Neurosteroids: Expression of Steroidogenic Enzymes and Regulation of Steroid Biosynthesis in the Central Nervous System

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I. Introduction
Steroid hormones, which are synthesized in the adrenal gland, gonads and placenta, exert a large array of biological effects on the nervous system. In particular, steroid hormones play an important role in the development, growth, maturation, and differentiation of the central nervous system (CNS) and peripheral nervous system (PNS) (for review, McEwen, 1994). Depending on their chemical nature and concentration, steroids can induce either protective or deleterious effects on nerve cells (Uno et al., 1989; Yu, 1989; Jones, 1993; Sapolsky, 1996; Green et al., 1997; Seckl, 1997; Kimonides et al., 1998). These actions had long been exclusively ascribed to steroids produced by endocrine glands, which can easily cross the blood-brain barrier to act on the CNS. However, a series of studies conducted by Baulieu and...
coworkers have shown that the rat brain is capable of synthesizing various steroid hormones such as pregnenolone (Δ⁵P) and dehydroepiandrosterone (DHEA) from cholesterol (Baulieu, 1981). These authors first demonstrated the existence of high amounts of Δ⁵P and DHEA in the brain of castrated and adrenalectomized rats (Corpéchot et al., 1981, 1983). Thereafter, they found that the cerebral concentrations of Δ⁵P and DHEA are not affected by administration of adrenocorticotropic hormone or suppression of circulating glucocorticoids by dexamethasone (Robel and Baulieu, 1985). They also showed that the levels of Δ⁵P and DHEA in the brain undergo circadian variations which are not synchronized with those of circulating adrenal steroids (Robel et al., 1986). Finally, the immunohistochemical localization of cytochrome P-450 side chain cleavage (scc) in rat oligodendrocytes and the observation that the enzyme was biologically active (i.e., capable of converting cholesterol into Δ⁵P) unambiguously demonstrated that steroids can be synthesized within the CNS (Le Goascogne et al., 1987). The term neurosteroids has been coined to designate steroids that are newly synthesized from cholesterol or another early precursor in the nervous system, and are thus still present in substantial amounts after removal of peripheral steroidogenic glands (Robel and Baulieu, 1994). Neurosteroids occur in the nervous system as unconjugated steroids, sulfated esters of steroids, or fatty acid esters of steroids (Jo et al., 1989). These various forms of steroids are involved in the control of metabolic, behavioral, and psychical processes including cognition, stress, anxiety, and sleep (Majewska, 1992; Baulieu and Robel, 1996).

Besides their actions at the transcriptional level (McEwen, 1994), neuroactive steroids may act on nerve cells via two types of membrane receptors. Steroids can exert allosteric modulation of receptors for neurotransmitters such as γ-aminobutyric acid (GABA)ₐ receptors (Majewska, 1992), nicotinic receptors (Valera et al., 1992), muscarinic receptors (Klangkalya and Chan, 1988), N-methyl-d-aspartate (NMDA) receptors (Wu et al., 1991), and α receptors (Monnet et al., 1995). In addition, it has been proposed that neuroactive steroids may act on nerve cells via proper membrane receptors coupled to G proteins (Orchikin et al., 1992) or through specific membrane sites using calcium as an intracellular messenger (Ramirez and Zheng, 1996). The recent demonstration that progesterone and 5β-dihydroprogestrone directly inhibit oxytocin receptor function suggests that neurosteroids may also interact with various membrane-bound neuropeptide receptors (Grazzini et al., 1998).

II. Biochemical Pathways of Steroid Biosynthesis in Endocrine Glands

All steroid hormones derive from cholesterol which is provided by blood as low-density and high-density lipoproteins. A small proportion of cholesterol can also be produced directly in steroidogenic cells from acetate. The biochemical reactions responsible for the synthesis of steroids are controlled by various families of enzymes including hydroxylases (desmolases), oxydo-reductases (dehydrogenases), sulfotransferases (sulfokinases), and sulfuryl transferases (Fig. 1). Molecular cloning of the enzymes responsible for biosynthesis of steroid hormones has revealed, for some of these enzymes, the existence of multiple isoforms which are differentially expressed in steroidogenic tissues (Miller, 1988; Labrie et al., 1994). It has also been shown that various peripheral organs, such as the digestive tract (Dalla-Valle et al., 1992; Le Goascogne et al., 1995), the liver (Martel et al., 1994), and the prostate (Belanger et al., 1989), can express at a low level the genes encoding several steroidogenic enzymes, suggesting that the production of bioactive steroids is not restricted to steroidogenic endocrine glands. The mechanisms regulating the expression of steroidogenic enzymes have been studied in great detail in the adrenal gland and gonads (Miller, 1988; Güse-Behling et al., 1992; Penning, 1997).

III. Cytochrome P-450scc

The scc of cholesterol leading to the formation of Δ⁵P is catalyzed by cholesterol-desmolase, an enzymatic complex composed of cytochrome P-450scc (P-450scc) which possesses a hydroxylasic activity, adrenodoxine or ferredoxine, and adrenodoxine reductase (Fig. 1). Using an antibody raised against bovine adrenal P-450scc, Le Goascogne et al. (1987) have shown the presence of immunoreactive elements in the white matter throughout the rat brain, an observation which is rightly considered as the fundamental discovery that paved the way for further research on neurosteroids. The fact that glial cells in primary culture are capable of converting cholesterol into Δ⁵P has subsequently demonstrated that the immunoreactive P-450scc localized in the rodent brain actually corresponds to an active form of the enzyme (Jung-Testas et al., 1989). In addition, the occurrence of the mRNAs encoding for P-450scc and adrenodoxine has been evidenced in the CNS of mammals by means of various approaches including reverse transcription-polymererase chain reaction (RT-PCR), ribonuclease protection assays, and in situ hybridization (Mellon and Deschepper, 1993; Compagnone et al., 1995a). The P-450scc gene is expressed at a particularly high concentration in the cerebral cortex and, to a lesser extent, in the amygdala, hippocampus, and midbrain. The distribution of P-450scc mRNA is similar in the brain of female and male rats (Mellon and Deschepper, 1993; Compagnone et al., 1995a). The P-450scc gene is also expressed in the nervous system of developing rodent embryos, specifically in the cell lineages derived from the neural crest and in sensory structures of the PNS (Compagnone et al., 1995a). Recently, Ukena et al. (1998) have shown the presence of P-450scc in Purkinje cells of neonatal and adult rats, indicating that the gene
is not only expressed in glial cells but also in neurons. The detection of relatively high amounts of $\Delta^5\text{P}$ in the frog hypothalamus (Mensah-Nyagan et al., 1994) and the localization of an active form of $P-450\text{scC}$ in the quail brain (Tsutsui and Yamazaki, 1995; Usui et al., 1995) have shown that the gene encoding $P-450\text{scC}$ is also actively expressed in the CNS of nonmammalian vertebrates.

Recent studies have revealed the existence of substantial differences between the transcriptional factors regulating the expression of $P-450\text{scC}$ in the C6 glial cell line and in steroid-secreting cells of endocrine glands (Mellon and Deschepper, 1993; Papadopoulos, 1993). Specifically, the steroidogenic factor SF-1, which plays a crucial role in the control of the expression of all steroid hydroxylase genes including $P-450\text{scC}$ (Clemens et al., 1994; Ikeda et al., 1994), and the basal transcriptional factor Sp1 are not expressed in C6 glioma cells (Zhang et al., 1995). In fact, SF-1 has been detected in discrete cerebral areas which do not contain $P-450\text{scC}$ mRNA (Mellon and Deschepper, 1993; Ikeda et al., 1994), indicating that SF-1 is not involved in the regulation of $P-450\text{scC}$ gene expression in neural tissues as it is in steroidogenic glands. In contrast, cAMP which controls steroid biosynthesis in peripheral tissues (Moore et al., 1990; Watanabe et al., 1994) appears to modulate neurosteroidogenesis in C6 glioma cells (Papadopoulos and Guarneri, 1994). These observations reveal that the mechanisms regulating $P-450\text{scC}$ gene expression in endocrine cells and nerve cells exhibit both differences and similarities.

Studies performed in rat indicate that the biological activity of $P-450\text{scC}$ may be controlled by peripheral-type benzodiazepine receptor (PBR) ligands in the CNS (Guarneri et al., 1992) as previously shown in classical steroidogenic tissues (Krueger and Papadopoulos, 1990; Papadopoulos et al., 1990). In fact, PBRs, which facilitate the translocation of cholesterol from the external surface of mitochondria to the internal membrane, cause indirect stimulation of $P-450\text{scC}$ activity (Papadopoulos, 1993). The observation that benzodiazepines activate neurosteroid biosynthesis has suggested that endogenous ligands of PBR (endozepines) could be involved in the regulation of $P-450\text{scC}$ activity (Papadopoulos et al., 1992; Korneyev et al., 1993). The major natural ligand of PBR, diazepam-binding inhibitor (DBI), is a 11-kDa polypeptide whose gene is highly expressed in steroid-secreting tissues (Rhéaume et al., 1990; Brown et al., 1992; Rouet-Smih et al., 1992; Toranzo et al., 1994) as well as in C6 glioma cells (Alho et al., 1994) and in astrocytes (Tong et al., 1991; Malagon et al., 1992, 1993; Slobodyansky et al., 1992; Libermann et al., 1994; Patte et al., 1995; Lamacz et al., 1996). Proteolytic cleavage of DBI generates several bioactive peptides including the triakontatetraneuropeptide (TTN) (Ferrero et al., 1986; Slobodyansky et al., 1994; Tonon et al., 1994; Patte et al., 1999). A study performed on isolated mitochondria from C6 glioma cells has revealed that DBI and TTN both stimulate the formation of $\Delta^5\text{P}$ (Papadopoulos et al., 1993).
1992). These results strongly suggest that endozeptines play an important role in the regulation of P-450scc activity in the nervous system.

IV. 3β-Hydroxysteroid Dehydrogenase

The enzymatic complex 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (3β-HSD), which catalyzes the conversion of Δ5-3β-hydroxysteroids into Δ4-3-ketosteroids, plays a crucial role in the biosynthesis of all classes of steroid hormones (Fig. 1). Molecular cloning of the cDNAs encoding 3β-HSD has revealed the existence in human of two isoforms of the enzyme: type I 3β-HSD which is mainly expressed in the placenta (Luu-The et al., 1989) and type II 3β-HSD which is predominantly expressed in the adrenal gland and gonads (Rhéaume et al., 1991). Four types of 3β-HSD cDNAs (types I-IV) have been characterized in the rat (Zhao et al., 1990, 1991; Mason, 1993) and six types (types I-VI) in the mouse (Simard et al., 1996; Abbassade et al., 1997). The rodent type III 3β-HSD isoform possesses the structural features common to all 3β-HSD but does not display the expected classical 3β-HSD activity; in fact, this isoenzyme behaves as a 3-ketosteroid reductase using NADPH as a cofactor, i.e., it is responsible for the conversion of saturated 3-ketosteroids into 3β-hydroxy metabolites (Labrie et al., 1992). The enzyme 3β-HSD is also present in various tissues such as the skin (Dumont et al., 1992), mammary gland (Rhéaume et al., 1991), and prostate (Bartsch et al., 1990).

The first data suggesting the existence of 3β-HSD in the CNS have been provided by Weidenfeld et al. (1980) who showed that homogenates of rat amygdala and septum are capable of converting Δ5 into progesterone. The formation of androstenedione from DHEA, which is also catalyzed by 3β-HSD (Fig. 1), confirmed the presence of the enzyme in the rat brain (Robel et al., 1986). The biological activity of 3β-HSD has also been detected in primary cultures of rodent oligodendrocytes (Jung-Testas et al., 1989) and neurons (Bauer and Bauer, 1989). The first immunohistochemical localization of 3β-HSD in the CNS has been performed in the European green frog Rana ridibunda by using an antiserum raised against type I human placental 3β-HSD (Mensah-Nyagan et al., 1994). This antiserum had been previously applied for the immunocytochemical localization of 3β-HSD in classical steroid-producing organs of mammals such as the adrenal, testis, ovary, and placenta (Dupont et al., 1990a-c). Although the antibodies were raised against type I human placental 3β-HSD (Luu-The et al., 1989), they also recognize other 3β-HSD isoforms, in particular, type II 3β-HSD (Dupont et al., 1990a-c) which is predominantly expressed in the adrenal and gonads (Lachance et al., 1991). It thus appears that the immunoreactive material detected in the frog brain may correspond to different variants of the 3β-HSD family.

The occurrence of large amounts of Δ4-3-ketosteroids (progesterone and 17-hydroxyprogesterone) in the frog brain and the capability of frog hypothalamic explants to catalyze the conversion of tritiated pregnenolone ([3H]Δ5P) into progesterone demonstrate that 3β-HSD-immunoreactive material detected in the CNS actually corresponds to an active form of the enzyme (Mensah-Nyagan et al., 1994). In situ hybridization studies have revealed that the mRNAs encoding for 3β-HSD in the rat brain are localized in the olfactory bulb, nucleus accumbens, hippocampus, area of medulla bordering the fourth ventricle as well as in the thalamus, hypothalamus, and cerebellum (Dupont et al., 1994; Guennoun et al., 1995). Immunocytochemical data have shown that, in the frog brain, the 3β-HSD gene is exclusively expressed in neurons (Fig. 2). Similarly, in the rat CNS, 3β-HSD mRNAs were only detected in neuronal cell bodies (Dupont et al., 1994; Guennoun et al., 1995) (Fig. 3). It should be noted however that the presence of 3β-HSD and its mRNAs has recently been found in rodent Schwann cells by immunocytochemistry and RT-PCR (Guennoun et al., 1997). In addition, 3β-HSD activity has been demonstrated in primary cultures of rat astrocytes and oligodendrocytes (Jung-Testas et al., 1989; Kabbadj et al., 1993). These observations indicate that glial cells, which do not possess 3β-HSD in situ, may acquire the ability of expressing the 3β-HSD genes when they are maintained in culture. Alternatively, it is possible that other 3β-HSD isoforms distinct from isotypes I and II are present in brain glial cells. To solve this question, it will be necessary to identify the mRNAs encoding for the different 3β-HSD isoforms in cultured rat astrocytes and oligodendrocytes.

The mechanisms of regulation of 3β-HSD gene transcription have been extensively studied in peripheral steroidogenic tissues (Labrie et al., 1994; Guérin et al., 1995; Mason et al., 1997). In contrast, until recently, nothing was known concerning the control of 3β-HSD activity in the CNS. The observation that, in the frog, numerous hypothalamic neurons contain simultaneously 3β-HSD- and PBR-like immunoreactivities (Do-Régo et al., 1998) suggested that the endogenous ligands of PBR may control 3β-HSD activity. As a matter of fact, it was found that the endozeptine TTN causes a dose-dependent stimulation of the conversion of Δ5P into 17-hydroxyprogesterone, indicating that TTN enhances 3β-HSD activity (Do-Régo et al., 1998). The effect of TTN was mimicked by the PBR agonist 4'-chlorodiazepam and inhibited by the PBR antagonist 1-(2-chlorophenyl)-N-methyl-N-(1-methyl-propyl)-3-isoquinoline carboxamide (PK11195; Benavides et al., 1984; Zavala and Lefrant, 1987; Costa et al., 1994). In contrast, flumazenil, a central-type benzodiazepine receptor antagonist (Brodgen and Goa, 1991), did not affect TTN-evoked neurosteroid secretion (Do-Régo et al., 1998) (Fig. 4). Altogether, these data indicate that TTN stimulates the biological activity of 3β-HSD in hypothalamic neurons through activation of PBR likely located at the plasma membrane level.
The enzymatic system 17α-hydroxylase/17,20 lyase (cytochrome P-450c17) is responsible for the transformation of C21 steroids (Δ5P, progesterone) into C19 steroids (DHEA and androstenedione, respectively) (Fig. 1). It is now clearly demonstrated that, in classical steroid-producing glands, these reactions are catalyzed by a single microsomal enzyme coupled to a cytochrome reductase,
cytochrome P-450c17 (P-450c17 or P-45017α), which possesses both 17α-hydroxylase and 17,20 lyase activities. The lyase bioactivity of this enzymatic complex is modulated by phosphorylation and depends on the lipidic environment (Nakajin et al., 1981; Miller, 1988; Namiki et al., 1988).

The early observation that the rat brain contains high concentrations of DHEA (Corpochet al., 1981) suggested the existence of P-450c17 activity in the CNS of mammals. However, biochemical and immunocytochemical studies aimed at demonstrating the presence of the enzyme in the brain have long remained unsuccessful (Baulieu and Robert, 1990; Le Goascogne et al., 1991; Mellon and Deschepper, 1993). In 1994, it was demonstrated that frog hypothalamic explants are capable of converting [3H]Δ5P into [3H]17α-hydroxyprogesterone (Mensah-Nyagan et al., 1994). This observation provided the first evidence for the presence of P-450c17 in the CNS. Subsequently, P-450c17 mRNAs have been detected by RT-PCR in the brain of rat embryos (Compagnone et al., 1995b). P-450c17-like immunoreactivity has also been observed in various neuronal populations of the pontine nucleus, the locus ceruleus and the spinal cord in mouse embryos. In contrast, conflicting data have been reported in adults: according to Compagnone et al. (1995b), P-450c17 gene is only expressed in the PNS of rat and mouse, whereas other studies have described the presence of P-450c17 mRNAs in various brain regions of adult rodents (Strömstedt and Waterman, 1995).

The regulation of P-450c17 gene expression in peripheral steroidogenic tissues is controlled by androgens (Burgos-Trinidad et al., 1997), insulin-like growth factor type I (Naseeruddin and Hornsby, 1990), catecholamines (Ehrhart-Bornstein et al., 1991; Güse-Behl ling et al., 1992; Haidan et al., 1998), cAMP, and protein kinase C activators (McAllister and Hornsby, 1988; Cheng et al., 1992). In contrast, the mechanisms regulating the expression of the P-450c17 gene in the brain of mammals have not yet been determined. In a recent study, it has been shown that, in the frog hypothalamus, TTN stimulates the conversion of [3H]Δ5P into [3H]17α-hydroxyprogesterone, indicating that endozepines can increase P-450c17 activity in nerve cells (Do-Régo et al., 1998).

**VI. 17β-Hydroxysteroid Dehydrogenase**

The enzyme 17β-HSD plays a pivotal role in the biosynthesis and the inactivation of sex steroid hormones by catalyzing the interconversion of 17-ketosteroids (androstenedione, estrone) and 17β-hydroxysteroids (testosterone, 17β-estradiol) (Fig. 1). Molecular cloning of the 17β-HSD cDNAs and biochemical characterization of the enzyme activity have revealed the existence of seven isoforms designated types I to VII (Andersson, 1995; Blomquist, 1995; Andersson and Moghramb, 1997; Biswas and Russell, 1997; Nokelainen et al., 1998). Type I, III, and V isoenzymes catalyze almost exclusively reductive reactions, leading to the formation of active steroids such as testosterone and 17β-estradiol. Conversely, the type II and IV isoenzymes, which preferentially catalyze the oxidative reaction, are responsible for the synthesis of androstenedione and estrone (Andersson, 1995). Type VI 17β-HSD oxidizes essentially 5α-androstane-3α,17β-diol to androsterone. This latter 17β-HSD isoform shares 65% sequence identity with retinol dehydrogenase 1 which catalyzes the oxidation of retinol to retinal (Biswas and Russell, 1997).

Type VII 17β-HSD, which catalyzes the conversion of estrone to estradiol, has been initially described as a prolactin receptor-associated protein because of a high (89%) sequence homology (Duan et al., 1996, 1997; Nokelainen et al., 1998). Recently, it has been shown that this protein, which is intimately linked to the development of cysts in the kidney and liver (Aziz et al., 1996), efficiently catalyzes the reduction of estrone and also the oxidation of estradiol and testosterone in an NAD-dependent manner, indicating that this is a potential eighth member of the 17β-HSD isozyme family (Fomitcheva et al., 1998). Five isoforms of 17β-HSD have been cloned in humans and their cDNAs structurally characterized. Type I 17β-HSD, which was isolated for the first time from a human placental library, has subsequently been identified in the ovary and mammary gland (Martel et al., 1992). The type II isoenzyme, which is isolated from prostate and placental cDNA libraries, is also present in the endometrium, liver, small intestine as well as in the kidney, pancreas, and colon (Casey et al., 1994). In contrast, the type III 17β-HSD gene is exclusively expressed in the testis (Geissler et al., 1994). Molecular cloning of human type IV 17β-HSD revealed that this isoenzyme is expressed in the liver, kidney and, to a lesser extent, in the endometrium and testis (Adamski et al., 1995). Recently, the cDNA encoding for the type V 17β-HSD isoenzyme has been characterized in humans using a placental cDNA library (Labrie et al., 1997). Different isoforms of 17β-HSD were also detected in various peripheral tissues of rodents (Normand et al., 1995) and pig (Adamski et al., 1992; Leenders et al., 1994a,b).

The existence of a 17β-HSD activity in the mammalian brain has long been known (Reddy, 1979; Resko et al., 1979) but it is only recently that the cellular distribution of the enzyme in the CNS has been described (Pelletier et al., 1995). Immunocytochemical mapping of 17β-HSD reveals that this enzyme is expressed in the brain, kidney, and, to a lesser extent, in the endometrium and testis (Adamski et al., 1995). Recently, it has been shown that this enzyme is expressed in the brain, kidney, and, to a lesser extent, in the endometrium and testis (Adamski et al., 1995). In the CNS of both mammals and amphibians, type I 17β-HSD is exclusively expressed in glial cells (Pelletier et al., 1995; Men sah-Nyagan et al., 1996a,b) (Fig. 5). In the rat brain, 17β-HSD-like immunoreactivity is widely distributed in ependymocytes and astrocytes of the hippocampus, cerebral cortex, thalamus, and hypothalamus, whereas, in
the frog brain, the immunoreactive material is only located in ependymocytes of the telencephalon. Whether these species differences reflect authentic variations in the anatomical distribution of 17β-HSD in the CNS of mammals and amphibians or whether they can be ascribed to the presence, in the frog brain, of distinct 17β-HSD isoforms which cannot be detected with the antibodies against type I 17β-HSD remains unknown. In this respect, it should be noticed that the five isoforms of 17β-HSD cloned in various vertebrate species do not exhibit the same cellular distribution or functional characteristics in peripheral steroidogenic tissues (Andersson, 1995; Andersson and Moghrabi, 1997; Puranen et al., 1997). In addition, in the CNS of mammals, 17β-HSD is mainly involved in the inactivation of sex steroid hormones (Reddy, 1979; Resko et al., 1979; Martini et al., 1996), whereas, in the brain of amphibians, this enzyme is responsible for the synthesis of testosterone (Mensah-Nyagan et al., 1996a,b). In the frog Rana ridibunda, a series of observations have demonstrated that biosynthesis of testosterone actually occurs in the CNS: 1) high amounts of testosterone have been detected in the medial pallium and the hypothalamus, and the concentrations of testosterone are not affected by castration; 2) endogenous testosterone extracted from the telencephalon has been chemically characterized by combining HPLC analysis, gas chromatography and mass spectrometry; and finally 3) synthesis of [3H]testosterone and [3H]5α-DHT from [3H]Δ5P by frog telencephalon explants has been demonstrated in vitro (Mensah-Nyagan et al., 1996a,b). Formation of androgens and estrogens from a distant precursor such as [3H]Δ5P or [3H]DHEA has also been shown in primary cultures of avian nerve cells (Vanson et al., 1996), indicating that 17β-HSD-like activity responsible for the synthesis of sex steroids is present in the CNS of various groups of vertebrates. Taken together, these data suggest the expression of new classes of 17β-HSD isoforms in the nervous system of nonmammalian vertebrates or the occurrence of a biological activity distinct from that of 17β-HSD isoenzymes present in the mammalian brain. Molecular cloning of the various 17β-HSD genes in representative submammalian species is clearly required to investigate their expression in the CNS. In peripheral tissues, the expression of 17β-HSD is regulated at the transcriptional level by sex steroid hormones (Peltoketo et al., 1996), growth factors (Ghersevich et al., 1994; Jantus-Lewintre et al., 1994), retinoic acid (Piao et al., 1995), and cAMP (Tremblay and Baudouin, 1993). Whether these different factors are also involved in the control of 17β-HSD gene expression in nerve cells has not yet been investigated.

VII. 5α-Reductase

The enzyme 5α-reductase (5α-R) is a microsomal NADPH-dependent protein which acts specifically on steroids possessing a C₄-C₅ double bond and a ketone group at the C₃ position. This enzyme catalyzes the transfer of two hydrogens from NADPH causing the reduction of the C₄-C₅ double bond and the formation of 5α-reduced metabolites. In particular, 5α-R catalyzes the conversion of testosterone, the main circulating androgen, into dihydrotestosterone (5α-DHT) and the transformation of progesterone into dihydropregester-
one (5α-DHP) (Fig. 1). In humans, two distinct cDNAs encoding type I and type II 5α-R have been cloned from a prostate library; these cDNAs exhibit an overall sequence identity of 60% (Andersson and Russell, 1990; Andersson et al., 1991). The 5αRI gene, located on chromosome 5, is mainly expressed in the skin (Luu-The et al., 1994), notably in the pubic skin and the scalp (Andersson and Russell, 1990; Jenkins et al., 1992). The 5αRII gene is predominantly expressed in the prostate and gonads (Thigpen et al., 1993; Luu-The et al., 1994; Mowszowicz et al., 1995). Deletion in the 5αRII gene causes male pseudohermaphroditism, indicating that 5α-RII is involved in the determination of the sexual phenotype during embryogenesis (Andersson et al., 1991). In rat, 5αRI and 5αRII cDNAs have been cloned from a prostate library but the two genes are actually transcribed in distinct cell types: 5α-RI mRNAs are localized in the basal epithelial cells whereas 5α-RII mRNAs are found in stromal cells (Andersson and Russell, 1990; Berman and Russell, 1993).

In vitro studies have shown the existence of 5α-R bioactivity in brain tissue and specially in primary cultures of nerve cells (Saitoh et al., 1982; Melcangi et al., 1993; Martin et al., 1996; Negri-Cesi et al., 1996a,b). Northern blot analysis has shown the occurrence of high concentrations of 5α-RI mRNAs but relatively low amounts of 5α-RII mRNAs in rat brain extracts (Normington and Russell, 1992; Lephart, 1993). The anatomical distribution of 5α-R in the rat brain was first investigated using an antibody raised against human 5α-RI (Luu-The et al., 1994). The presence of 5α-R-like immunoreactivity has been found in astrocytes, ependymocytes, and tanyocytes within various brain regions including the hypothalamus, thalamus, hippocampus, cerebral cortex, and circumventricular organs (Pelletier et al., 1994). At the ultrastructural level, the immunoreactive material appeared to be distributed throughout the cytoplasm of glial cells without any particular association with mitochondria (Pelletier et al., 1994). This observation is consistent with previous subcellular fractionation studies which had shown that 5α-R activity was mostly associated with the microsomal fraction (Lephart, 1993). Using an antibody raised against rat 5α-RI, Tsuruo et al. (1996) have recently reported that 5α-R-like immunoreactivity is mainly contained in oligodendrocytes of the white matter and in ependymocytes bordering the cerebral ventricles. Collectively, these data indicate that, in the CNS of mammals, the 5α-R gene is primarily expressed in glial cells. However, biochemical studies have shown that neurons from rat embryos in primary culture exhibit 5α-R activity (Melcangi et al., 1994). These observations suggest that the 5α-R genes may be transcribed in distinct cell types of the CNS according to development stages. Alternatively, the expression of the 5α-R genes may be up-regulated in cultured neurons.

In all classes of vertebrates, conversion of gonadal testosterone into 5α-DHT in the brain of male individual is necessary for the induction of various behavioral effects. Since the occurrence of 17β-HSD-like immunoreactivity has been demonstrated within glial cells in the frog brain (Mensah-Nyagan et al., 1996a,b), it would be interesting to investigate the cellular localization of 5α-R in the CNS of amphibians to examine whether the same cells may simultaneously synthesize testosterone and convert it into 5α-DHT. Concurrently, in the mammalian brain, the consecutive catalytic actions of 5α-R and 3α-hydroxysteroid dehydrogenase on progesterone leads to the formation of allopregnanolone (Baulieu et al., 1996), a potent modulator of GABAA receptors, which controls various psychical processes (Schumacher et al., 1996; Patchev et al., 1996). Expression of the 5α-R gene in nerve cells may thus have important implications in the control of neurophysiological functions in vertebrates.

Neuroanatomical studies have revealed that, in mammals, 5α-R is present in various regions of the CNS where androgen (Arnold and Gorski, 1984; Clark et al., 1988; Roselli et al., 1996a,b) and estrogen receptors are located (Pelletier et al., 1988; Balthazart et al., 1989; Torran-Allerand et al., 1992; Yuan et al., 1995). However, it is generally accepted that, in the brain, there is no sexual dimorphism in the expression of 5α-R; in addition, castration or sex steroid hormone administration does not affect 5α-R activity (Wilson, 1975; Celotti et al., 1983; Lephart, 1993; Negri-Cesi et al., 1996b). A remarkable exception has been reported in monkey in which castration induces a selective increase of the biological activity of 5α-R in the basolateral amygdala but not in other regions of the CNS (Roselli et al., 1987). These observations suggest that, in discrete brain areas, sex steroids may control 5α-R expression and/or activity as described in the rat adrenal gland (Lephart et al., 1991). Concurrently, suppression of hypothalamic (nor-) adrenergic neurotransmission by pharmacological blockers and surgical deafferentation of the hypothalamus do not affect 5α-R activity, indicating that the expression of the enzyme is not regulated by extrinsic neural inputs (Celotti et al., 1983). However, incubation of glial cells with 8Br-cAMP, but not phorbol esters, causes a significant increase of 5α-DHT formation (Celotti et al., 1992; Negri-Cesi et al., 1996b). These data suggest that a protein kinase A is involved in the regulation of 5α-R activity in nerve cells, although the neural factors responsible for the activation of this transduction pathway remain unknown.

VIII. Aromatase

The conversion of androgens into estrogens is catalyzed by aromatase (Fig. 1), an enzymatic complex which comprises two proteins, i.e., a specific form of cytochrome (cytochrome P-450aromatase) responsible for the binding of the C19 steroid substrate and the formation of the phenolic A-ring characteristic of estrogens, and a flavoprotein (NADPH-cytochrome P-450reduc-
tase) which transfers reducing equivalents from NADPH to any microsomal form of cytochrome (for review, Nelson et al., 1993). Aromatase activity occurs in various tissues including the placenta (Fournet-Dulguerov et al., 1987), ovary (McNatty et al., 1976; Lephart et al., 1995), testis (Fritz et al., 1976; Valladares and Payne, 1979; Levallet and Carreau, 1997), and adipoocytes (Simpson et al., 1989). The aromatase gene, which has been cloned in humans, is composed of 17 exons and is localized at the q21.1 level of chromosome 15 (Means et al., 1989; Harada et al., 1990; Toda et al., 1990). Molecular cloning of aromatase cDNAs in various vertebrate taxa has revealed the existence of a single enzyme in most species including trout (Tanaka et al., 1992), chicken (McPhaul et al., 1988), rat (Hickey et al., 1990), mouse (Terashima et al., 1991), bovine (Hinshelwood et al., 1993), and humans (Corbin et al., 1988; Harada, 1988). A remarkable exception has been reported in pig which possesses two distinct isoforms of aromatases (Corbin et al., 1995; Conley et al., 1997).

It has long been known that conversion of androstenedione into estrone occurs in the rat brain, indicating the presence of aromatase activity in the CNS (Naftolin et al., 1972, 1975; Roselli et al., 1985). Immunocytochemical studies have recently shown that aromatase is expressed in neurons and not in glial cells (Lephart, 1996). In the brain of birds, a good correlation has been observed between the localization of aromatase-like immunoreactivity and the distribution of aromatase activity. Particularly, in the Japanese quail, aromatase-positive neurons are located in the preoptic area where an intense enzymatic activity is also found (Balthazart et al., 1990a,b, 1991b, 1992). Conversely, in mammals, especially in rodents, mismatches have been reported between the localization of aromatase-positive neurons and the distribution of enzymatic activity in the CNS. For instance, high levels of aromatase activity are detected in the median preoptic area and the ventromedian nucleus of rat, two regions which are virtually devoid of aromatase-immunoreactive areas (Sanghera et al., 1991). It should be noted however that, during ontogenesis, aromatase-positive neurons have been visualized in the preoptic area, the ventromedian nucleus and the arcuate nucleus at embryonic day 13 (E13), E16, and E19, respectively. In these regions, the number of aromatase-positive neurons increases during gestation, peaks before birth, and decreases or vanishes during the two first postnatal weeks (Tsuruo et al., 1994). These data reveal the existence of spatio-temporal variations in the level of transcription of the aromatase gene during development.

The mechanisms controlling aromatase expression and bioactivity in the CNS have been investigated during ontogenesis and in the adult. Because of the high affinity of the enzyme for testosterone, various research groups have examined the effects of androgens on aromatase gene transcription during embryogenesis (Callard et al., 1980; Paden and Roselli, 1987; Lephart et al., 1992; Roselli and Resko, 1993). Their studies revealed that, in rodent embryos, neither testosterone nor 5α-DHT had any influence on aromatase gene expression in cultured hypothalamic neurons (Abe-Dohm ae et al., 1994; Negri-Cesi et al., 1996a). In contrast, aromatase activity in the CNS appears to be modulated by androgens, although controversial data have been reported in the literature: Lephart et al. (1992) have observed that androgens are capable of reducing aromatase activity in rat embryo hypothalamic explants, whereas Beyer et al. (1994b) have described a stimulatory effect of testosterone on estrogen formation in cultured mouse fetal diencephalic neurons. Depending on the species and/or the environmental milieu, androgens may thus exert opposite effects on aromatase activity in the developing brain. In adult individuals, androgens clearly play a crucial role in the regulation of aromatase gene transcription and aromatase activity in the CNS of amphibians (Moore et al., 1994), birds (Harada et al., 1992; Panzica et al., 1996), and mammals (Negri-Cesi et al., 1996a,b). In particular, it has been demonstrated that castration significantly reduces the amount of aromatase mRNAs and activity in the quail (Harada et al., 1992) and rat brain (Abdelgadir et al., 1994; Roselli et al., 1997). Reciprocally, administration of testosterone increases the level of aromatase mRNA and the number of aromatase-immunoreactive neurons (Harada et al., 1992; Abdelgadir et al., 1994), indicating the importance of testosterone in the regulation of aromatase expression in the CNS. Since estrogens stimulate the expression of androgen receptors and increase the duration of androgen receptor occupation in the rat brain (Roselli and Fasasi, 1992), it is conceivable that estrogens and androgens may exert a coordinate action in the control of aromatase gene expression in the CNS. Concurrently, an effect of dopamine on aromatase activity has been demonstrated in the quail preoptic area, indicating that neurotransmitters may regulate reproductive behavior by modulating estrogen formation in the brain (Baili en and Balthazart, 1997). Finally, the fact that a large population of aromatase-positive neurons are located in the preoptic-septal complex (Shinoda et al., 1989b; Balthazart et al., 1991a; Sanghera et al., 1991). It should be noted however that, during ontogenesis, aromatase-positive neurons have been visualized in the preoptic area, the ventromedian nucleus and the arcuate nucleus at embryonic day 13 (E13), E16, and E19, respectively. In these regions, the number of aromatase-positive neurons increases during gestation, peaks before birth, and decreases or vanishes during the two first postnatal weeks (Tsuruo et al., 1994). These data reveal the existence of spatio-temporal variations in the level of transcription of the aromatase gene during development.

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**IX. Sulfotransferase and Sulfatase**

Sulfate conjugation of steroids is catalyzed by sulfotransferases or sulfokinases, a family of cytosolic enzymes which transfer the sulfate moiety from the uni-
versal donor molecule 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to a hydroxyl group of the steroid substrates. In contrast, sulfatase is responsible for the hydrolysis of sulfated steroids leading to the formation of unconjugated steroids (Fig. 1). Hydroxysteroid sulfonates act as potent regulators of neuronal activity. In particular, pregnenolone sulfate (Δ⁵PS) and dehydroepiandrosterone sulfate (DHEAS) modulate the functions of GABA₄ receptors (Majewska, 1992), NMDA receptors (Wu et al., 1991; Weaver et al., 1997), α receptors (Monnet et al., 1995), and voltage-gated calcium channels (Ffrench-Mullen and Spence, 1991; Ffrench-Mullen et al., 1994). The fact that the inhibitory action of Δ⁵PS on calcium channel currents in pyramidal neurons is abolished after substitution of the sulfate moiety by an acetate (Ffrench-Mullen et al., 1994) demonstrates the importance of the sulfate group in the neurogenic activity of 3-hydroxysteroids.

Molecular cloning of sulfotransferase cDNAs has revealed the existence of multiple isoforms which have differential affinity for various steroid substrates and are expressed in a tissue-specific manner. The steroid sulfotransferase superfamily comprises four classes of enzymes: 1) hydroxysteroid sulfotransferases (HST) that act on primary and secondary alcohols of hydroxysteroids such as cholesterol, Δ⁵P and DHEA, 2) estrone sulfotransferases that transfer the sulfonate moiety on the 3-hydroxyl group of estrogens, 3) steroid sulfotransferases that have a broad specificity, and 4) cortisol sulfotransferases that act on the 21-hydroxyl group of glucocorticosteroids (for reviews, Webb, 1992; Strott, 1996).

The human sulfatase gene has been cloned and mapped to the Xp22.3 chromosome, proximal to the pseudoautosomal region (Ballabio and Shapiro, 1995). Recent molecular cloning studies have also characterized the complete gene of rat sulfatase (Li et al., 1996) and a mouse sulfatase cDNA (Salido et al., 1996). These results revealed that the overall genomic organization of rat and human sulfatasas is very similar, except that the insertion site for intron 1 in the rat is 26 bp upstream from that in humans.

The existence of sulfotransferase-like activity has long been demonstrated in the primate brain (Knapstein et al., 1968). Similarly, early studies have shown the occurrence of sulfatase bioactivity in the CNS of vertebrates including human (Kishimoto and Sostek, 1972; Iwamori et al., 1976). Consistent with these findings, high amounts of Δ⁵P, DHEA, and their sulfated esters (Δ⁵PS and DHEAS) have been detected in the brain of castrated and adrenalectomized rats, suggesting the presence of sulfotransferase and sulfatase activities in the CNS (Carpéchot et al., 1981, 1983). In vitro studies have confirmed the existence of sulfotransferase (Rajkowski et al., 1997) and sulfatase bioactivity (Park et al., 1997) in the mammalian brain. However, the anatomical localization of these enzymes in the brain of vertebrates has long remained unknown. Recently, the cellular distribution of sulfotransferase has been investigated in the CNS of the European green frog Rana ridibunda using an antiserum raised against rat liver HST (Beaujean et al., 1999). Two populations of HST-immunoreactive neurons have been detected in the anterior preoptic area and in the magnocellular preoptic nucleus of the hypothalamus. A dense bundle of HST-positive glial processes is also present in the ventral hemispheric zone. In addition, frog telencephalon and hypothalamus homogenates are capable of synthesizing Δ⁵PS and DHEAS (Fig. 6), as demonstrated by pulse-chase experiments using [³⁵S]PAP and [³H]Δ⁵P or [³H]DHEA as precursors (Beaujean et al., 1999). Concurrently, the presence of sulfatase mRNAs has been visualized in the cortex, hindbrain, and thalamus of mouse fetuses during the last week of gestation (Compagnone et al., 1997). In the adult bovine brain, sulfatase activity is particularly abundant in the midbrain and hypothalamus (Park et al., 1997), suggesting that the sites of expression of the enzyme in the CNS may vary during development and/or may differ from one species to the other. These studies indicate that brain neurons and/or glial cells express both sulfotransferase and sulfatase activities which play an important role in the regulation of the functions of neuroactive steroids.

X. 11β-Hydroxysteroid Dehydrogenase

The transformation of physiologically active glucocorticoids (cortisone, corticosterone) into inactive metabolites (cortisone, 11-dehydrocorticosterone) is catalyzed by 11β-hydroxysteroid dehydrogenase (11β-HSD), a microsomal NADP⁺-dependent enzyme. High levels of 11β-HSD activity are present in the kidney and salivary gland (Edwards et al., 1988; Monder et al., 1989), as well as in the liver, lung, and testis (Phillips et al., 1989). Molecular cloning of the cDNAs encoding 11β-HSD revealed the existence of two isoforms of the enzyme (type I 11β-HSD or 11β-HSDI and type II 11β-HSD or 11β-HSDII) in humans (Tannin et al., 1991; Albiston et al., 1994), sheep (Yang et al., 1992; Agarwal et al., 1994), rat (Agarwal et al., 1989; Zhou et al., 1995), and mouse (Rajan et al., 1995; Cole, 1995). Type I 11β-HSD isozyme utilizes NADPH as a cofactor and is capable of functioning (in addition of the classical 11β-HSD activity) as a 11β-reductase by regenerating active glucocorticoids in cultured cells (Agarwal et al., 1989; Duperrex et al., 1993; Low et al., 1994). Type II 11β-HSD is an exclusive glucocorticoid-inactivating enzyme whose bioactivity is NAD-dependent (Brown et al., 1993; Rusvai and Naray-Fejes-Toth, 1993).

The presence of 11β-HSD activity has been demonstrated in various areas of the CNS including the cerebellum, hippocampus, neocortex, amygdala, and brainstem (Grosser and Axelrod, 1968; Miyabo et al., 1973; Moisan et al., 1990, 1992; Lakshmi et al., 1991). Northern blot analysis, using a rat liver 11β-HSDI cDNA
probe has revealed the presence of a single mRNA band in the rat brain (Moisan et al., 1990, 1992). The 11β-HSDI gene is actively expressed in various neuronal populations of the cerebellum, hippocampus, cerebral cortex, and hypothalamus (Moisan et al., 1992). The location of 11β-HSDI mRNA in these brain areas coincides exactly with the regional distribution of the enzymatic activity (Lakshmi et al., 1991). Northern blot experiment using human kidney and placental 11β-HSDII cDNAs did not reveal the presence of type II 11β-HSD mRNAs in the whole human brain (Albiston et al., 1994; Brown et al., 1996). In contrast, a recent in situ hybridization study has revealed that the 11β-HSDII gene is expressed in discrete areas of the rat brain including the commissural portion of the nucleus tractus solitarius and the subcommissural organ (Roland et al., 1995). These data show the existence of important species differences in the expression of the 11β-HSD in the brain.

The regulation of 11β-HSD gene expression remains largely unknown. It has been recently reported that chronic treatment with dexamethasone and stress increase 11β-HSD activity in the hippocampus, but not in the kidney (Seckl et al., 1993), suggesting that 11β-HSD may contribute to the protection of hippocampal neurons against the deleterious effects provoked by glucocorticoid excess (for review, Seckl, 1997).

XI. Cytochrome P-45011β

The enzyme 11β-hydroxylase, or cytochrome P-45011β (P-45011β), catalyzes the formation of glucocorticosteroids (cortisol and corticosterone). The P-45011β gene is only expressed in the zona fasciculata reticularis of the adrenal cortex (Yabu et al., 1991; Ogishima et al., 1992; Ho and Vinson, 1993; Mitani et al., 1995; Erdmann et al., 1995).

The presence of P-45011β in the CNS was first demonstrated in rat by immunohistochemistry using polyclonal antibodies raised against purified bovine adrenocortical P-45011β. This study revealed that P-45011β-like immunoreactivity is selectively localized to the tracts of myelinated fibers throughout the brain (Ozaki et al., 1991). Enzymatic assays for P-45011β monoxygenase activity as well as the 11β-hydroxylation of [4-14C]11-deoxycorticosterone in brain homogenates demonstrated that the immunoreactive material detected in the CNS of rat actually corresponds to an active form of P-45011β (Ozaki et al., 1991). Recently, substantial amounts of P-45011β mRNA were detected in the neocortex and piriform cortex of the male rat by in situ hybridization, indicating that synthesis of corticosterone can occur in the CNS. Interestingly, the same regions of the brain which express the P-45011β gene also contain high concentrations of glucocorticoid receptors (Erdmann et al., 1996), suggesting a physiological role for brain-derived corticosterone in the neocortex.

XII. Other Enzymes Involved in the Synthesis or Metabolism of Steroids

Several other enzymatic activities involved in the synthesis or metabolism of steroid hormones have been evidenced in the CNS. However, the mapping of these enzymes in the brain has not yet been studied by immunocytochemistry, neither has the location of their mRNAs been investigated by in situ hybridization, so...
that the cellular distribution and the regulation of the
expression of these enzymes remain unknown.

A. 3α-Hydroxysteroid Dehydrogenase

The enzyme 3α-HSD is a member of the aldo-keto
reductase family, which is composed of various enzymes
including aldehyde reductase, aldose reductase, and di-
hydrodiol dehydrogenase (Bohren et al., 1989; Penning,
1997). 3α-HSD catalyzes the conversion of 5α-DHT into
3α-androstanediol and the conversion of 5α-DHP into
allopregnanolone (Fig. 7). The existence of multiple
mRNAs encoding various proteins structurally related to
3α-HSD has been reported in humans (Qin et al., 1993)
but, to date, only two functional 3α-HSD isoenzymes
(type I 3α-HSD and type II 3α-HSD) have been charac-
terized on the basis of their affinity for 5α-DHT (Khanna
et al., 1995a; Penning, 1997). In the rat, a 3α-HSD cDNA
has been cloned (Pawlowski et al., 1991) and the corre-
sponding gene has now been fully characterized (Lin and
Penning, 1995; Penning et al., 1996).

Fig. 7. Current knowledge concerning the biochemical pathways of neurosteroidogenesis. Solid arrows indicate that the biochemical reaction has been formally demonstrated, i.e., both the biological activity of the enzyme in neural tissue and the localization of the enzyme by immunohistochemistry or its mRNAs in situ hybridization in the nervous system are documented. Dashed arrows indicate that only the biological activity of the enzyme is documented in the nervous system. Multiple arrows indicate that the occurrence of the enzyme has not yet been found in the nervous system.
In vitro and in vivo experiments have demonstrated the presence of 3α-HSD bioactivity in the CNS of primates (Roselli et al., 1987; Clark et al., 1988; Bonsall et al., 1989, 1990) and rodents (Celotti et al., 1987, 1992; Krieger and Scott, 1989), indicating that 5α-DHT and 5α-DHP are metabolized in the brain (Martini et al., 1996). Intense 3α-HSD activity has been found in the olfactory bulb, olfactory tubercle, thalamus, caudate nucleus, cerebral cortex, and hypothalamus (Krieger and Scott, 1984, 1989; Khanna et al., 1995a). Northern blot analysis has shown that type II 3α-HSD is the predominant form in the human brain (Khanna et al., 1995b). In the rat, 3α-HSD activity is expressed in type 1 astrocytes while neurons, oligodendrocytes, and type 2 astrocytes contain 5α-R activity (Melcangi et al., 1993). The fact that the two interdependent 5α-R and 3α-HSD enzymatic activities are differently located indicates that some kind of coordination between neurons and glial cells must be necessary to ensure the metabolism of various sex steroids, particularly testosterone and progesterone.

B. Δ5-3β-Hydroxysteroid Acyltransferase

The enzyme Δ5-3β-hydroxysteroid acyltransferase (acyltransferase) catalyzes the saponification reaction which converts Δ5-3β-hydroxysteroids into lipoidal derivatives or fatty acid esters of steroids. The existence of acyltransferase activity in peripheral steroidogenic organs, including the adrenal gland and corpus luteum, has long been demonstrated (Mellon-Nussbaum and Hochberg, 1980).

In vitro studies have shown that incubation of hypotalamus, amygdala, or olfactory bulb explants with [3H]Δ5P or [3H]DHEA yields to the formation of fatty acid esters of [3H]Δ5P and [3H]DHEA (Robel et al., 1987; Baulieu et al., 1987; Jo et al., 1989; Vourc'h et al., 1992). These observations indicate that acyltransferase bioactivity is present in various regions of the CNS. However, the cellular localization of this enzyme remains unknown.

C. 7α-Hydroxylase

Hydroxylation of steroids on the C7 position is catalyzed by 7α-hydroxylase, an enzyme that is found in various tissues. In particular, it has been shown that incubation of a rat testicular microsomal fraction with radioactive testosterone or androstenedione yields to the formation of their respective 7α-hydroxylated metabolite (Inano and Tamaoki, 1971). In the liver, 7α-hydroxylase is responsible for the conversion of cholesterol into 7αOH-cholesterol (Noshiro et al., 1989).

The existence of a 7α-hydroxylase activity in the CNS has been inferred from the observation that rat brain microsomal preparations can convert 3β-androstanediol into a 7α-hydroxylated derivative (Warner et al., 1989). It should be noted however that the presence of endogenous 3β-androstanediol has never been demonstrated in the rat brain. Subsequently, it has been shown that rat brain microsomes are capable of converting the neurosteroids Δ5P and DHEA into 7α-hydroxy-Δ5P and 7α-hydroxy-DHEA, respectively (Akwa et al., 1992), confirming the existence of 7α-hydroxylase bioactivity in the CNS of rodents. The cDNA encoding a new isoform of cytochrome P-450 has been recently cloned from rat and mouse hippocampal libraries; the corresponding protein exhibits 39% sequence identity with liver cholesterol 7α-hydroxylase (Cyp7a) (Stapleton et al., 1995). The mRNAs encoding this 7α-hydroxylase-related enzyme (Cyp7b) are primarily located in the CNS, particularly in the hippocampus, whereas the Cyp7a gene is mainly expressed in the liver (Jelinek et al., 1990; Noshiro and Okuda, 1990; Stapleton et al., 1995). The recombinant Cyp7b protein possesses a high affinity for DHEA and Δ5P that are respectively converted by this enzyme into 7α-hydroxy-DHEA and 7α-hydroxy-Δ5P (Rose et al., 1997).

D. Cytochrome P-450-Aldosterone Synthase

The last step of the aldosterone biosynthesis pathway is catalyzed by an enzymatic complex called cytochrome P-450-aldosterone synthase (P-450aldo) which exerts three distinct activities (11β-hydroxylation, 18-hydroxylation, and 18-oxidoreduction) responsible for the conversion of 11-deoxycorticosterone successively into corticosterone, 18-hydroxycorticosterone, and aldosterone (Fig. 1). In the rat adrenal cortex, P-450aldo is exclusively located in the two or three outermost cell layers of the zona glomerulosa, whereas P-45011β is only present in the zona fasciculata reticularis (see section XI). The occurrence of P-450aldo activity has been reported in various tissues including the aortic endothelium, vascular smooth muscles, and myocardial tissue (Hatakeyama et al., 1994; Silvestre et al., 1998).

The initial investigations aimed at demonstrating the existence of P-450aldo in the CNS, using ribonuclease protection assays, were unsuccessful (Mellon and Deschepper, 1993). In contrast, these studies have identified the mRNAs encoding for P-450c11β in various areas of the rat brain, namely, in the amygdala, cortex, cerebellum, and hippocampus. Recently, Gomez-Sanchez et al. (1997) have demonstrated by RT-PCR/Southern blot analysis the presence of P-450aldo in various regions of the CNS of the rat, including the hypotalamus, hippocampus, amygdala, and cerebellum. These authors have also shown that hippocampic, hypothalamic and cerebellar explants are capable of converting [3H]11-deoxycorticosterone into [3H]corticosterone, [3H]18-hydroxycorticosterone, and [3H]aldosterone. It thus appears that the enzyme detected in the rat brain is a biologically active form of P-450aldo.

XIII. Conclusion and Clinical Implications

Neuroanatomical and biochemical studies have now firmly established that several key enzymes of steroido-
genesis such as P-450sc, 3β-HSD, cytochrome P-450c17, 17β-HSD, 5α-R, and aromatase are present in the brain of vertebrates (Fig. 7). The occurrence of sulfotransferase and sulfatase which catalyze the formation and deconjugation of sulfated esters of steroids, respectively, has also been demonstrated (Fig. 7). The cellular localization of these enzymes indicates that various types of nerve cells, either neurons or glial cells or both, participate in the biosynthesis of unconjugated and sulfated neurosteroids. Other enzymatic activities involved in the synthesis or metabolism of steroid hormones, such as 3α-HSD, acyltransferase, 7α-hydroxylase, and P-450aldo have also been detected in the CNS (Fig. 7) but the anatomical distribution of these enzymes remains to be determined.

To date, little is known concerning the involvement of steroidogenic enzymes expressed by nerve cells in the physiopathology of the nervous system. Therefore, the possible pharmacological implications are currently a matter of speculation. The decrease in the concentration of Δ4PS in the rat hippocampus during aging (Robel et al., 1995) suggests the existence of a correlation between the levels of sulfated neurosteroids and neurodegenerative processes. A promising therapeutic application would be to compensate the decline of the DHEAS level in aging subjects by administering moderate amounts of DHEA, a lipophilic substrate of HST which can easily cross the blood-brain barrier to be converted, in the CNS, into DHEAS (Baulieu and Robel, 1996, 1998). Another pharmacological approach would be to develop novel psychotropic agents which may selectively control, in nerve cells, the expression and/or activity of enzymes involved in the biosynthesis of potent neuroactive neurosteroids such as allopregnanolone, DHEA, Δ5P, and their sulfated derivatives.

Neurosteroids, which are involved in the regulation of stress responses, anxiety, sleep, neurodegenerative processes, aggressive behavior, and cognitive activities, are now considered as key factors of chemical neurotransmission. Because most of the biochemical pathways of neurosteroidogenesis are now elucidated (Fig. 7), the main questions which have to be answered during the next years concern the role of classical neurotransmitters and neuropeptides in the control of the expression of steroidogenic enzyme genes and activities in the brain.

XIV. Summary

Steroid hormones exert important functions in the control of growth, maturation, and differentiation of the central and peripheral nervous systems. These actions have long been attributed exclusively to steroid hormones secreted by endocrine glands, i.e., adrenal, ovary, and testis. However, during the last decade, it has been shown that nerve cells (both neurons and glial cells) are capable of synthesizing bioactive steroids, now called neurosteroids, which also participate in the control of various functions in the CNS. One of the major criteria supporting the concept of neurosteroidogenesis is based on the occurrence of steroidogenic enzymes in nerve cells. Immunocytochemical and in situ hybridization techniques have made it possible to determine the neuroanatomical distribution of key enzymes such as P-450sc, 3β-HSD, cytochrome P-450c17, 17β-HSD, 5α-R, aromatase, sulfotransferase, and sulfatase. Concurrently, the presence of enzymatic activities for steroid biosynthesis has been demonstrated in neurons and/or glial cells, thus indicating that the isoforms expressed in nerve cells actually correspond to active forms of the steroidogenic enzymes. Recent studies concerning the control of the expression and activity of key steroidogenic enzymes in the CNS strongly suggest that neurosteroidogenesis may be regulated by adrenal and gonadal steroids as well as by neuropeptides of the enkephalin family.

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