well as P450c17 protein were detected in the brain of rat embryos using ribonuclease protection assays and immunocytochemistry.<sup>70</sup> In adults the data reported have been conflicting: Compagnone and co-workers<sup>70</sup> reported expression of P450c17 mRNA only in the peripheral nervous system of rats and mice; others demonstrated the presence of P450c17 mRNA in various brain regions of adult rodents.<sup>61</sup>

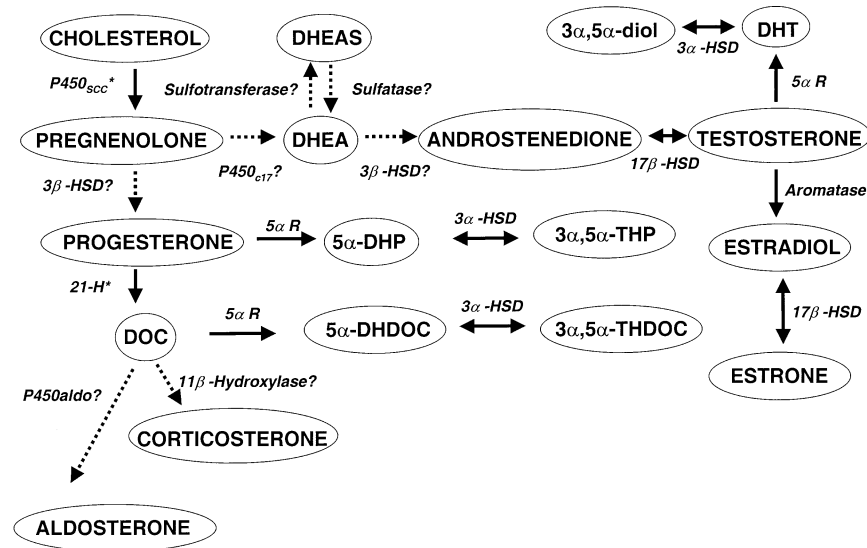
### CLINICAL IMPLICATIONS

The presence of the above-mentioned steroidogenic enzymes cytochrome P450<sub>SCC</sub>, aromatase, 5 $\alpha$ -reductase, 3 $\alpha$ -hydroxysteroid dehydrogenase, and 17 $\beta$ -hydroxysteroid dehydrogenase in human brain has now been firmly established by molecular biological and biochemical studies. These findings provide evidence that neuroactive steroids can be produced within the human brain. However, the (patho)physiological significance of these findings remains to be elucidated. FIGURE 1 presents 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. These reproductive and neuroendocrine actions of steroids via intracellular receptors, which regulate transcriptionally directed changes in protein synthesis generally occur within hours or days. In addition to the classic sites of steroid synthesis, neurosteroids can rapidly alter the excitability of the central nervous system by modulating neurotransmitter-gated ion channels such as  $\gamma$ -aminobutyric acid A (GABA<sub>A</sub>) and *N*-methyl-D-aspartate (NMDA) receptors.<sup>5,6,69</sup>

GABA, a major inhibitory neurotransmitter, mediates fast synaptic inhibition by activating ligand-gated chloride channels. Binding of 3 $\alpha$ -reduced neurosteroids to GABA<sub>A</sub> receptors results in either inhibition or potentiation of the inhibitory effects of GABA (FIG. 2). Therefore, anticonvulsive, anaesthetic and anxiolytic effects of neuroactive steroids are mediated by their capacity to positively modulate GABA<sub>A</sub> receptor function (i.e., these substances act to increase GABA-ergic effects by increasing frequency and duration of chloride channel openings).<sup>5,6</sup> On the other hand, inhibition of GABA<sub>A</sub> receptor function, which is mostly documented for the neurosteroids pregnenolone sulfate and DHEAS, produces effects ranging from anxiety and excitability to seizure susceptibility.<sup>71-73</sup>

Other neurosteroid actions have been described in the brain including the inhibition of *N*-methyl-D-aspartate (NMDA) receptor function as well as the modulation of other receptors, such as nicotinic, muscarinic, serotonin (5-HT<sub>3</sub>), kainate, glycine and sigma receptors.<sup>74-79</sup> In summary, neurosteroids exert both genomic and nongenomic effects, and regulate neuronal function via their concurrent influence on gene



**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<sub>scc</sub>* 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, deoxicorticosterone; DHT, dihydrotestosterone; 5α-DHP, 5α-dihydroprogesterone; 3α,5α-THP, 3α,5α-tetrahydroprogesterone (allopregnanolone); 5αR, 5α-reductase; 3α-HSD, 3α-hydroxysteroid dehydrogenase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 21-H, 21-hydroxylase.

expression and transmitter-gated ion channels. These actions suggest that neurosteroids play a crucial role in mediating many brain functions.

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

As early as 1941,<sup>80</sup> the potential anaesthetic properties of neuroactive steroids had been suggested. This led to the development of steroid anaesthetics such as alphaxalone.<sup>81</sup> However, side effects have hindered the development of steroid anaesthetics for routine clinical use.<sup>71</sup>

Apparently, the observation that epileptic seizures in cycling women are less frequent in the luteal phase, when circulating levels of progesterone are high, is associated with cyclical variations in the metabolism of progesterone to allopregnanolone in the brain.<sup>6,82,83</sup> Progesterone and 3α-reduced neuroactive steroids have potent anticonvulsant effects.<sup>84,85</sup> Synthetic derivatives of neuroactive steroids are under investigation for treatment of epilepsy disorders. Some preliminary investigations in healthy volunteers and in patients with medically intractable epi-

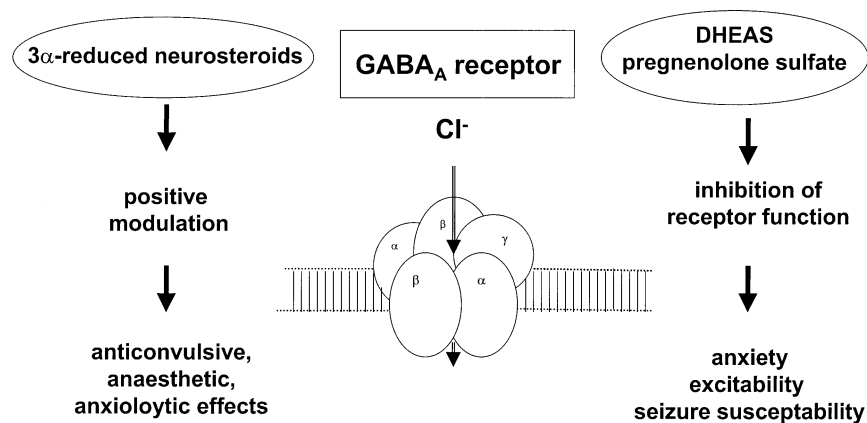


FIGURE 2. Effects of neurosteroids on GABA<sub>A</sub> receptor function.

lepties have already been undertaken. Ganaxolone, for example, showed a promising pharmacokinetic profile and was well tolerated in a trial with healthy volunteers.<sup>86,87</sup> In clinical studies in patients with epilepsy it was also shown to be effective.<sup>88</sup> Although promising, potential side effects call for caution. For example, progesterone and 3α,5α-THP have benzodiazepine-like effects,<sup>84,85</sup> and progesterone withdrawal may lead to an increase in seizure susceptibility.

Through the development of sensitive assays to measure cerebral fluid or blood neurosteroid concentrations researchers have been enabled 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 to healthy control subjects. Also, clinically effective antidepressant treatment was accompanied by an increase of 3α,5α-tetrahydroprogesterone in the plasma of these patients.<sup>89</sup>

Neuroactive steroids may also be involved in physiological conditions where fluctuations of the hormonal balance occur. For example, increased fatigue during pregnancy may be the result of higher concentrations of progesterone and GABA agonistic 3α-reduced neuroactive steroids such as 3α,5α-THP.<sup>90</sup> On the other hand, a rapid decline in these substances may lead to the premenstrual syndrome or postpartum depression.<sup>91,92</sup> 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 postpartum period, and around menopause.

DHEA and DHEAS are the most abundant circulating steroid hormones in humans. Their concentrations decrease with age and under stress.<sup>93,94</sup> It was hypothesized that DHEA and DHEAS may be neuroprotective agents as both age and stress are associated with neuronal vulnerability to degeneration. Indeed, neuroprotection by DHEA and DHEAS was observed *in vivo* in hippocampal structures.<sup>95</sup> The mechanisms by which DHEA and DHEAS act are still unknown. In patients with Alzheimer's disease and multi-infarct dementia, decreased DHEAS concentra-

tions have also been reported.<sup>96–98</sup> So far 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.<sup>99–101</sup> However, high-quality trials are required with the duration of DHEA treatment in excess of a few weeks and with a large enough number of participants to detect possible effects. In such trials, the outcome measures must include objective tests of cognitive function.

## CONCLUSIONS

It has been firmly established by molecular biological and biochemical studies that several key enzymes of steroidogenesis, namely cytochrome P450<sub>SCC</sub>, aromatase, 5 $\alpha$ -reductase, 3 $\alpha$ -hydroxysteroid dehydrogenase, and 17 $\beta$ -hydroxysteroid dehydrogenase, are present in human brain (FIG. 1). Whether and how the steroidogenic enzymes are involved in the pathophysiology of the nervous system we still do not know. However, clinical investigations in humans are now providing evidence for an involvement of neuroactive steroids in conditions such as fatigue during pregnancy, premenstrual syndrome, postpartum depression, catamenial epilepsy, and depressive 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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