

## Neurosteroids: Biosynthesis and Function of These Novel Neuromodulators

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Over the past decade, it has become clear that the brain is a steroidogenic organ. The steroids synthesized by the brain and nervous system, given the name *neurosteroids*, have a wide variety of diverse functions. In general, they mediate their actions, not through classic steroid hormone nuclear receptors, but through ion-gated neurotransmitter receptors. This paper summarizes what is known about the biosynthesis of neurosteroids, the enzymes mediating these reactions, their localization during development and in the adult, and their function and mechanisms of action in the developing and adult central and peripheral nervous systems. The expression of the steroidogenic enzymes is developmentally regulated, with some enzymes being expressed only during development, while others are expressed during development and in the adult. These enzymes are expressed in both neurons and glia, suggesting that these two cell types must work in concert to produce the appropriate active neurosteroid. The functions attributed to specific neurosteroids include modulation of GABA<sub>A</sub> and NMDA function, modulation of  $\sigma$  receptor function, regulation of myelination, neuroprotection, and growth of axons and dendrites. Neurosteroids have also been shown to modulate expression of particular subunits of GABA<sub>A</sub> and NMDA receptors, providing additional sites at which these compounds can regulate neural function. The pharmacological properties of specific neurosteroids are described, and potential uses of neurosteroids in specific neuropathologies and during normal aging in humans are also discussed. © 2000 Academic Press

### WHAT IS A NEUROSTEROID?

The concept that steroids could be synthesized *de novo* in the brain derived from observations made in the 1980s by Baulieu and colleagues. They found that steroids such as pregnenolone, DHEA,<sup>1</sup> and their sulfate and lipoidal

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<sup>1</sup> Abbreviations used: Steroidogenic enzymes. P450<sub>scc</sub>, cytochrome P450 side chain cleavage; P450<sub>cx</sub>, cytochrome P450 x hydroxylase ("x" refers to the carbon position at which the hydroxylation occurs); P450<sub>c11AS</sub>, aldosterone synthase; P450<sub>aro</sub>, aromatase; StAR, steroidogenic acute regulatory protein; b5, cytochrome b5; HSD, hydroxysteroid dehydrogenase; STS, steroid sulfohydrolase or steroid sulfatase; HST, hydroxysteroid sulfotransferase. Steroids. Allopregnanolone, 3 $\alpha$ , 5 $\alpha$ -tetrahydroprogesterone or 5 $\alpha$ -pregnan-3 $\alpha$ -ol-20-one; DHEA, dehydroepiandrosterone; DHEAS,

esters were present in higher concentrations in tissue from the nervous system (brain and peripheral nerve) than in the plasma. Furthermore, steroids remained in the nervous system long after gonadectomy or adrenalectomy (43, 44). These results suggested that steroids either might be synthesized *de novo* in the CNS and PNS or might accumulate in those structures. Such steroids were named "neurosteroids" to refer to their unusual origin and to differentiate them from steroids derived from more classical steroidogenic organs, such as gonads, adrenals, and placentae. The finding of steroids in the brain was striking. To test whether steroids were actually made in the brain or if they accumulated specifically in tissue from the nervous system, several laboratories, including ours, determined directly if enzymes known to be involved in steroidogenesis adrenals, gonads, and placentae could be responsible for neurosteroids synthesis. Results from several laboratories over the past decade have established unequivocally that the enzymes found in classic steroidogenic tissues are indeed found in the nervous system. However, the nervous system contains additional steroid modifying enzymes as well.

#### **Enzymes Involved in Neurosteroidogenesis: Biochemistry and Molecular Biology**

Enzymes involved in neurosteroidogenesis can be classified in two main groups: the cytochrome P450 group and the non-P450 group (Table 1). Neurosteroidogenic enzymes, P450s and non-P450, are mitochondrial or microsomal.

Cytochromes P450 are oxidases which all function in an identical manner. While they can be defined as heme-binding monooxygenases, they are able to catalyze the oxidative conversion of many steroids, lipids, and a variety of xenobiotics and environmental toxins [reviewed in (152)]. Steroidogenic P450s are unusual, in that they have limited, and specific, steroidal substrates. They reduce atmospheric oxygen with electrons from NADPH, which requires the action of specific cofactors, adrenodoxin reductase and adrenodoxin for mitochondrial P450s and P450-reductase and b5 for microsomal P450s.

Most of the enzymes present in the adrenals, gonads, and placenta have been found in the brain by measuring their enzymatic activity and/or their mRNA transcript level and/or their protein expression. The biosynthetic pathway of

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dehydroepiandrosterone sulfate DHP, 20 $\alpha$ -dihydroprogesterone or 4-pregnen-20 $\alpha$ -ol-3-one; DOC, 11-deoxycorticosterone; DHT, 5 $\alpha$ -dihydrotestosterone. Hormones and neurotransmitters. ACTH, adrenocorticotrophic hormone; GABA,  $\gamma$ -aminobutyric acid; NMDA, *N*-methyl-D-aspartic acid. Chemicals and enzymes. CNPase, 2',3'-cyclic nucleotide 3'-phosphodiesterase; D-AP5, D(-)-2-amino-5-phosphonopentanoic acid; MAP2, microtubule-associated protein type 2; MK801, (5R,10S)-(+)-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; PAPS, 3'-phosphoadenosine 5'-phosphosulfate. Other abbreviations used. CNS, central nervous system; CSF, cerebrospinal fluid; DRG, dorsal root ganglia; EPSCs, evoked excitatory postsynaptic currents; GR, glucocorticoid receptor; HPA/HPG, hypothalamic-pituitary-adrenal axis and hypothalamic-pituitary-gonadal axis; LPT, long term potentiation; MR, mineralocorticoid receptor; PMS, premenstrual syndrome; PNS, peripheral nervous system.

TABLE 1

## Classification of Steroidogenic Enzymes

Cytochrome P450	Cofactors	Non-P450 enzymes
Mitochondrial		
P450 <sub>scc</sub>	Adrenodoxin	3 $\beta$ -HSD
P450 <sub>11<math>\beta</math></sub>	Adrenodoxin reductase	11- $\beta$ HSD
P450 <sub>c11AS</sub>		17 $\beta$ -HSD
Microsomal		
P450 <sub>c17</sub>	b5	5 $\alpha$ -Reductase
P450 <sub>c21</sub>	P450-Reductase	3 $\alpha$ -HSD
P450 <sub>aro</sub>		STS
P450 <sub>7<math>\alpha</math>-Hydroxylase</sub>		HST
20 $\alpha$ -Hydroxylase		

neurosteroids is presented in Fig. 1. The synthesis of specific steroid hormones in the adrenals, gonads, placenta, and brain is dependent on the tissue-, cell-, and developmentally specific expression of these various enzymes. For example, P450<sub>c11 $\beta$</sub>  and P450<sub>c11AS</sub> are expressed in the adrenals and not in the gonads or placenta, resulting in glucocorticoid and mineralocorticoid production, while expression of P450<sub>c17</sub> in the testes results in androgen production, and P450<sub>aro</sub> expression in the gonads results in estrogen production.

As is evidenced in Fig. 1, many of the steroid hydroxylases have multiple enzymatic activities. However, purification of the proteins and cloning of the cDNAs encoding these proteins have rigorously demonstrated that these activities indeed reside within single proteins.

In the nervous system, there is not only region-specific expression of the steroidogenic enzymes, but there is also cell type-specific and developmental regulation of these enzymes, indicating a more complex scheme than that depicted in the figure. The synthesis of neurosteroids probably proceeds through some similar and some different pathways than those used in the adrenals, gonads, and placenta. The brain contains additional steroid metabolizing enzymes, including sulfotransferases and sulfohydrolases, that convert classic steroid hormones to a variety of neuroactive compounds.

We have modified the original definition of neurosteroids to include both neuroactive compounds produced *de novo* and steroids metabolized to neuroactive compounds in the brain but derived from circulating precursors. Albumin-bound steroids are freely cleared by the brain on a single pass (173, 174, 176). However, globulin-bound steroids, like testosterone or estradiol bound to the sex hormone-binding globulin or corticosterone bound to the corticosteroid-binding globulin, are not transported into the brain. However, progesterone bound to guinea pig progesterone-binding globulin (PBG) can be transported into the brain, suggesting that the half time of progesterone dissociation from its binding globulin (1.8 s) is similar to the brain capillary transit time (0.1 to 1.0 s) (175). Testosterone binds to the progesterone-binding globulin even less tightly than does progesterone. Thus, a substantial fraction of plasma proges-



terone (10%) or testosterone (25%), bound to PBG, can still be transported into the brain. Gonadal steroids, such as progesterone, 17-hydroxyprogesterone, testosterone, and estradiol, are sequestered in the brain, since their brain/plasma concentrations are greater than 1 (134, 177). Unlike gonadal steroids, corticosterone is not sequestered in the brain. There may also be a regional brain distribution of steroids derived from the plasma (256). Neuroactive steroids are considered inactive metabolites in the adrenals, gonads, and placentae and include  $5\alpha$ -DHP and allopregnanolone, which could be derived from both brain-synthesized and gonadal progesterone, as well as DHEA. They meet the definition of neuroactive hormones in the CNS and PNS.

Other enzymatic activities giving rise to steroidal compounds have been described in the brain of several species. Hydroxylation and accumulation of  $20\alpha$ - and  $7\alpha$ -hydroxylated metabolites of progesterone ( $20\alpha$ ) and pregnenolone ( $20\alpha$  and  $7\alpha$ ) have repeatedly been observed in the fetal and adult rodent brain (2, 201). In most cases, the expression of the steroidogenic enzymes in the CNS and PNS is developmentally, regionally, and cell-specifically regulated, ensuring the regulated synthesis of specific neurosteroids.

A previous review in *Frontiers in Neuroendocrinology* summarized the existing data that demonstrated that the brain expressed steroidogenic enzymes and synthesized steroids (257). At that time, the sites of expression of only a few enzymes were known, and functions for neurosteroids included only modulation of GABA<sub>A</sub> function. In the 4 years since that publication, the sites of expression and ontogeny of most of the neurosteroidogenic enzymes have been determined, and many more novel functions for neurosteroids have been demonstrated. Still lacking, however, is a rigorous demonstration of the particular steroids synthesized within a specific brain region and at particular times in development. We will review what is known about each of the steroidogenic enzymes that are crucial for the synthesis of the neuroactive steroids (progesterone, pregnenolone, DHEA, allopregnanolone, and their sulfated moieties) in different species and at different developmental stages.

### *P450scc*

The first, rate-limiting, and hormonally regulated step in the synthesis of all steroid hormones is the conversion of cholesterol to pregnenolone. This reaction is catalyzed by the mitochondrial enzyme cholesterol side chain cleavage, P450scc, in three successive chemical reactions:  $20\alpha$ -hydroxylation,  $22$ -hydroxylation, and scission of the  $c20$ – $c22$  carbon bond cholesterol. The products of this reaction are pregnenolone and isocaproic acid. The same P450scc is found in all steroidogenic tissue and in the brain (146, 147).

P450scc is the rate-limiting step in steroidogenesis and is one of the slowest enzymes known, with a  $V_{\max}$  of 1 mol cholesterol/mol enzyme/s. The slowest part of this reaction may be the entry of cholesterol into the mitochondria and its binding to the active site of P450scc.

The human and rat genome contains a single gene encoding P450scc (36, 137,

162, 171), which is about 20 kb long, contains nine exons, and in humans is located on chromosome 15. This gene encodes a mRNA of about 2.0 kb, which encodes a 521-amino-acid protein. This protein is proteolytically cleaved, removing a 39-amino-acid leader peptide that directs the protein to the mitochondria.

### *Adrenodoxin Reductase/Adrenodoxin*

P450<sub>scc</sub> functions as the terminal oxidase in a mitochondrial electron transport system. As described above, electrons from NADPH are first accepted by a flavoprotein, adrenodoxin reductase, which is located in the mitochondrial matrix and is loosely associated with the inner membrane (98, 164, 169). Adrenodoxin reductase transfers the electrons to an iron/sulfur protein, adrenodoxin, located in the mitochondrial matrix. Adrenodoxin first forms a complex with adrenodoxin reductase, dissociates after oxidation, and then binds to P450<sub>scc</sub> (or to the other mitochondrial P450s, P450c11 $\beta$ , P450c11AS, and P450c11B3). These proteins are also often called "ferredoxin oxidoreductase" and "ferredoxin." In humans, there is one gene encoding adrenodoxin reductase, found on chromosome 17, and multiple functional adrenodoxin genes on chromosome 11, encoding identical mRNAs and proteins, and two nonfunctional adrenodoxin pseudogenes on chromosome 20. Adrenodoxin, but not adrenodoxin reductase, is transcriptionally regulated by tropic hormones, acting through cAMP.

### *StAR*

A novel protein, steroidogenic acute regulatory protein, StAR, was identified as important for movement of cholesterol into the mitochondria in an acute steroidogenic response (37, 230). In the adrenal and gonads StAR expression and function are critical for steroidogenesis since individuals who are homozygous for mutations that inactivate StAR have a marked impairment in adrenal and gonadal steroidogenesis, but not in placental steroidogenesis (20, 120). Patients with congenital lipoid adrenal hyperplasia do not appear to have neurological defects, suggesting that StAR is not necessary for neurosteroidogenesis, as it is unnecessary for placental steroidogenesis (204). Nevertheless, StAR has been recently identified in the rodent brain, suggesting that it may play a role in regulating neurosteroidogenesis (64).

### *3 $\beta$ -HSD*

Pregnenolone produced from cholesterol can undergo one of two conversions: it may be 17 $\alpha$ -hydroxylated to 17 $\alpha$ -hydroxypregnenolone by P450c17 (see below) or converted to progesterone by the enzyme 3 $\beta$ -HSD. 3 $\beta$ -HSD has two distinct enzymatic activities: 3 $\beta$ -dehydrogenation and isomerization of the double bond from C5,6 in the B ring ( $\Delta$ 5-steroids) to C4,5 in the A ring

( $\Delta 4$ -steroids) (121, 123, 238). This enzyme is encoded by multiple distinct genes, located on chromosome 1, that are expressed in a tissue-specific manner. There are at least two forms of human  $3\beta$ -HSD and at least four forms of rodent  $3\beta$ -HSD. The human  $3\beta$ -HSD type I gene is expressed in the placenta, skin, mammary gland, and other tissues, including the brain, while a distinct human type II  $3\beta$ -HSD gene is expressed in adrenals and gonads. In rats, it is unknown whether the type I  $3\beta$ -HSD isoform alone or additional isoforms are expressed in the brain. The enzymes can be classified in two groups: those that function as dehydrogenase/isomerases and those that function as  $3$ -ketosteroid reductases.

### *P450c17*

Pregnenolone and progesterone may undergo  $17\alpha$ -hydroxylation to  $17\alpha$ -hydroxypregnenolone and  $17\alpha$ -hydroxyprogesterone. These steroids may then undergo scission of the c17,20 bond to form DHEA and androstenedione. All these four reactions are mediated by a single P450c17 enzyme. P450c17 is bound to the smooth endoplasmic reticulum and accepts electrons from P450-reductase. Since P450c17 has both  $17\alpha$ -hydroxylase and 17,20-lyase activities, it catalyzes a key branch point in steroidogenesis. In the human adrenal, the regional expression of P450c17 directs steroidogenesis to the production of mineralocorticoids, glucocorticoids, or sex steroids. In the human zona glomerulosa, P450c17 is not expressed and pregnenolone is metabolized to mineralocorticoids; in the human zona fasciculata, P450c17 is expressed but the majority of the activity is  $17\alpha$ -hydroxylase activity, and hence pregnenolone is directed to glucocorticoids; and in the zona reticularis, P450c17 has both  $17\alpha$ -hydroxylase and c17,20-lyase activities, and hence pregnenolone is metabolized into sex steroids. Several factors are important in determining whether a steroid will undergo 17,20 bond scission after 17-hydroxylation, such as the presence of the cofactors and the potential competition for substrate between P450c17 and  $3\beta$ -HSD. In the human adrenal,  $3\beta$ -HSD mRNA and activity are low in the zona reticularis and high in the zona fasciculata (46, 58, 253). In addition, there appears to be a gradient of *b5* expression in the human adrenal, with the highest concentration in the zona reticularis, indicating that P450c17 expressed in this zone would have greater lyase activity (see *b5* section below) (268). The  $17\alpha$ -hydroxylase reaction occurs more readily than the 17,20-lyase reaction. P450c17 prefers  $\Delta 5$  substrates, especially for 17,20 bond scission, accounting for the large concentrations of DHEA in the human adrenal.

The single human gene encoding P450c17 is located on chromosome 10 and contains eight exons (138).

### *P450-Reductase*

Both P450c17 and P450c21 receive electrons from a mitochondrial membrane-bound flavoprotein, P450 reductase, which is distinct from adrenodoxin reduc-

tase. P450-reductase receives electrons from NADPH and transfers them one at a time to the microsomal P450. The second electron can also be provided by cytochrome b<sub>5</sub> (see below). The amount of microsomal P450-reductase is less than that of both P450c<sub>21</sub> and P450c<sub>17</sub>, resulting in competition for this protein. Therefore, factors that influence the association of a specific P450 with the reductase will likely influence the pathway that will be followed, e.g., c<sub>17</sub> vs c<sub>21</sub> hydroxylation of progesterone or c<sub>17</sub>,<sub>20</sub>-lyase vs c<sub>21</sub>-hydroxylation. Electron abundance also influences the activity of P450c<sub>17</sub>; increasing electron abundance favors both 17 $\alpha$ -hydroxylase and 17,<sub>20</sub>-lyase activities, while limiting electron abundance favors only 17 $\alpha$ -hydroxylase activity. Therefore, the ratio of P450-reductase to P450c<sub>17</sub> seems to be critical in determining the pathway of steroidogenesis (120, 265, 267).

### *Cytochrome b<sub>5</sub>*

Cytochrome b<sub>5</sub> is a small heme-containing protein which supplies electrons for many cytochrome P450-catalyzed reactions in the liver and for the reduction of methemoglobin in erythrocytes. Cytochrome b<sub>5</sub> is found both in a soluble form, necessary for its function in blood cells, and as a microsomal electron donor, playing the role of cofactor for microsomal P450s. The cytochrome b<sub>5</sub> is of crucial importance in the biosynthesis of DHEA since it may control the 17,<sub>20</sub>-lyase activity of P450c<sub>17</sub> (101, 170). Recent work has shown that cytochrome b<sub>5</sub> specifically augments the 17,<sub>20</sub>-lyase activity of P450c<sub>17</sub> *in vitro* and that this augmentation is produced by an allosteric modulation of P450c<sub>17</sub>, not through electron donor (7). These results suggest that expression of b<sub>5</sub> may be a mechanism by which c<sub>17</sub>,<sub>20</sub>-lyase activity, and hence DHEA production, is regulated in specific regions of the brain. Recent studies have also indicated that cytochrome b<sub>5</sub> expression in the human adrenal may be zone-specific, as b<sub>5</sub> expression appears to be greater in the zona reticularis than in the zona fasciculata (268). This differential expression may account for zone-specific synthesis of glucocorticoids in the zona fasciculata versus c<sub>19</sub> steroids in the zona reticularis.

Cytochrome b<sub>5</sub> deficiency is associated with the development of two different type of diseases. In the Type I form, cyanosis is the only symptom, and the soluble enzyme is defective in red blood cells. In the Type II form, cyanosis is associated with severe mental retardation and neurologic impairment. The enzymatic defect is systemic involving both soluble and membrane-bound isoforms. Several mutations in cytochrome b<sub>5</sub> gene have been isolated from patients carrying the Type II methemoglobinemia (131, 252). Mental retardation in Type II methemoglobinemia was associated with a disruption of fatty acid metabolism in the brain, resulting in a lower content of cholesterol in the white matter (80). In addition to a diminution of the necessary cholesterol precursor for neurosteroidogenesis, one could also hypothesize that a b<sub>5</sub> deficiency in regions of the developing brain where P450c<sub>17</sub> is expressed could result in diminution of DHEA synthesis. This may result in abnormal axonal

growth in specific regions where we previously showed that DHEA promoted targeted axonal growth (see section Neurosteroids Modulate NMDA Receptors 40). If proven correct this hypothesis could explain the mental retardation not only by disturbance of fatty acid metabolism but also by the selective disruption of neurosteroidogenesis.

#### *P450c21 (and Extraadrenal 21-Hydroxylase Activity)*

Both progesterone and 17-hydroxyprogesterone can be hydroxylated at c21 to yield 11-deoxycorticosterone and 11-deoxycortisol by P450c21. This enzyme has been of great clinical interest because mutations in this enzyme result in congenital adrenal hyperplasia. P450c21 is found in the smooth endoplasmic reticulum. There are two P450c21 genes that lie in the middle of the HLA locus on human chromosome 6. Only one gene is functional in both human beings and mice, but both genes are expressed in cows.

21-Hydroxylase activity has been shown in a large number of extra-adrenal tissues, especially in the fetus and in pregnant women, resulting in the conversion of progesterone to 11-deoxycorticosterone (29, 30). Tissues with this activity include kidney, testis, ovary, skin, urinary bladder, pancreas, thymus, spleen, aorta, and brain. Analysis of RNA from various human fetal tissues using RNase protection assays demonstrated that P450c21 mRNA is not found in these tissues (149). Thus this activity is not mediated by P450c21. The presence of an additional enzyme with 21-hydroxylase activity is demonstrated by persistence of this activity even in those humans who lack a functional P450c21 gene. The enzyme responsible for this activity has not yet been identified.

#### *P450c11*

The final steps in the synthesis of glucocorticoids and mineralocorticoids are mediated by two distinct adrenocortical enzymes, P450c11 $\beta$  and P450c11AS, encoded by two different genes located on human chromosome 8. The conversion of 11-deoxycorticosterone and 11-deoxycortisol to corticosterone and cortisol is mediated by the mitochondrial 11 $\beta$ -hydroxylase P450c11 $\beta$ . This enzyme is found specifically in the zona fasciculata/reticularis, not in the zona glomerulosa, and is regulated by ACTH. P450c11AS, also referred to as aldosterone synthase, is found exclusively in the zona glomerulosa and has three distinct activities: 11 $\beta$ -hydroxylase, 18-hydroxylase, and 18-oxidase. It therefore converts 11-deoxycorticosterone to aldosterone. This enzyme is mainly regulated by the renin/angiotensin system. A third P450c11 gene, called P450c11B3, has been isolated from rat adrenals. P450c11B3 mRNA is only expressed during the early neonatal period and is not expressed in the fetal or adult adrenal. Like P450c11 $\beta$ , P450c11B3 is expressed in the zona fasciculata/reticularis and is regulated by ACTH. However, P450c11B3 has enzymatic activity intermediate between P450c11 $\beta$  and P450c11AS. It has both 11 $\beta$ - and 18-hydroxylase activi-

ties, but has no 18-oxidase activity and therefore can synthesize both corticosterone and 18-OH DOC (from DOC) and 18-OH corticosterone (from corticosterone). A gene corresponding to P450c11B3 has not been found in human beings (270) and has not been reported in other species.

### *17-Ketosteroid Reductase / 17 $\beta$ -HSD*

In the adrenal, DHEA is converted to  $\Delta$ 5-androstenediol and  $\Delta$ 4-androstenedione is converted to testosterone by 17-ketosteroid reductase (17KSR). In the ovary, estrone is converted to estradiol by similar mechanisms. These reactions are reversible, but although the reverse reactions are mediated by the same enzyme, they are given the name 17 $\beta$ -HSD. Like the multiple 3 $\beta$ -HSD enzymes, the three 17-KSR/17 $\beta$ -HSD reactions are catalyzed by more than one enzyme [reviewed in (105)]. So far, five types of 17 $\beta$ -HSD have been cloned and have been named Types I–V. Type I mainly catalyzes the reductive conversion of estrone to estradiol but also catalyzes the conversion of DHEA to androstenediol (122, 184, 242), while Type IV mainly catalyzes the oxidative conversion of estradiol to estrone (1, 27, 114). Type II catalyzes the conversion of testosterone to androstenedione, androstenediol to DHEA, and estradiol to estrone. Types III and V are “androgenic,” as they mainly catalyze the conversion of androstenedione to testosterone.

17 $\beta$ -HSD is an NADPH-dependent, non-P450 enzyme that is bound to the endoplasmic reticulum. It is widely found in both steroidogenic and nonsteroidogenic tissues [reviewed in (105)].

### *P450aro*

The aromatization of c18 estrogenic steroids from c19 androgenic steroids is mediated by the enzyme aromatase P450aro, found in the endoplasmic reticulum. P450aro converts androgens to estrogens by two hydroxylations at the c19 methyl and a third hydroxylation at c2. These three hydroxylations result in the loss of c19 and aromatization of the A ring of the steroid. These reactions, all occurring on a single active site of P450aro, utilize three pairs of electrons, donated by three molecules of NADPH and P450-reductase (239, 240).

The gene for P450aro has been cloned, is over 75 kb, and is located on human chromosome 15 (33, 126, 141). This gene encodes two mRNAs that differ in the length of their 3' untranslated regions (141).

### *11 $\beta$ -HSD*

The conversion of cortisol to cortisone is mediated by 11 $\beta$ -HSD, and the reverse reaction is mediated by an 11-oxidoreductase activity. While enzymo-

logic studies suggest that these reactions are mediated by two different proteins, cloning and expression of  $11\beta$ -HSD cDNA showed that one protein has both activities [reviewed in (26)]. Two isoforms of  $11\beta$ -HSD have been described.  $11\beta$ -HSD-1 is a low-affinity NADH(H)-dependent enzyme (159, 229) and is found mainly in human liver, decidua, lung, gonad, pituitary, and cerebellum (234, 262). By contrast,  $11\beta$ -HSD-2 is an NAD-dependent enzyme and is localized to the placenta and to mineralocorticoid target tissues (kidney, colon, and salivary gland) (3, 24, 25, 229, 262). Since  $11\beta$ -HSD converts cortisol to cortisone, it is thought to protect mineralocorticoid receptors, which can be bound by both glucocorticoids and mineralocorticoids, from occupation by glucocorticoids. It is also thought to protect glucocorticoid receptors from occupation by glucocorticoids.

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

Testosterone is converted to the more potent androgen DHT by the enzyme  $5\alpha$ -reductase. In the brain, progesterone is converted to  $5\alpha$ -DHP, and  $11$ -deoxycorticosterone is likewise reduced at the  $5\alpha$  position. This membrane-bound, non-P450 is found mainly in peripheral target tissues, such as genital skin and hair follicles.

Cloning and expression studies have demonstrated the existence of two  $5\alpha$ -reductase genes (4, 5). The gene for Type I is found on human chromosome 5, is about 35 kb, and encodes a 29-kDa protein found in the scalp, but does not encode the protein that causes a form of male pseudohermaphroditism in which genetic males differentiate as phenotypic females (88). The gene for Type II is found on human chromosome 2 and has the same intron/exon structure as the gene for the Type I enzyme (235). Mutations in the gene for  $5\alpha$ -reductase Type II cause classic  $5\alpha$ -reductase deficiency (236). The human Type I and II genes are differentially regulated during development (237). The Type I isozyme is not detectable in the fetus, is only transiently expressed in the newborn skin and scalp, and is permanently expressed in the skin from the time of puberty. The Type II isozyme is transiently expressed in skin and scalp of newborns and is the predominant form in fetal genital skin, male accessory sex glands, and in the normal prostate and in prostatic hyperplasia and adenocarcinoma tissues. In rats, the Type I mRNA is expressed in basal epithelial cells, while the Type II mRNA is expressed in the stromal cells of regenerating ventral prostate. In the rodent brain, both Type I and II mRNAs are expressed in a developmentally regulated fashion (186, 187) (see below).

### *3 $\alpha$ -HSD*

cDNA cloning experiments revealed that most HSDs belong to one of two families: the short chain dehydrogenase/reductase family (also known as short-

chain alcohol dehydrogenases) and the aldoketo reductase family [reviewed in (185)]. Mammalian  $3\alpha$ -HSDs are members of the aldoketo reductase family. The reactions catalyzed by  $3\alpha$ -HSD are stereospecific and involve the interconversion of a carbonyl with a hydroxyl group. In the prostate,  $3\alpha$ -HSD is involved in inactivating dihydrotestosterone, by conversion to the weak androgen  $3\alpha$ -androstenediol, while in the nervous system,  $3\alpha$ -HSD is involved in activating  $5\alpha$ -reduced steroids, such as  $5\alpha$ -DHP, to the potent neurosteroid allopregnanolone. Thus, this enzyme is a key regulator of both steroid hormone receptor and ion-gated receptor occupancy and action.

Most biochemical studies have used the rat liver enzyme for purification, biochemical analysis, generation of antibodies, cDNA cloning, and enzyme structure determination [reviewed in (185)]. A single cDNA species from rat liver was cloned (34, 181, 231, 247), had an open reading frame of 966 nucleotides, and predicted a protein of 322 amino acids. Rat liver  $3\alpha$ -HSD cDNA has high sequence identity (>70%) with the clone for human liver Type I  $3\alpha$ -HSD (DD4 or chlordecone reductase), human liver DD1 (which is both a  $3\alpha$ - and  $20\alpha$ -HSD), and human liver DD2 (human bile acid-binding protein) (53); (75). A human liver Type II  $3\alpha$ -HSD (96) and human prostatic  $3\alpha$ -HSD cDNAs have also been cloned. Human Type I and Type II  $3\alpha$ -HSDs differ in their  $K_m$  values for  $5\alpha$ -DHT, with the Type I enzyme having a lower  $K_m$ . All  $3\alpha$ -HSD cDNAs share high sequence identity. The genes encoding the human Type I and II enzymes spanned approximately 20 and 16 kb, respectively, and both contain nine exons of the same size and intron/exon boundaries (96).

## HST

Sulfation of free  $3\beta$ -hydroxy steroids is a major enzymatic reaction of metabolism and excretion and homeostasis of steroids and bile acids [reviewed in (81)]. Both sulfation and sulfohydrolyation activities have been reported in lung, kidney, adrenal, and testis and have been described as crucial during development. 16-Hydroxy DHEA sulfate originating from the liver, DHEA sulfate originating from the adrenal, and DHEA originating from the placenta serve as precursors for the production of estrone, estradiol, and estriol in the developing human placentae (10, 87, 179). Sulfotransferases are a family of cytosolic enzymes that conjugate steroid and phenolic substrates with inorganic sulfate derived from an active donor, PAPS (50, 163). The hydroxysteroid sulfotransferase specifically uses  $\Delta^5$ -steroid substrates that are hydroxylated at carbon 3, 5, 17, or 21 in the A or B rings (50). The resulting steroid sulfate esters are hydrophilic and are therefore more easily secreted from the cell. However, sulfation has a broader role than facilitating secretion, since it can also change the pharmacological activity of steroids, such as changing the way in which pregnenolone binds to the GABA<sub>A</sub> receptor [reviewed in (127, 128)]. HST is mainly found in adrenal in humans and in liver in other mammals (168).

## STS

The steroid sulfohydrolase is a sterol sulfate sulfohydrolase also known as steroid sulfatase or steroid-3-sulfatase. It specifically hydrolyzes sulfate groups in the 3 $\beta$  position of  $\Delta^5$ -steroids, such as pregnenolone, DHEA, and androstenediol. STS is an important enzyme in steroid metabolism since its activity increases the pool of precursors that can be metabolized by other steroidogenic enzymes to produce biologically active sex steroids (50, 219). The human STS gene has been cloned and mapped to chromosome Xp22.3, proximal to the pseudoautosomal region (PAR), and the genetic aspect of this enzyme has been widely documented (8). Deficiency of STS activity results in severe ichthyosis caused by accumulation of steroid sulfates in the stratum corneum of the skin (153, 218). The rat and mouse STS have recently been cloned and are fairly dissimilar. The mouse STS cDNA is 75% identical to the rat STS cDNA and only 63% identical to the human STS cDNA (119, 206).

## Other Neurosteroidogenic Enzymes

Other enzymatic activities that modify steroid hormones have been identified in the brain, but the activities of the resulting neurosteroid products have not yet been characterized. Those enzymes are the 7 $\alpha$ -hydroxylase (201), 20 $\alpha$ -hydroxylase, and 26 $\alpha$ -hydroxylase (193).

7 $\alpha$ -Hydroxylase is a microsomal P450 that hydroxylates steroid at the 7 $\alpha$  position. Its activity was originally described in liver protein extract, where 7 $\alpha$ -hydroxylated bile acids are produced for detoxification. Several recent papers have reported the identification of a brain 7 $\alpha$ -hydroxylase that shares only 39% sequence identity with the hepatic cholesterol 7 $\alpha$ -hydroxylase (214, 228). This enzyme is predominantly found in the brain and is regionally expressed in the hippocampus (228). The hippocampal isoform of the 7 $\alpha$ -hydroxylase showed a restricted substrate specificity compared to the hepatic 7 $\alpha$ -hydroxylase and preferentially modifies pregnenolone and DHEA (201), although it could also hydroxylate cholesterol (133, 214). Abundant 7 $\alpha$ -hydroxylated derivatives of pregnenolone were found in brain extracts and in pure glial primary cultures (2), but it is still unknown if these compounds are inactive metabolites or if they have a neuroactive function.

### **Enzymes Involved in Neurosteroidogenesis: Distribution in the Brain and Developmental Regulation**

Evidence of the presence of functional steroidogenic enzymes in the brain has been established using enzymatic activity measurements, mRNA expression (using RT-PCR, ribonuclease protection assays, or *in situ* hybridization), and protein expression (using Western blotting or immunocytochemistry). Studies by several laboratories have been conducted mainly in the adult rat brain but

have also been done in the adult frog, bird, and guinea pig. There are only a limited number of reports that document steroidogenic enzyme expression during embryogenesis in rodents (38, 39, 41, 110, 111). However, such studies are crucial, since neurosteroids have functions during development. We have compiled the results from many papers and present the data summarized in a table format (Table 2). Specific cellular localization and regulation of the enzymes are discussed in the text. The regional localizations of steroidogenic enzymes in the adult and developing brain are also summarized in two schematic representations of an adult and embryonic rodent brain (Figs. 2 and 3). As in the first section, distribution of the neurosteroidogenic enzymes will be presented first followed by that of the neurosteroidogenic enzymes themselves.

### *Expression of P450scc in the Adult Rat Brain*

Initial support for the hypothesis that the brain is a steroidogenic organ derives from experiments demonstrating conversion of radioactive cholesterol to pregnenolone [reviewed in (12)]. While these experiments were suggestive that the brain had steroidogenic capacity, the demonstration that CNS tissue and neuronal cells contained P450scc mRNA and protein was hampered by the extremely low amount of this mRNA. However, using RT/PCR and Southern blotting, we were able to show that P450scc mRNA was regionally expressed (147). In the adult rat, it was found most abundantly in the cortex and to a lesser extent in the amygdala, hippocampus, and midbrain of both male and female rats. Purification of mixed primary glial cultures showed that Type 1 astrocytes synthesized P450scc mRNA (147). Western blotting and immunocytochemistry showed that P450scc protein was almost as abundant in neonatal cultures of forebrain astrocytes as in mouse adrenocortical Y-1 cells, while P450scc mRNA was orders of magnitude less abundant, suggesting that the protein was stable in the brain (38).

### *Developmental Regulation of P450scc Expression in the CNS and PNSs*

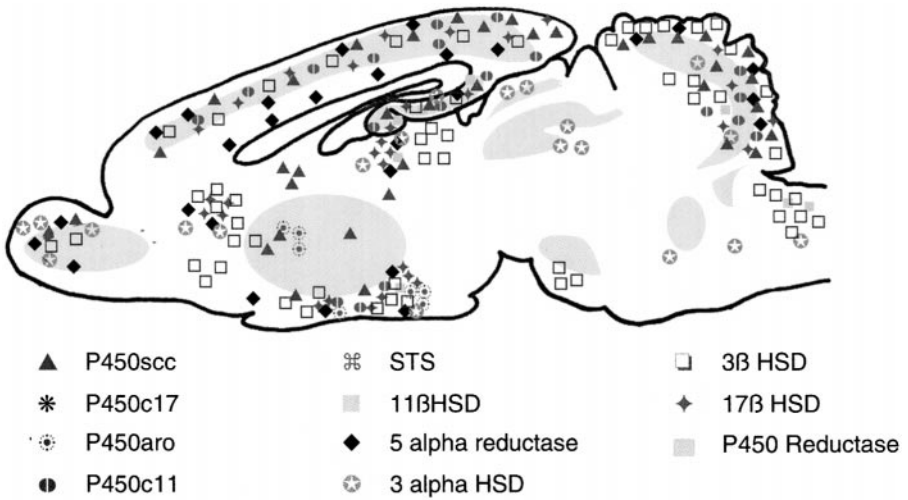
P450scc mRNA and protein are expressed very early in development. P450scc mRNA was present as early as embryonic day 7.5 (E7.5) and its amount increased until E9.5. This increased expression was due to increased expression in the placenta (38, 57). While P450scc mRNA could be readily detected in the developing gonads and adrenals, it could not be easily detected in the developing nervous system. However, by analyzing P450scc protein by immunocytochemistry, we demonstrated P450scc expression as early as E9.5 in the mouse (10.5 in the rat) in cells in the neural crest (38). Expression of P450scc continued mainly in structures derived from the neural crest during embryogenesis and was found in the neuroepithelium, the retina, the trigeminal and dorsal root ganglia, the neuroectoderm, and the thymus (38). Consistent with a neural crest origin, cells expressing P450scc belong to several different cell

**TABLE 2**

Distribution of Steroidogenic Enzymes in the Brain Independent of Developmental Stage or Species

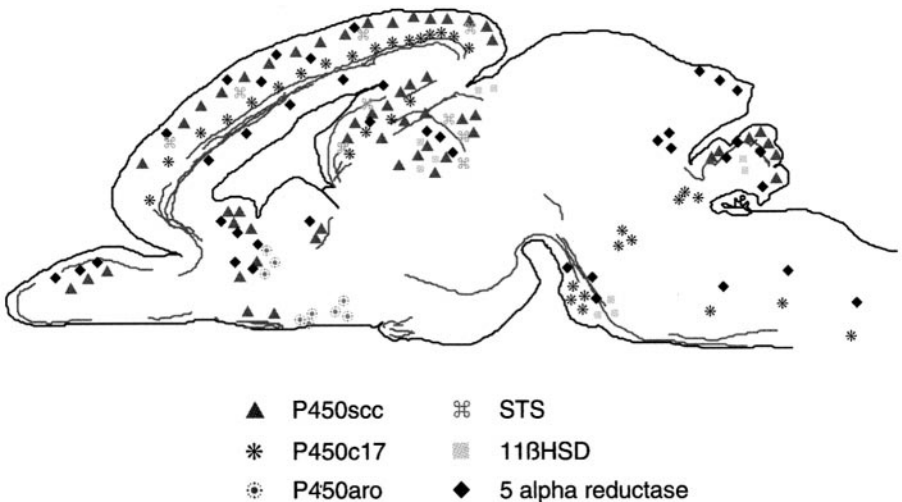
	Cortex	Hippo- campus	Olfactory bulb	Basal ganglia	Hypothal- amus	Thala- mus	Tectum/ tegmen- tum	Cere- bellum	Pons	Medulla	Pitui- tary	Spinal cord	PNS	REF
P450scc	++ P mRNA EA	+	+	++ P, EA	+	+	+	+			+	+	++	(38, 83, 89, 147)
P450c17	++ P, mRNA	++	-	+(f) P	-	+	+(f) P	+(f) P	++ P	++ P	-	++ P	++ P	(39)
3β-HSD	+	+	+	mRNA	+	+	+	mRNA	++ mRNA	++ mRNA	-	-		(56, 72, 150, 208, 260)
P450c11β	+++ mRNA	+	+	+(f) P, EA +++ mRNA	+	+	+	-	+	-	+	+		(69, 147)
P450c11AS	++ mRNA	+	+	+++ mRNA EA	+	+	+	+	+	+	+	+		(69)
17β-HSD	+	+	+	mRNA	+	+	+	+	-	-	-	-		(151, 183, 194, 196)
P450aro	+	+	+	+	+	+	+	+	+	+	+	+		(68, 110, 117, 124, 207)
11β-HSD (Types I and II)	+(I) mRNA	+(1) mRNA	+	+(I, II) mRNA	+(I, II) mRNA	+(II) mRNA	+	+(II) mRNA EA	+(II) mRNA	+(II) mRNA	+(I) mRNA	+		(24, 106, 154, 155, 198, 199, 272)
5α-Reduc- tase	+	+	+	+	+	+	+	+	+	+	+	+		(31, 111, 143, 144, 165, 182)
3α-HSD	+	+	++	+	+	+	+	+	+	+	+	+		(74, 93, 96, 143)
STS	+	+	-	-	-	+	-	-	-	-	-	-	+	(41)

Note. (±) Expression of the corresponding enzyme in the corresponding brain region; (+/++/+++) indicates the level of expression of the enzyme in the region; (f) expression of the enzymes observed only in *fibers* and not in cell bodies in the particular region. Type of detection: mRNA; P, protein (immunocytochemistry), Western blotting); EA, enzymatic activity.



**FIG. 2.** Schematic representation of an adult brain showing regional expression of enzymes involved in neurosteroidogenesis. The symbols for the different steroidogenic enzymes are shown at the bottom of the figure. In this figure, data from several species, including rodent, primates, and amphibians, are represented.

lineages. P450scc was found in neurons of the DRGs, trigeminal ganglia, and neuroectoderm and was found in glial lineages in the CNS. P450scc was also found in cells not derived from the neural crest, such as motor neurons (38), Purkinje cells in the cerebellum (245), oligodendrocytes (83), and astrocytes (147) in various regions of the brain from late embryogenesis to adulthood. In



**FIG. 3.** Schematic representation of an E18.5 rodent brain showing regional expression of enzymes involved in neurosteroidogenesis. The symbols for the different steroidogenic enzymes are shown at the bottom of the figure.

the PNS P450scc was expressed from E10.5 in condensing DRGs and in cranial ganglia (38). Other groups have also reported activity and expression of P450scc protein in peripheral nerves (160). Thus, P450scc is expressed in a variety of cell types in both the CNS and the PNS. Its expression is initiated in the developing neural tube and in the neural crest, before organogenesis of the adrenal or of the gonads, suggesting a role for neurosteroidogenesis in neural development. Its expression in restricted areas of the brain do not seem to be developmentally regulated, as P450scc expression persists during adulthood.

### *Expression of Adrenodoxin and Adrenodoxin Reductase in the Brain*

Adrenodoxin mRNA was found in virtually all rodent tissues examined (148), including the brain. This is not surprising, as this protein functions with all mitochondrial P450s, not just the steroidogenic P450s. Similarly, as expected, adrenodoxin protein has also been found in brain homogenates (167). The exact site of expression of these proteins has not been systematically examined either in the adult brain or during development. The expression of adrenodoxin reductase has not yet been documented in the CNS or PNS.

### *Developmental Regulation of P450c17 Expression in the Nervous System*

Initial studies failed to detect expression of P450c17 mRNA in any region of the adult rat brain, using highly sensitive RT-PCR analyses (147). Those studies also indicated that the steroidogenic enzyme proteins may be more easily detected than their mRNAs, since using an antibody directed against human P450c17, we determined the pattern of expression of P450c17 in developing rodent embryos (39). Data are summarized in Table 2 and in Fig. 3. P450c17 expression is restricted in neurons in specific regions of the developing brain. We first observed P450c17 in E10.5 cells migrating from the neural crest and condensing in DRGs. Immunopositive cells were also observed in the neural tube in the position of the lateral motor column. Neuronal cell bodies immunopositive for P450c17 were restricted to the hindbrain mesopontine system (from E14.5), the thalamus, and the neocortical subplate (from E16.5). Fibers extended in the areas of projections of these nuclei. We also found fibers coming from the trigeminal ganglion and the retina.

In all the CNS regions P450c17 expression was transient and P450c17 immunostaining gradually disappeared in the first week of life. In the neonatal mouse and rat (days 0 to 7), P450c17 was mainly detected in the brainstem and in the cerebellum, internal capsule, olfactory tract, hippocampus, stria terminalis, and thalamus. Few immunopositive cell bodies could be detected in any region of the adult rat brain, although fiber tracts could still readily be observed. In the PNS, P450c17 expression persisted in the adult, in both cell bodies and fibers. Thus, unlike P450scc, P450c17 is expressed in the CNS only during development, but persists in the PNS throughout life.

DHEA has been one of the first neurosteroids identified in the adult rodent brain, although at low concentrations. However, P450c17 mRNA and protein are not found in cells of the CNS in adult animals. We propose that in the rodent, peripheral expression of P450c17 provides the brain with DHEA found in adult rat brains. An alternative pathway has been proposed for DHEA generation in the brain involving an Fe<sup>2+</sup>-sensitive chemical reaction independent of P450 (28). However, it is unlikely that such a process occurs *in vivo* since the concentrations of Fe<sup>2+</sup> necessary to induce production of DHEA (in the 10 mM range) in cells lacking P450c17 is far out of a physiological range. We thus believe that, in the adult, DHEA is delivered to the spinal cord and brain via the peripheral nerves and terminals which express P450c17 and synthesize DHEA.

### *Expression and Activity of P450-Reductase and b5 in the Brain*

P450-reductase activity, protein (166), and mRNA (166, 222) have been reported in the brain. Cytochrome P450-reductase immunoreactivity was detected mainly in neurons, but also in some glial populations, and it appears to be expressed widely in the rat CNS (166) (see Table 2 and Fig. 3). Although no studies specifically addressed the colocalization of P450-reductase and P450c17 in the same cells, the regional distribution of these proteins suggests that P450c17 may have functional activities in the cortical thalamic and pontine regions, where we described its expression. The expression of b5 has not yet been documented in the CNS or PNS.

### *3 $\beta$ -HSD Expression in the Adult Nervous System*

Demonstration of the conversion of pregnenolone to progesterone in cultured rat oligodendrocytes (83, 89), astrocytes (91), cultured rodent glia and neurons (11), and in discrete regions of the rat and monkey brain (197, 260) suggested that 3 $\beta$ -HSD is expressed in those regions. Expression of 3 $\beta$ -HSD protein and mRNA has been studied in brains from rats (55, 72, 244) and in considerable detail in frogs (150), demonstrating that this enzyme is expressed in the brains from both mammalian and nonmammalian vertebrates. There are conflicting reports as to whether 3 $\beta$ -HSD Type I alone (72) or whether Types I, II, and IV (208) are the isoforms that are expressed in the adult rat brain. While 3 $\beta$ -HSD activity has been reported in both neurons and glia, 3 $\beta$ -HSD mRNA and protein appear to be expressed only in neurons (55, 72, 244). 3 $\beta$ -HSD mRNA was found in the brain in much lower amounts than in the ovary, adrenal, and liver. The regional distribution of 3 $\beta$ -HSD is summarized in Table 2 and Fig. 2. Results from different laboratories are conflicting and relate to the specificity of the probe/antibody to a particular subtype of the enzyme and to the sensitivity of the technique used (55, 72, 244). An extensive study using immunocytochemistry in the frog showed the restricted expression of 3 $\beta$ -HSD in neurons, in

regions consistent with the pattern of expression of  $3\beta$ -HSD Type I mRNA in the rat (72), except in the cerebellum, and in the cortex, where only  $3\beta$ -HSD mRNA was reported (72, 244). However, reports of  $3\beta$ -HSD activity did not always correlate with expression of  $3\beta$ -HSD protein. The lack of significant activity in the rat cerebellum and cortex (197, 260) correlates with the lack of  $3\beta$ -HSD protein in these regions in the frog (150), but not with the presence of  $3\beta$ -HSD mRNA in the rat (72). A recent report described a transient  $3\beta$ -HSD activity in cerebellum slices obtained from neonates (244). High levels of  $3\beta$ -HSD activity have been reported in the rat amygdala and hippocampus (260).  $3\beta$ -HSD mRNA was detected in the rat hippocampus but  $3\beta$ -HSD protein was not detected in this region in the frog.

#### *P450c11 $\beta$ and P450c11AS Expression in the Adult Nervous System*

Analysis of RNA from different regions of the adult rat brain indicated that P450c11 $\beta$ , but not P450c11AS, was region-specifically expressed in the brain (147). The abundance of P450c11 $\beta$  mRNA was greater than that of P450c11AS mRNA, since it could be detected readily by RNase protection assays. We showed that P450c11 $\beta$  mRNA was found in virtually all regions analyzed (amygdala, cerebellum, cortex, hippocampus, hypothalamus, midbrain), with the greatest amount in the cortex. We also found that there may be differences between male and female rats in the expression of P450c11 $\beta$  mRNA in the hippocampus, as it appears to be found in greater amounts in female.

Unlike P450c11 $\beta$ , P450c11AS mRNA was not detected in any region of the adult rat brain, analyzing RNA by both RNase protection assays and RT-PCR (147). A recent report by others has shown expression of P450c11AS mRNA in adult rat brains and has shown aldosterone synthase activity in the same regions (69). Thus, whether P450c11AS is expressed in the adult rat brain is not completely resolved.

#### *Expression of 17 $\beta$ -HSD in the Brain*

Demonstration of 17 $\beta$ -HSD activity in the brain of rats and monkeys (195, 196) indicated that 17 $\beta$ -HSD protein and mRNA would also be present. Recent analysis of both rat and frog brains has demonstrated that 17 $\beta$ -HSD Type I is expressed in the brain (151, 183). In adult rat brains, 17 $\beta$ -HSD Type I protein was detected in nonneuronal cells (GFAP-positive cells) (183). In the frog brain, 17 $\beta$ -HSD Type I was also found in glia (151). Regional distribution of 17 $\beta$ -HSD protein and mRNA in the rodent and in the frog are consistent, and the pattern of expression of 17 $\beta$ -HSD is summarized in Table 2 and Fig. 2. No data on the expression and distribution of other forms of 17 $\beta$ -HSD in the brain are available.

### *Expression of P450aro in the Brain*

There are several lines of evidence that demonstrate that testosterone is aromatized to estradiol in the brain of many species, from frogs and songbirds to human beings [reviewed in (84, 115)]. Aromatase activity in the rat brain was shown to be limited to discrete regions (202). Aromatase activity was first detected on embryonic day 15, increased up to embryonic day 19, and then declined to adult levels (68, 117, 124).

While three distinct P450aro mRNA species of 2.7, 2.2, and 1.7 kb exist in many tissues, only the 2.7-kb P450aro mRNA was found in the brain (117). In human beings, the major transcript in the hypothalamic preoptic area and amygdala contained exon I-f, referred to as the "brain-specific exon I" (82), while a minor transcript contained exon I-b (82, 241). In rat cortex, an additional novel P450aro transcript was identified, which contained exons IV through X, but initiated transcription within intron III and did not contain exons I, II, or III (94). Thus the rat brain contains P450aro transcripts that initiate at three different sites. It is not clear if the three different P450aro transcripts correspond to different sites of transcription initiation or if they are variants that lack the heme-binding domain (116).

The regulation of P450aro mRNA expression during rodent development was studied by *in situ* hybridization (110). In neurons, P450aro mRNA appeared to parallel aromatase activity but P450aro mRNA was also found in regions not previously associated with activity. The regional distribution of P450aro mRNA was restricted to the preoptic area/hypothalamic area on embryonic day E16 and was more widely distributed by E18 to E20, but was still absent from the cortex, midbrain, and hindbrain structures. From postnatal day 2 to adulthood, P450aro mRNA abundance decreased in the preoptic area, but remained constant in other areas. In the adult P450aro mRNA was still present with a similar pattern of expression (Table 2, Fig. 2). Female rats had the same distribution of P450aro mRNA, but the number of cells expressing P450aro mRNA in each region was less than that in males rats (254).

The regions of the brain that contained P450aro mRNA, but did not contain activity or were not examined, include the mediodorsal thalamus, subfornical organ and the cingulate cortex, and hippocampus. P450aro immunoreactivity has been demonstrated in the same areas in which P450aro mRNA has been detected (207), indicating that the lack of aromatase activity may be due to the sensitivity of the assay.

### *Expression of 11 $\beta$ -HSD in the Brain*

Both 11 $\beta$ -HSD-1 (106, 155) and 11 $\beta$ -HSD-2 (25, 199, 272) activities and mRNA have been detected in adult rat brains (Table 2, Fig. 2).

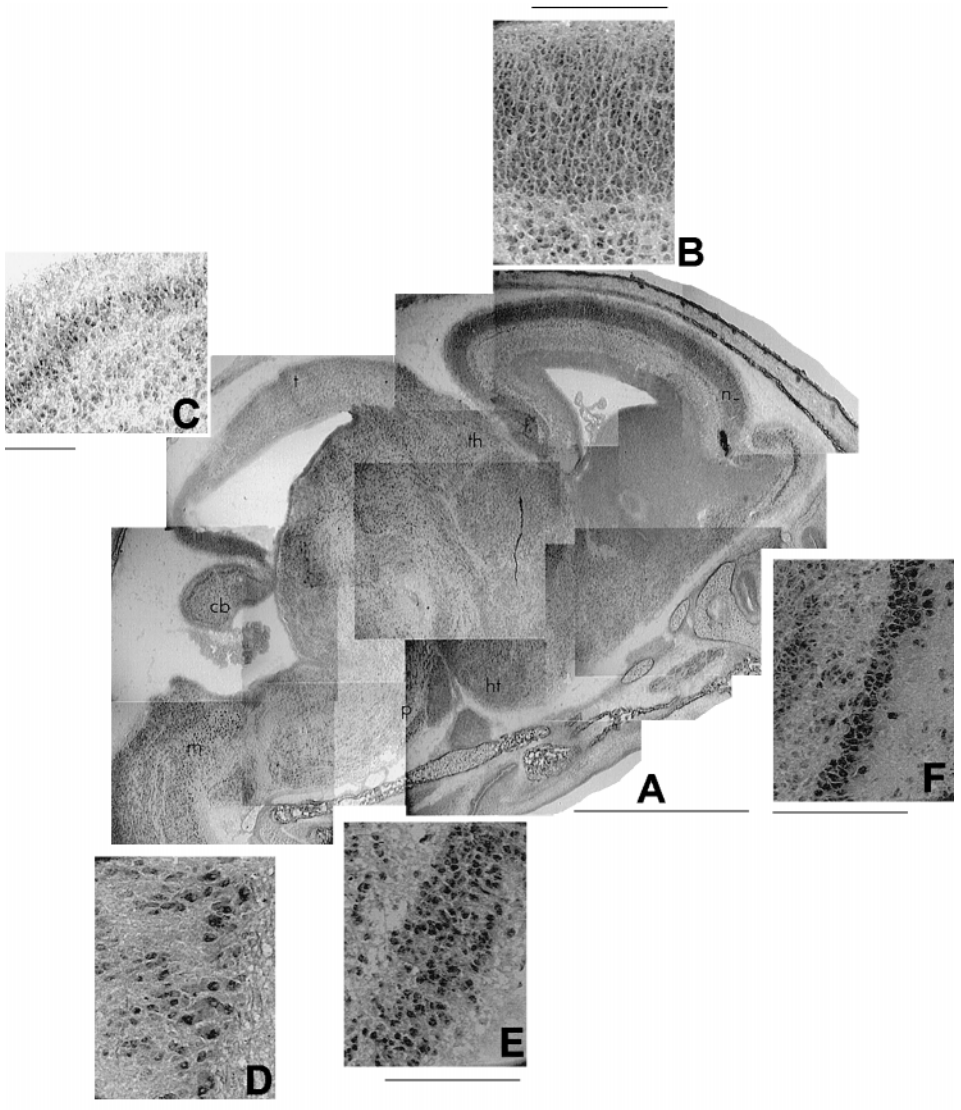
During development, 11 $\beta$ -HSD-2 mRNA was widely expressed in the CNS throughout the neuroepithelium, thalamus, and spinal cord up to embryonic

day 12.5. It declined thereafter (25). Expression in the thalamus and cerebellum persisted postnatally.

### *Expression of 5 $\alpha$ -Reductase in the Brain*

The rat CNS is capable of converting progesterone into 5 $\alpha$ -reduced metabolites [reviewed in (31)], indicating that this enzyme is present in the CNS. 5 $\alpha$ -Reductase activity, protein, and mRNA have been detected in neurons, astrocytes, and glia (31, 111, 143, 144, 182), and the predominant isoform is Type I (165). 5 $\alpha$ -Reductase Type I mRNA is more abundant during development than in the adult, but its overall level of expression during development remains relatively constant (125, 187). Expression of 5 $\alpha$ -reductase Type II mRNA in the brain is, however, developmentally regulated (186, 187). RT/PCR analysis of embryonic and neonatal rat brain mRNAs demonstrated that whereas Type I mRNA was constitutively expressed in the rat brain throughout late gestation, neonatal period, and adulthood, the Type II mRNA was transiently expressed only at the end of gestation (embryonic day 18) through the early postnatal period, dramatically falling by postnatal day 14. This pattern of expression of Type II mRNA correlated with testosterone synthesis in the fetal testis and may also suggest that 5 $\alpha$ -reductase Type II may be involved in regulating brain sex differentiation at a critical period. In the adult rat, immunocytochemical staining detected 5 $\alpha$ -reductase type I protein throughout the brain, with no differences between male and female rats.

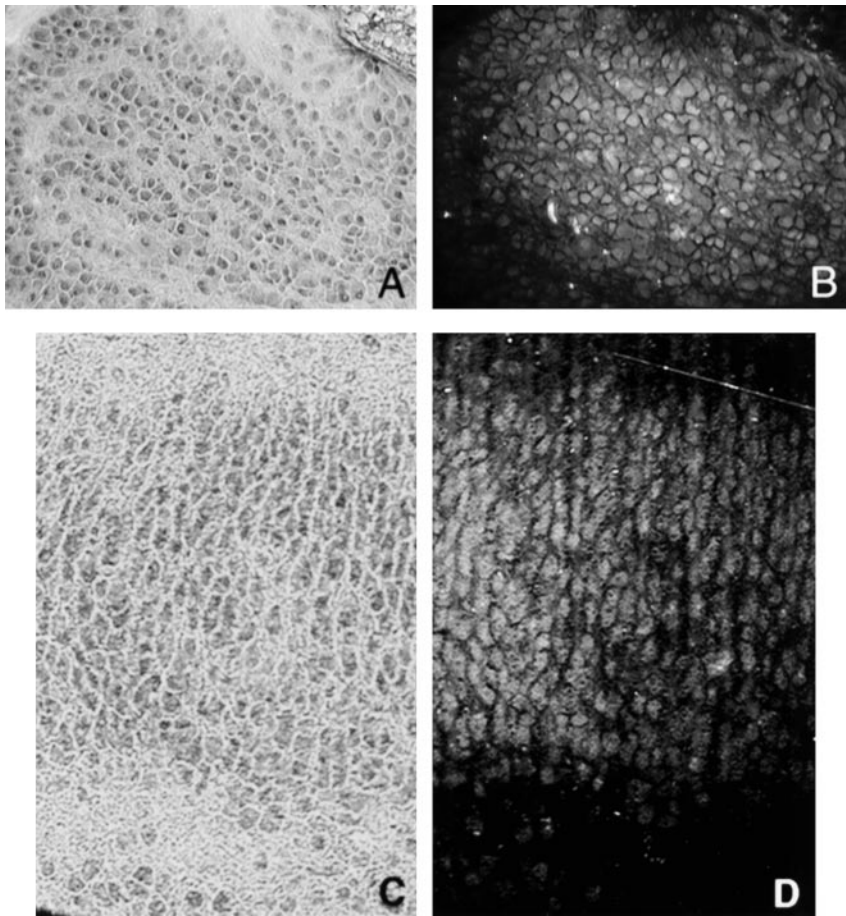
We assessed the expression of 5 $\alpha$ -reductase Type I during development by *in situ* hybridization and immunocytochemistry (Fig. 4) and confirmed the results established by others who mapped the distribution of 5 $\alpha$ -reductase Type I mRNA (111). The pattern of expression of 5 $\alpha$ -reductase during embryogenesis was greatly regulated during development. First observed at E12 in the spinal cord and neuroepithelial walls of the brain ventricular system, its distribution widened to highly proliferative zones of the CNS from E14 to E16. At embryonic day 16.5, 5 $\alpha$ -reductase mRNA and protein were detected in the cortex, thalamus, cerebellum, medulla, spinal cord, and peripheral ganglia (Fig. 4). 5 $\alpha$ -Reductase mRNA was found in lesser amounts in the trigeminal ganglia and inferior ganglia of the nodose-petrosal nerves (IX and X) than in the spiral ganglia (111). However, 5 $\alpha$ -reductase Type I protein expression was found at similar levels in all peripheral ganglia (trigeminal, DRGs, nodose-petrosal, or spiral ganglia). In late embryogenesis (E18 to E20), 5 $\alpha$ -reductase Type I mRNA expression and protein were similar in distribution, but decreased in all areas except in the trigeminal ganglia and the ventricular zones of striatum and amygdala and the thalamus. In the neonate, expression of 5 $\alpha$ -reductase was higher in the PNS (trigeminal ganglia, DRGs) than in the CNS, and by 2 weeks of age, 5 $\alpha$ -reductase mRNA expression changed completely and was evenly distributed throughout the brain. At this time, white matter structures (e.g., optic chiasma, lateral olfactory tract, internal capsule, corpus callosum) were highly positive for 5 $\alpha$ -reductase mRNA, but other regions (hippocampus, stri-



**FIG. 4.** Immunostaining for  $5\alpha$ -reductase Type 1 in E16.5 mouse brain. A composite picture of the whole brain representing the wide distribution of this enzyme in the developing brain (Bar under A for composite, 1 mm). nc, neocortex; h, hippocampus; th, thalamus; ht, hypothalamus; t, tectum; p, pons; cb, cerebellum; m, medulla. (B–F) Higher magnification of locations where  $5\alpha$ -reductase Type 1 immunostaining is the strongest (bar under C, for B, C, and F, 100  $\mu$ m). (B) Neocortex, (C) cerebellum, Purkinje cell layer, (D) ventral part of the spinal cord, (E) pontine nucleus, (F) olfactory bulb (bar under D, for D and E, 100  $\mu$ m).

tal neuroepithelium) were also positive (111). Our results demonstrate that  $5\alpha$ -reductase is expressed in the same location as its mRNA. We and others further established a transient expression of  $5\alpha$ -reductase in the proliferative zones of the developing CNS, suggesting that  $5\alpha$ -reduced steroid may have a role in neuronal proliferation.

Expression of  $5\alpha$ -reductase is summarized in Table 2 and Figs. 2 and 3. Like P450scc,  $5\alpha$ -reductase is expressed in several cell types, including neurons in the hindbrain, cerebellum, spinal cord, and PNS and in a majority of glial cells in the other regions of the CNS. We thus tested the colocalization of  $5\alpha$ -reductase and P450scc.  $5\alpha$ -Reductase and P450scc are colocalized in the same cells in the developing neocortex (Fig. 5A). In other locations, such as the neuroepithelium, the spinal cord, and the peripheral ganglia, where both



**FIG. 5.** Dual staining for  $5\alpha$ -reductase Type 1 (A, C) and P450scc (B, D). Magnification of A and B is ( $\times 20$ ) and in C and D is 48 ( $\times 40$ ). Comparison between A, C and B, D shows a colocalization of  $5\alpha$ -reductase Type 1 and P450scc in the neocortex (C, D) and only partial colocalization of P450scc and of  $5\alpha$ -reductase Type 1 in DRGs (A, B).

P450scc and 5 $\alpha$ -reductase Type I were expressed, P450scc and 5 $\alpha$ -reductase were not always found in the same cells (Fig. 5B). In the adult DRGs, 5 $\alpha$ -reductase was also found in cells expressing P450c17 but not P450scc. The colocalization of P450c17 and 5 $\alpha$ -reductase in the regions of the developing brain where they both are expressed remains to be tested. However, the expression of both enzymes in those regions suggest that steroids other than 5 $\alpha$ -DHP or allopregnanolone may be synthesized in regions such as the hind-brain, the hippocampus, and the spinal cord. Further detection of 17 $\beta$ -HSD and P450aro in those same regions could argue for local androgen and/or estrogen production during embryogenesis in sexually dimorphic regions.

Brain-expressed 5 $\alpha$ -reductase Type I may be crucial for regulation of estrogen synthesis during pregnancy. Recent studies created a transgenic mouse that had the 5 $\alpha$ -reductase type I gene ablated (125). About half of homozygous mutant mice died in midgestation between embryonic days 10.75 and 11, due to estrogen excess. Administration of exogenous estrogens to normal wild-type mice also caused fetal death and antagonists of estrogen action could prevent fetal demise in the homozygous knockout animals, demonstrating that estrogens, rather than a lack of 5 $\alpha$ -reduced steroids, was causative of fetal death. Thus, 5 $\alpha$ -reductase Type I plays a major role in the fetus in inactivating testosterone, thereby reducing available substrate for estrogen synthesis. The results from the 5 $\alpha$ -reductase Type I knockout mouse also indicate that it is the inactivation of testosterone mediated by 5 $\alpha$ -reductase Type I, and not by 5 $\alpha$ -reductase *Type II*, that is crucial for fetal development and survival. 5 $\alpha$ -Reductase Type I mRNA is induced in the brain with pregnancy. Its increased expression in the brain may regulate estrogen levels in normal wild-type animals, perhaps through feedback mechanisms involving the hypothalamic pituitary gonadal axis.

#### *Expression of 3 $\alpha$ -HSD in the Brain*

Human 3 $\alpha$ -HSD Type II mRNA was found in the brain, while human 3 $\alpha$ -HSD Type I mRNA was found only in the liver (96). Extensive biochemical studies demonstrated 3 $\alpha$ -HSD activity in various regions of the rodent brain and its regulation by estrogens and lactation [reviewed in (93)]. 3 $\alpha$ -HSD activity was found mainly in Type I astrocytes, but was also detected in oligodendrocytes (144). Distribution of 3 $\alpha$ -HSD is summarized in Table 2 and Fig. 2. Western blots of proteins extracted from different brain regions demonstrated that the olfactory bulb contained the greatest abundance of 3 $\alpha$ -HSD protein (96).

#### *Expression of HST in the Brain*

The presence of abundant quantities of steroid ester sulfates in the brain of gonadectomized and adrenalectomized animals have been noticed since the early 1980s, when the concept of neurosteroids was proposed (43, 44). We and

others have observed that several neurosteroids and their respective sulfate esters have different pharmacologic properties [reviewed in (127, 128)] and different biological effects in the developing brain (40), suggesting that both steroid sulfotransferase and steroid sulfohydrolase activities may play crucial roles in the modulation of the physiological effects of neurosteroids. However, very little information is available on the expression and enzymatic activity of steroid sulfotransferases in human or rodent fetal or adult brains.

Despite the presence of higher concentration of steroid ester sulfate than concentration of free steroids in the brain, and despite the ubiquitous presence of PAPS, a cosubstrate of the steroid sulfotransferase, there is no conclusive evidence for expression of HST in the brain. Several studies failed to show HST activity in the human fetal brain (87). Immunocytochemistry did not reveal any expression of HST in fetal or adult human brains nor in adult rat brains (179, 220). However, using human fetal brain slices, HST activity was detected (99). More recently, others have characterized brain and liver HST activities from immature rats (192). These data demonstrated that the brain HST activity (kinetics, pH, and specificity for substrate) was not comparable to the liver HST activity, suggesting that brain and liver sulfotransferases may be different isozymes. These authors found that the brain HST activity was relatively high in late embryogenesis, remained high until puberty, and then decreased in the adult. This ontogeny of HST activity in the brain is the opposite of the ontogeny in the liver, where HST activity is absent during embryogenesis and rises following birth (192).

### *Activity and Expression of STS in the Brain*

Previous studies have demonstrated steroid sulfatase activity in the brain as well as in a variety of tissues other than its major site of expression in the placenta (87). We demonstrated the sites of expression of STS mRNA using a mouse STS riboprobe (41) (see Table 2 and Fig. 3). The sites of STS expression provide more precise indications of the target tissues in which active, nonsulfated steroid hormones resulting from STS activity may act during embryogenesis. In addition to the known roles of STS in the placenta and in the skin, our results emphasized other possible roles for STS in the function of other organs during embryogenesis. In the CNS and PNS, the expression of mSTS mRNA may be related to the expression of steroidogenic enzymes involved in neurosteroid synthesis. We previously showed the expression of P450c17 in restricted areas of the developing nervous system (39), particularly in the neocortex, and demonstrated a role for both DHEA and DHEAS in neocortical neurons differentiation. While DHEA promoted axonal growth, DHEAS promoted dendritic growth and cell clustering (40). The expression of STS in the neocortex, hippocampus, and thalamus may regulate the DHEA:DHEAS ratio. Furthermore, as the thalamus has been shown to influence the survival of cortical subplate (188), where we detected P450c17 expressing neurons, the ratio of these two steroids may play crucial roles in the controlling axonal versus

dendritic growth of corticothalamic fibers during neocortex organization. The afferent and efferent connections of the cerebral neocortex develop simultaneously toward the end of embryogenesis.

### *Present State of Research in Neurosteroidogenesis and Future Directions*

Although many enzymes involved in neurosteroidogenesis have been described in the brain, the main information still lacking is a detailed understanding of which steroids are synthesized at a particular region and time in the brain. The brain, unlike the adrenal, gonad, and placenta, cannot be considered one functional unit whose role is to produce steroids. Steroid production in the brain is highly relevant to neuromodulation and to the behavior associated with the neurotransmitter systems modulated by putative neurosteroids. It is apparent that neurosteroidogenesis involves the cooperation of several cell types within particular regions of the CNS. However, because of the lack of a detailed understanding of steroid production in specific regions and developmental stages, it is currently not possible to produce a model of neurosteroidogenesis. To date, very few data are available on the cellular colocalization of enzymes involved in the production of any particular neurosteroid in a specific regional and temporal pattern. Similarly, there are no data on the specific synthesis or abundance of neurosteroids in particular regions of the brain at particular times. Such experiments, however, are crucial to allow one to develop hypotheses predicting the potential roles of particular neurosteroids in the developing or adult brain, as well as to identify potential regulators of their function.

### **HOW DO THEY FUNCTION?**

Neurosteroids exert several biological actions in the brain during embryogenesis as well as in adults. These have been reported in several species, including human beings. One of the earliest observations of the neural action of neurosteroids was the report of the remarkable anesthetic property of progesterone (217). Mechanisms by which progesterone and its derivatives act in the nervous system include both genomic actions mediated by nuclear steroid receptors and nongenomic actions, mediated by neurotransmitter receptor (e.g., GABA<sub>A</sub>) [reviewed in (107, 130)]. Neurosteroids derived from P450c17 activity, DHEA, and DHEA sulfate have not been reported to act on nuclear steroid receptors but have rather been shown to modulate activity from GABA<sub>A</sub>, NMDA, and sigma receptors (60, 129, 156, 226, 246). The nongenomic actions of neurosteroids in the nervous system have been covered by several reviews (12, 107, 127, 146). We will focus on discussing the most recent progress in understanding the interaction between neurosteroids and specific neurotransmitter receptors.

**Neurosteroids Exert Their Action via Nuclear Steroid Receptors***Progesterone*

The action of steroid hormone in the regulation of the HPA/HPG axes to control both stress and sexual functions have been well characterized [reviewed in (49, 269)]. In this paper we will focus on less documented actions of progesterone in the functional maturation of the CNS and PNS.

Progesterone produced *in situ* in the nervous system was initially reported to have genomic effects in the repair of injured sciatic nerves in rodent, by promoting myelination (100). In the paradigm of cryolesioned sciatic nerves, blockade of endogenous progesterone synthesis or of progesterone receptor activation reduced both nerve regeneration and progesterone-induced myelination, demonstrating that progesterone was binding to its nuclear receptor to improve myelination (100). Two genes involved in enhancing proteins involved in myelination, the peripheral myelin protein -22 and the protein zero (Po) genes, were tested as potential target genes for progesterone action (51). Both genes could be regulated by progesterone in Schwann cells, but not in other progesterone receptor containing cells, suggesting that these genes were regulated by progesterone in a cell-specific fashion. The progesterone antagonist RU486 did not abolish the progesterone-mediated response, suggesting an alternative signaling mechanism.

Other studies suggested that reduced progesterone derivatives, rather than progesterone, modulate expression of proteins involved in myelination (145). In a different animal model, investigators have shown that there is an age-dependent decrease in the expression of myelin basic protein and in Po. Treatment of animals *in vivo* with dihydroprogesterone restored the levels of Po without affecting myelin basic protein. Furthermore, both 5 $\alpha$ -reduced and 3 $\alpha$ , 5 $\alpha$ -reduced derivatives of progesterone increased Po mRNA expression in cultured Schwann cells more effectively than progesterone. These results therefore suggest that reduced derivatives of progesterone, and not progesterone, regulate expression of Po during aging.

In another recent study, investigators used a mixed coculture of Schwann cells and sensory neurons to study the effect of steroids on myelin formation (32). They found that both dexamethasone and progesterone increased the rate of myelin formation but did not increase the total amount of myelin. This effect was mediated by the progesterone receptor, since the effect was blocked by RU486, and was mimicked by the progesterone agonist RU5020. The authors correlated myelination of sensory axons *in vitro* with the increase in mRNA expression for the myelin basic protein, P450scc, 3 $\beta$ -HSD, and the progesterone receptor, suggesting that the initiation of myelination was correlated with an increased expression of endogenous enzymes crucial for the *de novo* synthesis of progesterone. In addition, progesterone receptor mRNA was increased during the process of myelination in the cocultures. P450scc, 3 $\beta$ -HSD, and progesterone receptor mRNA were found to be expressed in oligodendrocytes once differentiated, but not in their undifferentiated state, suggesting that the

expression of steroidogenic enzymes in Schwann cells can be modulated in relation to the cell's fate. Schwann cell differentiation *in vivo* is largely dependent upon oligodendrocyte–sensory neuron interactions. Further studies are necessary to elucidate the mechanisms by which the cellular interactions are necessary for Schwann cell maturation and myelination of sensory axons. The regulation of endogenous steroidogenesis in both maturing oligodendrocytes and in extending sensory axons may be key regulators of this process.

### *Glucocorticoids*

Other neurosteroids such as corticosterone may have genomic effects mediated by both glucocorticoid and mineralocorticoid receptors. Both glucocorticoids (corticosterone) and mineralocorticoids (aldosterone) can bind with an equally high affinity to mineralocorticoid receptor (MR), while corticosterone binds to glucocorticoid receptors (GR) with a low affinity. A stress-induced elevation of glucocorticoids in maternal blood during pregnancy has been correlated with a dysfunction of the HPA axis in offspring (140, 261). Clinical dysfunction of the HPA axis results in depression, eating disorders, anxiety, and age-related cognitive dysfunctions, disorders that have been associated with elevated glucocorticoid concentrations in the developing brain (92, 216).

High concentrations of glucocorticoids in the developing brain may result not only from glucocorticoid elevation in maternal blood crossing the placenta, but also from modification of *de novo* synthesis of corticosterone or *in situ* modulation of corticosterone-mediated activation of GR and MR. We and others have shown that the final enzyme required for glucocorticoid synthesis, P450c11 $\beta$ , was expressed in the adult brain in a tissue- and sex-specific manner (147), and the region-specific expression of P450c11AS, the final enzyme necessary for the production of mineralocorticoids, has also recently been detected in adult rat brain (69). In addition, access to GR and MR is modulated by the presence of tissue-specific 11 $\beta$ -HSDs that catalyze the interconversion of active corticosterone (11 $\beta$ -HSD Type 1) and inactive 11-dehydrocorticosterone (11 $\beta$ -HSD Type 2) (215). Both enzymes are expressed in specific regions of the developing brain (54). The temporal and spatial pattern of expressions of 11 $\beta$ -HSD Types 1 and 2 are very different and correlate with the expression of MR and GR, respectively, defining regions and windows of time in which the potent inactivation of GR by the activity of 11 $\beta$ -HSD Type 2 can occur under normal physiological conditions. Neither MR nor 11 $\beta$ -HSD Type 1 is expressed in the early gestational stages, but their expression increases around birth. By contrast, both 11 $\beta$ -HSD Type 2 and GR are expressed in early gestation in the neuroepithelium, and their expression becomes more restricted during embryogenesis. One of the postulated roles for activated GR was to inhibit the cell division based on its ability to increase the length of the cell cycle *in vitro* in nonneuronal cells. Expression of 11 $\beta$ -HSD Type 2 in the developing brain was proposed to attenuate effects of glucocorticoids profoundly during the stage of CNS development, as its expression in the placenta was thought to protect the fetus from the

deleterious effect of high concentrations of maternal glucocorticoids. Both 11 $\beta$ -HSD Type 2 and GR were expressed in the neuroepithelium in the developing nervous system. Their expression in highly proliferating cells in the developing CNS thus suggested that glucocorticoids may play a role in differentiation/maturation events (cell birth, migration, cell cycle arrest, differentiation) during fetal brain development, and that the levels of glucocorticoids can be modulated by neurally expressed 11 $\beta$ -HSD.

### Neurosteroids Are Allosteric Modulators of GABA<sub>A</sub> Receptors

Although early observation of rapid anesthetic effect of steroids (217) indicated that these effects may result from alternative mechanisms, the genomic mechanism of steroid action has been an unquestioned dogma for the explanation of steroid hormone action for the past 4 decades. Evidence for nongenomic steroid effects is now coming from all fields of steroid research, and mechanisms of agonist action are being studied with regard to the membrane receptor and the second messenger involved. One of the most documented examples of such a nongenomic action of steroid is the ability of several progesterone derivatives to bind and activate GABA<sub>A</sub> receptors.

Beginning in the mid 1980s (76, 130) a series of papers have shown that the neurosteroid allopregnanolone (5 $\alpha$ , 3 $\alpha$ -tetrahydroprogesterone) is a potent GABA<sub>A</sub> receptor modulator [reviewed in (127)]. 5 $\alpha$  Derivatives of progesterone like allopregnanolone and dihydroprogesterone, as well as pregnenolone and pregnenolone sulfate, bind to GABA<sub>A</sub> receptors at a site different from benzodiazepine and barbiturates (67, 243) and are allosteric agonists for GABA<sub>A</sub>, inducing chloride currents in different *in vitro* and *in vivo* models [reviewed in (108)]. In different neuronal populations, distinct neurosteroids may act as positive or negative modulators of GABA<sub>A</sub> and display different binding characteristics (67, 102, 109, 130, 189, 190, 209, 221). Steroids active at GABA<sub>A</sub> receptors are active as sedative-hypnotics, anticonvulsants, and anxiolytics in animal models. However, it has been postulated that the heterogeneity of GABA<sub>A</sub> receptor subunit composition in different regions of the brain may explain the different sensitivity of the evoked chloride currents to different neurosteroids, as well as explain the different pharmacologic profiles shown by barbiturates, benzodiazepines, and neurosteroids [reviewed in (107)].

### *In Vitro Studies*

Using functional expression of recombinant GABA<sub>A</sub> receptor subunits in nonneural cells, incredible progress has been made in understanding interactions of neurosteroids at the GABA<sub>A</sub> receptor. Although there is no absolute specificity for neurosteroid modulation of GABA<sub>A</sub> receptors, it was demonstrated that the presence of  $\alpha$  and  $\gamma$  subunits in the pentameric structure of

GABA<sub>A</sub> receptors affect GABA<sub>A</sub> neuromodulation by neurosteroids acting as positive GABA<sub>A</sub> modulators (allopregnanolone) and negative GABA<sub>A</sub> modulators (pregnenolone sulfate). Elegant electrophysiological studies showed that the presence of an  $\alpha 1$  subunit in a recombinant pentameric GABA<sub>A</sub> receptor doubles the efficacy of allopregnanolone. Other combinations of recombinant  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits, except for the  $\alpha 6$ ,  $\beta 1$ ,  $\gamma 2$  receptors, are less sensitive to allopregnanolone (189, 190, 273). Others have reported that chloride currents did not occur when the recombinant receptor contained  $\alpha 6$ ,  $\beta 3$  subunit heteromers (211), but this result was not reproduced using in a different cell type (273). Using potentiation of [<sup>3</sup>H]flunitrazepam binding, investigators found that the greatest binding of allopregnanolone was observed with an  $\alpha 3$ ,  $\beta 1$ ,  $\gamma 2$  receptor complex, whereas  $\alpha 1$ ,  $\beta 1$ ,  $\gamma 2$  and  $\alpha 2$ ,  $\beta 1$ ,  $\gamma 2$  complexes showed less than 100% enhancement of binding (109). As spinal cords were devoid of  $\alpha 1$  and rich in  $\alpha 3$  subunits, these authors suggested that the specific expression of the subunit  $\alpha 3$  in the spinal cord accounted for the greater potentiation of neurosteroids in this location.

GABA<sub>A</sub> receptors containing the  $\delta$  subunit have been reported to have distinct pharmacological characteristics in response to GABA (modification of EC<sub>50</sub>) as well as in response to other allosteric modulators, such as benzodiazepine, barbiturates, and lanthanum-induced potentiation (211). It was recently shown that the presence of  $\delta$  subunits inhibited neurosteroid modulation at GABA<sub>A</sub> receptors (273). That study supported the idea that the presence of  $\delta$  subunit in GABA<sub>A</sub> receptor did not block direct interaction of neurosteroids (3 $\alpha$ ,5 $\alpha$ -tetrahydro-11-deoxycorticosterone, allopregnanolone, and pregnenolone sulfate) with GABA<sub>A</sub>, but abolished the modulation resulting from this interaction. The  $\delta$  subunit mRNA is differentially distributed in various regions of the brain and is developmentally regulated, suggesting that neurosteroid effects on GABA<sub>A</sub> receptors may be developmentally regulated (62). The  $\delta$  subunit was specifically abundant in the adult cerebellum and hippocampus (112, 113, 263), regions in which selective sensitivity of GABA<sub>A</sub> receptors for neurosteroids has been reported in different *in vitro* models (209, 210, 212, 213). The abundant expression of  $\delta$  subunit mRNA in the cerebellum paralleled the development of a subset of granule neurons in culture (13, 21) and correlated with decreased modulation of GABA currents by neurosteroids. This event also correlated with the increase of the  $\alpha 6$  subunit mRNA in the cerebellum (112, 135, 271), which was proposed to result in decreased sensitivity of GABA<sub>A</sub> to neurosteroids. Others have correlated  $\alpha 6$  subunit incorporation into the cerebellar GABA<sub>A</sub> receptor with its benzodiazepine insensitivity (212). Interestingly, results from recombinant receptors experiments showed that allopregnanolone enhanced the effects of GABA in receptors containing  $\alpha 1$ ,  $\beta 2$ ,  $\gamma 2$  and  $\alpha 6$ ,  $\beta 2$ ,  $\gamma 2$  subunits, while the action of allopregnanolone without added GABA was more pronounced in receptors containing the  $\alpha 6$  subunit than in receptors containing the  $\alpha 1$  subunit (102). In cotransfection studies,  $\delta$  subunit incorporation into  $\alpha 6$ , $\beta 1$  or  $\alpha 5$ ,  $\beta 2$  GABA<sub>A</sub> heteromers significantly reduced the ability of tetrahydrodeoxycorticosterone to induce GABA<sub>A</sub> potentiation, suggest-

ing a critical role for  $\delta$  subunit in the assembly of neurosteroid-insensitive GABA<sub>A</sub> receptors (273). This developmentally regulated event may be of particular importance in light of the role of allopregnanolone and other progesterone derivatives as neuromodulators for GABA during embryogenesis in particular regions of the nervous system. Taken together, these results showed that the stoichiometry of GABA<sub>A</sub> receptor subunits and their functional cooperation is important for neurosteroid binding and for the neurosteroid-evoked modulation of GABA<sub>A</sub> activity. Despite all the pharmacology and electrophysiology performed with recombinant GABA<sub>A</sub> receptors, a consensus for which subunit or which combination of subunits are necessary and sufficient to trigger neurosteroid effects at GABA<sub>A</sub> receptors has not yet emerged.

### *In Vivo Studies*

The functional relevance of the diversity of GABA<sub>A</sub> receptor subunit composition has also been addressed *in vivo*. GABA<sub>A</sub> receptors in different regions of the brain may be differentially affected by the same neurosteroid. While the hippocampus and cerebellum both possess active GABA<sub>A</sub> receptors, the hippocampal GABA<sub>A</sub> receptor is expressed in noradrenergic neurons and is sensitive to benzodiazepine agonists, whereas the cerebellar receptor is expressed in glutamatergic (granule) neurons and is insensitive to benzodiazepine (212). Hippocampal GABA<sub>A</sub> receptors can evoke norepinephrine release only when the membrane on which they are localized is under resting potential (18, 63), while the cerebellar GABA<sub>A</sub> receptors are silent under resting potential and mediate glutamate release when depolarized (66, 118). These results suggest that the hippocampal and cerebellar synaptosome preparations could provide a model for studying the differential affinity of two native GABA<sub>A</sub> receptors for neurosteroids. In cerebellar synaptosomes, allopregnanolone was able to evoke D-aspartate release in the absence of GABA and potentiated D-aspartate release induced by GABA binding to its receptor. By contrast, allopregnanolone did not evoke norepinephrine release from hippocampal synaptosomes in the absence of GABA, but was still able to potentiate GABA norepinephrine release (212). These results suggest that allopregnanolone may act at two sites of the GABA<sub>A</sub> receptor in the cerebellum: (a) a high-affinity site mediating allosteric potentiation of GABA effects and (b) a low-affinity site mediating direct GABA-like effects, in the absence of GABA. The lack of effect of allopregnanolone in hippocampal synaptosomes suggested that the low-affinity, bicuculline-sensitive site may not be present in the GABA<sub>A</sub> receptors expressed in the hippocampus. Results also suggested that another neurosteroid, DHEAS, acted as an antagonist of the allosteric effects elicited by allopregnanolone, but not by GABA, in cerebellar synaptosome preparations, suggesting that DHEAS may not be an allosteric modulator of GABA but rather may elicit its effects by antagonizing allopregnanolone allosteric high-affinity binding.

*Neurosteroid Modulation of GABA<sub>A</sub> Receptor Subunit Composition*

GABA<sub>A</sub> receptor composition changes in response to brief or long-lasting exposure to endogenous physiological stimuli or long-term pharmacological treatments. Thus, animal models have been used to correlate GABA<sub>A</sub> receptor composition and altered GABA sensitivity to neurosteroids, barbiturates, or benzodiazepine. A recent study correlated the plasma and CSF concentrations of pregnenolone, progesterone, allopregnanolone, and tetrahydrodeoxycorticosterone in relation to changes in GABA<sub>A</sub> receptor composition during pregnancy and after delivery in female rats (42). Changes in allopregnanolone concentrations in the cortex of pregnant females at different stages during pregnancy coincided with changes in the sensitivity of the GABA<sub>A</sub> receptor to muscimol and to the decrease in the expression of that GABA<sub>A</sub> receptor subunit  $\gamma 2$ . Inhibition of allopregnanolone production reversed the changes of GABA<sub>A</sub> receptors in the hippocampus, both for its pharmacological sensitivity to muscimol and for the variations observed in its subunit composition. These data thus suggested that fluctuations of cortical neurosteroid concentrations modulated GABA<sub>A</sub> receptor subunit composition and that these changes altered the sensitivity of the receptor toward the neurosteroid.

Another *in vivo* study showed that ethanol dependency and/or withdrawal modified the composition of GABA<sub>A</sub> subunit assembly in rats (52). While ethanol dependency increased  $\alpha 1$  and decreased  $\alpha 4$  levels, ethanol withdrawal increased  $\beta 2$  and  $\gamma 1$  subunit mRNAs. Changes in GABA<sub>A</sub> receptor composition during ethanol withdrawal were correlated with a sensitization to the anxiolytic and anticonvulsant effects of neurosteroids and tolerance to ethanol, barbiturates, and benzodiazepine (52). These results suggested that the changes in the GABA<sub>A</sub> receptor assembly during ethanol withdrawal would produce a GABA<sub>A</sub> receptor more sensitive to neurosteroids and suggested a therapeutic potential for neurosteroids in the treatment of alcoholism.

Animal models of progesterone withdrawal also suggest that neurosteroids modulate GABA<sub>A</sub> receptor subunit composition and sensitivity to neurosteroid. Allopregnanolone withdrawal, but not progesterone withdrawal in rats, resulted in increased expression of  $\alpha 4$  subunit protein and mRNA in the hippocampus (45, 65, 224). Incorporation of the  $\alpha 4$  subunit into the hippocampal GABA<sub>A</sub> receptor altered its pharmacological sensitivity to allopregnanolone, eliminating the anti-anxiolytic effect of this neurosteroid. The increase in the  $\alpha 4$  subunit in ethanol dependency can thus be interpreted as a loss of function of endogenous allopregnanolone in the hippocampus of alcoholic animals, rather than by a lack of tolerance to neurosteroid effects during sensitization to ethanol. The allopregnanolone-induced GABA<sub>A</sub> receptor subunit changes during progesterone withdrawal, a physiologic situation occurs during the menstrual cycle or after delivery, may also explain catamenial epilepsies and increased anxiety and seizure susceptibility occurring in premenstrual syndrome and postpartum (224, 225).

Thus, differences in the subunit composition of GABA<sub>A</sub> receptors could explain the regional sensitivity to neurosteroids at the level of both GABA

potentiation and GABA<sub>A</sub> allosteric neuromodulation. These novel and exciting data have greatly increased our understanding of the interactions of neurosteroids, such as allopregnanolone and DHEAS, at the GABA<sub>A</sub> receptor. These data have also initiated functional analysis of the neuromodulation of neurosteroids at GABA<sub>A</sub> receptors and suggest several physiological situations in which neuromodulation of GABA<sub>A</sub> receptors by specific neurosteroids may occur in humans under both normal and pathologic conditions.

### Neurosteroids Modulate NMDA Receptors

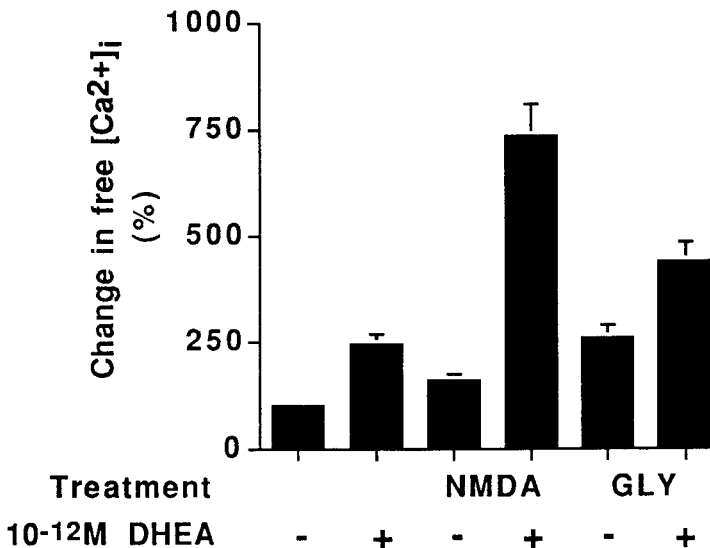
Neuroactive steroids, such as pregnenolone sulfate, DHEA, and DHEA sulfate, have been shown to modulate NMDA receptor activity (22, 40, 59, 178, 264). Allopregnanolone sulfate, but not allopregnanolone, acts as a negative allosteric modulator of NMDA receptors (178), while DHEA, pregnenolone, and their sulfate esters are considered 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 interaction sites have been described. Molecular cloning has demonstrated the presence of five NMDA receptor subunits, called  $\zeta$ 1 in the mouse (NR1 in the rat), and four  $\epsilon$  subunits (1–4) in the mouse (NR2A–D in the rat) (85, 104, 142, 158, 161, 266). The  $\zeta$ 1 subunit is obligatory for channel function and different splice variants in its mRNA have been found (161). The  $\epsilon$  subunit mRNAs are expressed in a varying regional and developmental distribution. This suggests that differences in the NMDA receptor composition in various regions of the brain and during early postnatal development in the cerebellum may result in different pharmacologic profiles.

In a pure neuronal primary culture of mouse embryonic neocortical neurons, we showed that DHEA, but not DHEAS, could evoke an increase in free intracellular calcium in the absence of KCl or NMDA (40). DHEAS potentiated glutamate- and NMDA-mediated increases in free intracellular calcium concentrations in similar primary neuronal chick cultures (59). DHEA-mediated increases in free intracellular calcium concentrations occurred at low concentrations of DHEA (from  $10^{-12}$  M) in high-density cell suspensions (40) and from  $10^{-8}$  M in low-density primary neuronal cultures. We also previously showed that DHEA, as others had shown for pregnenolone sulfate, greatly potentiated NMDA-induced increases in free intracellular calcium concentrations. Increased intracellular calcium mediated by DHEA was abolished in a dose-dependent manner by both MK801, a noncompetitive antagonist of NMDA, and by D-AP5, a competitive inhibitor of NMDA receptor, suggesting a direct interaction of DHEA at the NMDA receptor. These data demonstrated, for the first time, the ability of DHEA to act as a neuromodulator at NMDA receptor. In our experiments, embryonic cortical neurons were also mildly responsive to glycine since  $10 \mu\text{M}$  glycine increased the free intracellular calcium concentration by 2.6-fold. DHEA had an additive and not a potentiating effect on glycine-mediated increases in free intracellular calcium concentrations, suggest-

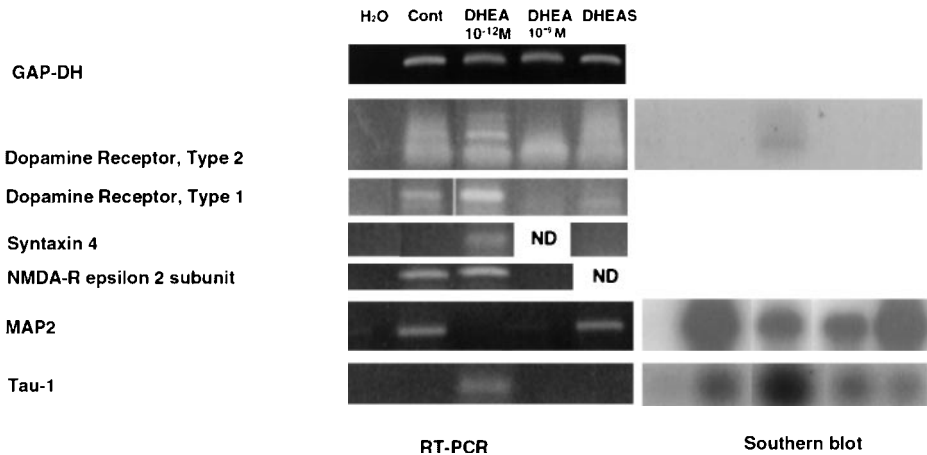
ing that DHEA and glycine acted independently at the NMDA receptor (see Fig. 6).

The rather low affinity of the NMDA receptors for glycine and their sensitivity for D-AP5 suggested the presence of the subunit  $\epsilon 1$  in our embryonic neocortical cultures (6, 95). We confirmed the expression of both  $\epsilon 1$  and  $\epsilon 2$  in our cultures using RT-PCR. NR2A expression was shown to be developmentally regulated in neuronal cortical cultures from different ages of neonatal rats (95). The late expression of NR2A was correlated with the appearance of a markedly reduced affinity for glycine around the 20th postnatal day.

In the cerebellum, the expression of NR2 subtype mRNA correlated with the developmental migration and postmigratory maturation of granule cells. During migration and immediately after arrival in the internal granular cell layer, NR2B was the only subunit expressed, and its expression declined to undetectable levels in the adult (157, 258), while expression of NR2A mRNA correlated with the formation of synaptic contacts in the cerebellum and was heavily expressed during synaptic pruning. More recently, it was shown that receptors containing both  $\epsilon 1$  and  $\epsilon 3$  subunits could be present at the synapse from granule cells in cerebellar slices (232). Expression of  $\epsilon 1$  subunits resulted in a relatively fast decay of the NMDA receptor-mediated EPSCs, whereas late expression of  $\epsilon 3$  subunit in the developing cerebellum contributed to a developmental reduction in the voltage-dependent block of the EPSCs by magnesium. Our results obtained from semiquantitative RT-PCR suggested that the  $\epsilon 2$



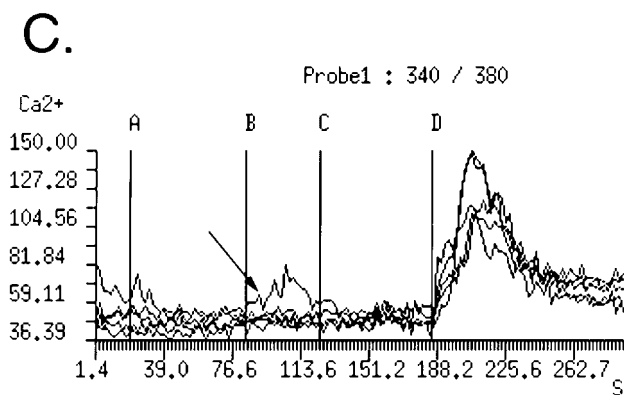
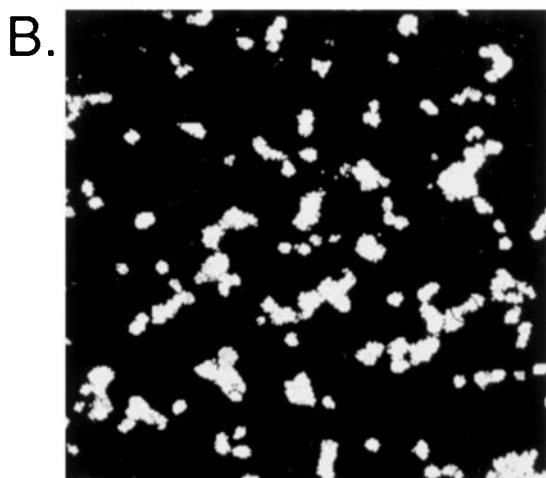
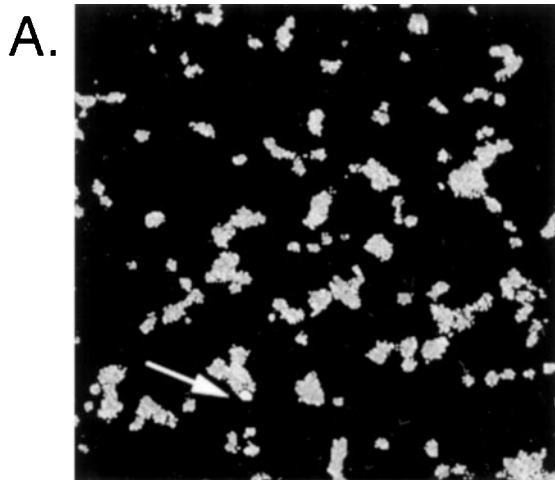
**FIG. 6.** Effect of NMDA and glycine on the increase of  $[Ca^{2+}]_i$ -mediated by DHEA. Each bar shows the mean percentage of  $[Ca^{2+}]_i$  increase over baseline. Error bars are  $\pm$ SEM of six separate measurements. NMDA (10  $\mu$ M) and glycine (10  $\mu$ M) were added to the cell suspension before addition of DHEA. NMDA increased  $[Ca^{2+}]_i$  above baseline values, and DHEA synergistically potentiated this response; glycine increased  $[Ca^{2+}]_i$ , and DHEA increased this response. These results support the involvement of the NMDA receptor in the DHEA-mediated increase in  $[Ca^{2+}]_i$ .



**FIG. 7.** Semiquantitative RT-PCR of total RNA extracted from individual wells of neuronal primary cultures obtained from E16.5 neocortex in control or after 16 h incubation with DHEA ( $10^{-10}$  M and  $10^{-12}$  M) or DHEAS ( $10^{-10}$  M). The quantification of RNA level was obtained in comparison to the level of the ubiquitously expressed GAPDH mRNA in the linear amplification of the PCR reaction (32 cycles). When a probe corresponding to the amplified region of the targeted mRNA was available, the nature of the amplified fragment was ensured by Southern blotting. DHEA increases mRNAs for Tau-1, NMDA receptor  $\epsilon 2$  subunit, Syntaxin-4, and both dopamine receptors Type 1 and 2 in a dose-dependent fashion, correlating the morphological data previously obtained. DHEAS, however, increases mRNA for MAP2 and seems to downregulate the dopamine receptor Type 2 mRNA.

subunit was downregulated by increased concentrations of DHEA (Fig. 7). Thus DHEA may be involved in inducing plasticity in NMDA receptor subunit composition.

In a manner similar to agonist- and antagonist-induced changes in the subunit composition of the GABA<sub>A</sub> receptors (see previous section in this review), other pharmacologic agents have been shown to produce changes in NMDA receptor subunit composition. Chronic ethanol treatments increased the expression of NMDA receptor number both *in vitro* and *in vivo* (103). Furthermore, chronic ethanol treatments *in vivo* selectively increased NR2B mRNA in the cortex without altering NR1 mRNA. Cultured cortical neurons treated continuously with ethanol (50 mM, 5 days) stabilized NR2A mRNA without altering NR1 mRNA, suggesting that the regulation of NR2B mRNA *in vivo* may be at the transcriptional level, while ethanol may act posttranscriptionally, since the level of NR1 mRNA did not change. Similarly, treatment with the NMDA antagonist D-AP5 produced changes in NMDA receptor subunit composition in cultured mouse cortical neurons. D-AP5 selectively increased NR2B mRNA, modifying the ratio of NR2B/2A proteins. It also increased NR1 protein without modifying NR1 mRNA levels (61). It is likely that other molecules that bind directly to NMDA receptors or potentiate its effects will be shown to participate in the regulation of the subunit composition of NMDA receptors in a particular location, thus modifying the modulation of the effects driven by NMDA at the synaptic level.



Behavioral effects of neurosteroids, such as pregnenolone sulfate and DHEAS, in enhancing spatial memory in mice are thought to result from their positive modulatory action at the NMDA receptor (136). This effect may result from changes in NMDA receptor subunit composition. Both  $\epsilon 1$  and  $\epsilon 2$  NMDA receptor subunits are expressed in hippocampal CA1 (205) and CA3 neurons (86). In  $\epsilon 1$  knockout mice, impairment of  $\epsilon 1$  expression caused loss in LTP at the hippocampal CA1 synapses associated with deficiency in spatial learning (205) and resulted in a reduction of NMDA EPSCs and LTP in response to the commissural/associational CA3 synapses without significantly modifying the EPSCs originating from fimbrial CA3 synapses (86).  $\epsilon 2$  Impairment, however, produced reversed effects in CA3 neurons, reducing NMDA EPSCs and LTP in fimbrial CA3 synapses with no appreciable modification of the commissural/associational CA3 synapses. These results suggest that in addition to changes in NMDA subunit composition, the function of NMDA receptor is regulated in a synapse selective manner.

#### Are Neurosteroids Endogenous Ligands for $\sigma$ Receptors?

Sigma receptors are membrane-associated protein of unknown physiological function. Radioligand-binding assays have indicated that there are at least three receptors, denoted  $\sigma 1$ ,  $\sigma 2$ , and  $\sigma 3$ , respectively (19, 78, 191). Recently, a protein with the binding profile of  $\sigma 1$  receptor has been cloned from guinea pig liver microsomes (73). A structurally diverse range of compounds bind to  $\sigma$  receptors without a clear selectivity for a particular isoforms (48, 203, 255). Thus, it has been difficult to attribute biochemical, physiological, and behavioral effects to each  $\sigma$  receptor. One example of poor specificity is the direct interaction between  $\sigma 1$  and  $\sigma 2$  ligands and NMDA receptors (47, 233). Neurosteroids like DHEA and DHEAS have been shown to induce NMDA-mediated norepinephrine release via  $\sigma 1$  receptor activation in hippocampal slices (156), suggesting that neurosteroids acting as potentiators of NMDA receptor may be endogenous ligands for  $\sigma$  receptors. Several studies have reported that the behavioral effect of DHEAS on improvement of memory are related to a pharmacological action at  $\sigma$  sites (15, 139). Being reported as both NMDA-

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**FIG. 8.** Fluorescence ratio imaging of neuronal primary culture cells derived from embryonic neocortex preincubated with Fura-2. Recording of the dynamic changes of  $[Ca^{2+}]_i$  were obtained by applying a wavelength ratio of 340/380. Digitalized images processed by a MetaFluor system (Universal Imaging Corp.) are presented in A and B, and quantified  $[Ca^{2+}]_i$  changes are presented in C. (A) Stimulation of the cultured cells with  $10^{-8}$  M DHEA in the perfusion medium (magnification is  $\times 200$ ); the white arrow points to the responsive cells in the field. (B) The same field was perfused with  $10^{-4}$  M veratridine, a potent  $Na^{2+}$  channel activator, to ensure the neuronal nature and the viability of the cultured cells at the end of the recording period (magnification is  $\times 200$ ). (C) Quantification of  $[Ca^{2+}]_i$  plotted against the recording time. The black arrow shows the response of the cell to DHEA. Vertical lines correspond to addition of drugs to the perfusion medium: (A) DHEA  $10^{-10}$  M; (B) DHEA  $10^{-8}$  M; (C) DHEA  $10^{-6}$  M; (D) Veratridine  $10^{-4}$  M.

positive allosteric modulators and nonselective  $\sigma$  antagonists, neurosteroids such as pregnenolone sulfate and DHEA may finely temper NMDA activity.

## WHAT IS THE PHYSIOLOGICAL RELEVANCE OF NEUROSTEROID PRODUCTION?

### Developmental Role in Organogenesis

#### *DHEA and DHEAS Are Modulators of Cytoarchitectural Organization in the Developing Brain*

We and others have previously shown that DHEA and DHEAS are potent neuroactive steroids which selectively increase neurite outgrowth in regions where P450c17 is expressed (17, 40). We further determined that, in the developing neocortex, DHEA promoted axonal growth and morphological indices of synaptic contacts, while DHEAS promoted dendritic growth and branching, suggesting that these two neurosteroids are separate hormones active in the same process. Thus, the local ratio of DHEA versus DHEAS may regulate specific neurite growth in the developing neocortex, thereby shaping projections and synapses during embryogenesis. We also hypothesized that the sulfohydrolase (STS) and sulfotransferase (HST) activities in the developing brain are key regulators of DHEAS effects in the developing brain. We identified STS mRNA expression in the thalamus, a site where P450c17 fibers are terminating and where P450c17 is active during embryogenesis (41). The thalamus was identified as a region involved in pioneering thalamic axons to the neocortex (188). The presence of the sulfohydrolase in this region is consistent with the presence of axonal growth promoting cues in the thalamus. Others have shown that allopregnanolone induced axonal regression in the developing hippocampus (23), suggesting that neurosteroids produced *de novo* in the nervous system may work in concert to promote, guide, and refine axonal growth and synaptic connections in the developing neocortex. Furthermore, DHEA, but not DHEA-S, increased free intracellular calcium concentrations in a selected population of neocortical neurons via an NMDA receptor pathway, as determined in both microfluorimetry and calcium imaging (Fig. 8). The small number of responsive cells within the cultures suggested that DHEA was active on only a few neurons in our culture, and these may be subplate neurons that express P450c17.

To identify downstream events induced by DHEA/DHEAS in primary neocortical cells we performed semiquantitative RT-PCR in control cells and in cells treated with DHEA ( $10^{-12}$  M) or DHEAS ( $10^{-10}$  M) for 16 h (Fig. 7). As anticipated, DHEA induced expression of Tau mRNA, a microtubule-associated protein segregated in axons, while DHEA-S induced Map-2 mRNA, a microtubule-associated protein segregated in dendrites. Furthermore, DHEA induced the dopamine receptor Type 1 and 2 mRNAs, while DHEAS reduced the expression of both these mRNAs to undetectable levels. With both treatments,

SNAP 25 mRNA was increased above the control level. These data suggest that both DHEA and DHEAS enhance the differentiation of the cultured neocortical neurons, but each steroid produces specific downstream events. Taken together, our results suggest that DHEA is strongly involved in axonal growth and functional activation of specific neuronal networks and that at least some of these effects are mediated via NMDA receptor activation. We believe that DHEA may serve a similar role in all the regions where P450c17 is expressed and active. We are currently identifying which neuronal networks are specifically targeted by DHEA.

### *Progesterone May Participate in Oligodendrocyte Differentiation*

In addition to regulating myelin formation (see previous section on genomic actions of neurosteroids), progesterone was reported to improve the myelination of injured nerves *in vivo* (100) and to facilitate myelination *in vitro* (32). Recent data have demonstrated that progesterone, estradiol, and insulin, as well as the combination of progesterone and insulin, increased expression of myelin basic protein and CNPase (90), suggesting that these factors induced oligodendrocyte maturation *in vitro*. The pathway by which progesterone and insulin mediate this effect is not resolved. Increases in mRNAs for both P450<sub>sc</sub> and 3 $\beta$ -HSD, enzymes which together convert cholesterol into progesterone, paralleled the differentiation of oligodendroglial precursors into myelin-forming oligodendrocytes in both glial cultures from the CNS and cocultures of dorsal root ganglial neurons and Schwann cells from the PNS (32). These data suggest that *de novo* synthesis of progesterone may participate in or result from the differentiation of oligodendroglial precursors in both the CNS and PNS. However, further studies are necessary to determine if progesterone is essential to induce oligodendroglial differentiation in peripheral nerves and in the CNS or if expression of steroidogenic enzymes in oligodendrocytes results from the acquisition of their differentiated state. Others have shown that in a model of mixed oligodendroglial precursors and mature oligodendrocytes, progesterone was not able to rescue oligodendrocytes from apoptosis induced by a phosphatidylinositol 3-kinase (251). These data strongly suggest that endogenously produced neurosteroids may have a generic role in the differentiation of their target cells in the developing CNS in a regional and temporal fashion.

### **Neuroprotection**

In human beings, DHEA and DHEAS are the most abundant circulating steroids during development and after adrenarche, and their levels decline with aging (97, 172). Certain types of illness (77, 227) or stress (70, 79) also decrease the circulating levels of both steroids. As both age and stress are associated with neuronal vulnerability to degeneration, several investigators have hypothesized that DHEA and DHEAS may be active in protecting the

brain from insults due to neurodegeneration and/or ischemia. Glutamate and its agonists mimic neural damage after cerebral ischemia, since release of glutamate directly results from ischemic insults (14, 35, 223). Neuroprotection by DHEA and DHEAS was observed in pyramidal neurons and also in glial cells both *in vitro* and *in vivo* in hippocampal structures (97) and isolated retina (71). Other sulfated neurosteroids, such as allopregnanolone sulfate, were also reported to be potent neuroprotectors of hippocampal structures challenged by the glutamate agonist NMDA (259).

The mechanism(s) by which DHEA and DHEAS exert their neuroprotective effects is not known. DHEAS, but not DHEA treatment, was associated with activation of NF $\kappa$ B transcriptional activity (132). Manipulation of NF $\kappa$ B-mediated transcriptional activity was shown to be sufficient to mimic neuroprotective effects of other known neuroprotectors (such as antioxidants) against amyloid  $\beta$  toxicity (9). The mechanisms by which DHEA acts are still unknown. Two nonexclusive hypotheses are that DHEA may reduce or alter free intracellular calcium concentrations after a massive NMDA exposure, or that DHEA acts as a glucocorticoid antagonist (97).

In the retina, exposure to NMDA modified the formation of pregnenolone and pregnenolone sulfate in a dose-dependent manner (71). This effect was mediated by NMDA receptors and was primary to any cell damage. There was a clear correlation between the increase in neurosteroidogenesis and the glutamatergic insult to retinal explants, which was independent of GABA<sub>A</sub> receptor activation. These results suggested that modulation of neurosteroidogenesis is an endogenous mechanism by which regions of the nervous system that are vulnerable to NMDA-induced cell damage may counteract the potential insult resulting from an excess or a long exposure to glutamate. Thus, the NMDA-mediated stimulation of neurosteroid synthesis could be considered an early response to acute excitotoxicity, designed to delay/protect from neuronal cell death.

#### **GABA<sub>A</sub> Modulation and PMS; Anti-anxiolytic Effects in Depression (Postpartum Depression)**

Allopregnanolone may play a varied role in the regulation of behavior in human beings and in several rodent models of stress, epilepsy, and depression. It has been reported to be protective against adverse early life events when administered to neonatal rats (180). It abolished alterations in central mechanisms controlling the HPA axis in pups that were subjected to intermittent maternal deprivation in early postnatal life, suggesting that this neurosteroid exerts persistent stress-protective effects in the developing brain. Thus, neurosteroid treatment may be a therapeutic intervention that may prevent mental disorders associated with early maternal deprivation, such as major depression.

Alterations in allopregnanolone concentrations *in vivo* may also be associated with affective disorders in human beings. A recent study showed increases

in allopregnanolone concentrations in the cerebrospinal fluid of patients successfully treated for unipolar major depression treated with certain selective serotonin reuptake inhibitors (SSRIs) (200, 249). Normalization of CSF allopregnanolone concentrations in depressed patients was sufficient to mediate the anxiolytic and antidysphoric action of fluoxetine or fluvoxamine, suggesting an additional mechanism of action of these SSRIs. A similar study by the same group showed identical results in adrenalectomized and gonadectomized rats treated with fluoxetine, suggesting that the increase in allopregnanolone concentration in the CSF was due to *de novo* endogenous synthesis of allopregnanolone in the brain of those animals (248).

Others have related endogenous allopregnanolone biosynthesis/metabolism rate during pregnancy with changes in GABA<sub>A</sub> receptor subunits (42), suggesting that the pharmacological changes in GABA<sub>A</sub> receptor subunits and their associated behaviors are regulated, at least in part, by local production of allopregnanolone. These results are consistent with a study that showed that withdrawal of allopregnanolone was responsible for the relative insensitivity of GABA<sub>A</sub> to neurosteroid neuromodulation and induced increased anxiety (225). Such a paradigm may be related to the premenstrual syndrome and postpartum or postmenopausal dysphoria characterized by an unstable mood and tendency to anxiety, in its mildest form, and associated with major depression as its worst symptom.

Several laboratories, including ours, are thus demonstrating the importance of endogenous neurosteroid biosynthesis and its regulatory mechanisms in both induction of specific neuronal networks and in neuromodulation of those pathways in the adult. Neurosteroids are of particular importance in situations requiring adaptation to stress. The dysregulation of their biosynthesis is now recognized as associated with pathologic neurologic responses. However, neurosteroid content in CSF and in plasma is highly variable in individuals. If neurosteroidogenesis declines with age, little has been done to correlate DHEA/DHEAS, pregnenolone/pregnenolone sulfate, or allopregnanolone concentrations in the elderly with their mental and physical status. In rats, high pregnenolone and pregnenolone sulfate concentrations have been correlated with higher performances in both the Y maze and the Morris water maze, suggesting that PREG/S protects spatial memory acquisition and/or maintenance (250). In human beings aging is accompanied by a progressive decline in the secretion of the dehydroepiandrosterone and DHEA sulfate. A randomized trial of a replacement dose of DHEA involving a limited number of "old" (40 to 70 years old) men (13) and women (17) was associated with a remarkable increase in perceived physical and psychological well-being for both men (67%) and women (84%). This study, however, lacked an evaluation of the neurological profile of the subjects before and after restoration of DHEA levels and did not characterize changes in memory during the replacement period. Another study on a French community-based sample of elderly associated low DHEAS levels in plasma in women (over 65 years of age) with functional limitations, including confinement, dyspnea, depressive symptomatology, poor subjective perception of health and life satisfaction, and usage of various medication (16). In men,

lower DHEAS was significantly associated with increased short-term mortality, death occurring after a period of 2 to 4 years after baseline measurement. Thus, there may be indications that circulating levels of neurosteroids may be predictive for mental health.

### CONCLUSION

Important findings are beginning to correlate the modulation of neurosteroid biosynthesis and the regulation of endogenous neurosteroidogenic enzymes with functional neuropathologies. More studies are necessary to identify the neuronal circuits that are modulated by each neurosteroid. Such studies need to be augmented with the cellular and molecular mechanisms by which the neurosteroids exert their effects.

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