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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)[2] 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

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[2] Abbreviations: CNS, central nervous system; $GABA_A$, type A γ-aminobutyric acid receptors; NMDA, *N*-methyl-D-aspartate receptors; DBI, diazepam-binding inhibitor; DHEA, dehydroepiandrosterone; Δ^5P, pregnenolone; DHEAS, dehydroepiandrosterone sulfate; Δ^5PS, pregnenolone sulfate; ODN, octadecaneuropeptide (DBI[33-50]); PAPS, 3′-phosphoadenosine,5′-phosphosulfate; PBR, peripheral-type benzodiazepine receptor; PK11195, 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methyl-propyl)-3-isoquinoline carboxamide; PNS, peripheral nervous system; TTN, triakontatetraneuropeptide (DBI[17–50]); P-450scc, cytochrome P-450 side chain cleavage; P450c_{17}, cytochrome P-450c_{17}; P-45011β, cytochrome P-45011β; P-450aldo, cytochrome P-450-aldosterone synthase; 3α-HSD, 3α-hydroxysteroid dehydrogenase; 3β-HSD, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase; HST, hydroxysteroid sulfotransferase; 5α-R, 5α-reductase; 5α-DHT, 5α-dihydrotestosterone; 11β-HSD, 11β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase.

coworkers have shown that the rat brain is capable of synthesizing various steroid hormones such as pregnenolone (Δ^5P) and dehydroepiandrosterone (DHEA) from cholesterol (Baulieu, 1981). These authors first demonstrated the existence of high amounts of Δ^5P and DHEA in the brain of castrated and adrenalectomized rats (Corpéchot et al., 1981, 1983). Thereafter, they found that the cerebral concentrations of Δ^5P 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 Δ^5P 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 Δ^5P) 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-asparate (NMDA) receptors (Wu et al., 1991), and σ 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 (Orchinik 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-dihydroprogesterone 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 (Bélanger 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 Δ^5P 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 Δ^5P 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-polymerase 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

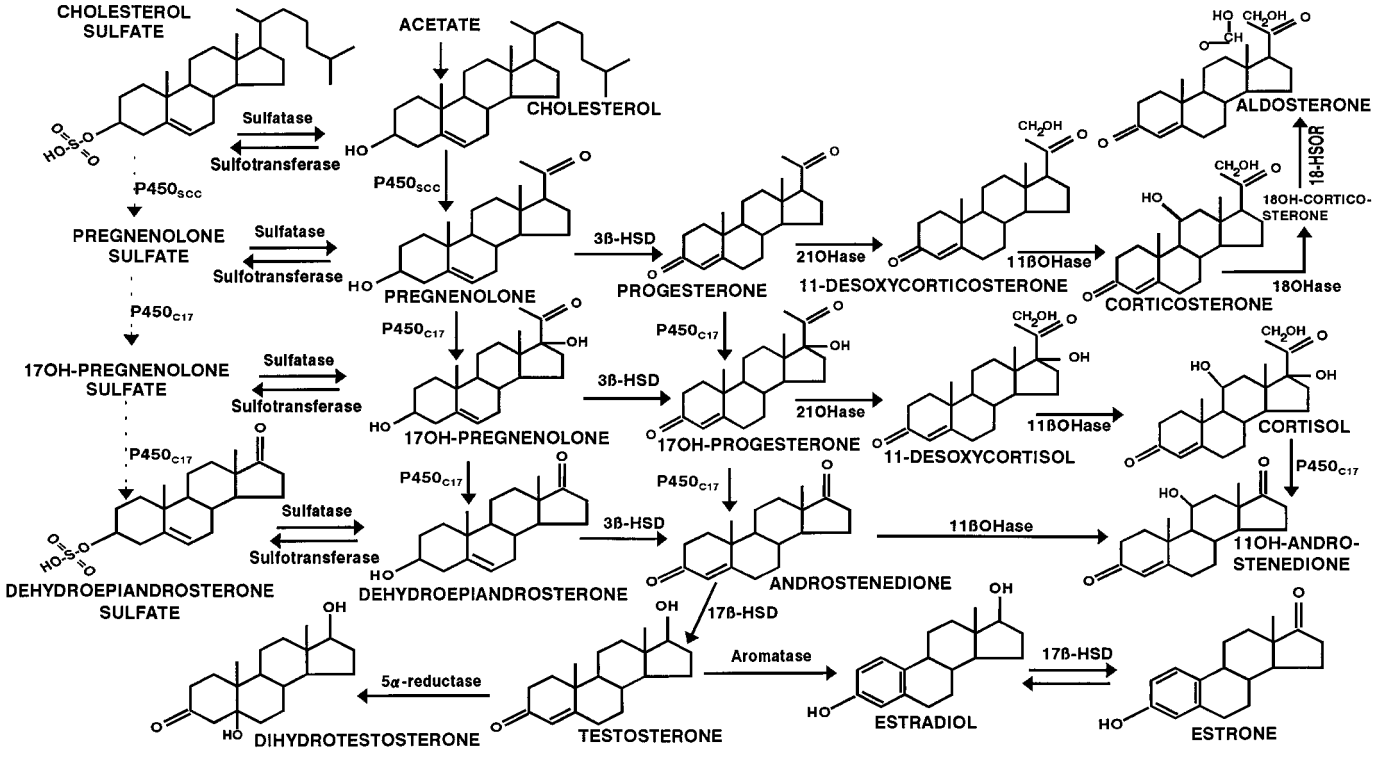

FIG 1. Biosynthesis of steroid hormones in endocrine glands. P-450scc, P450c$_{17}$, 3β-HSD, 17β-HSD, 11βOHase, 11β-hydroxylase; 18βOHase, 18-hydroxylase; 18-HSOR, 18-hydroxysteroid oxidoreductase; 21OHase, 21-hydroxylase.

is not only expressed in glial cells but also in neurons. The detection of relatively high amounts of Δ^5P in the frog hypothalamus (Mensah-Nyagan et al., 1994) and the localization of an active form of P-450scc in the quail brain (Tsutsui and Yamazaki, 1995; Usui et al., 1995) have shown that the gene encoding P-450scc 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-450scc 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-450scc* (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-450scc mRNA (Mellon and Deschepper, 1993; Ikeda et al., 1994), indicating that SF-1 is not involved in the regulation of *P-450scc* 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-450scc* gene expression in endocrine cells and nerve cells exhibit both differences and similarities.

Studies performed in rat indicate that the biological activity of P-450scc 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-450scc 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-450scc 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; Lihrmann et al., 1994; Patte et al., 1995; Lamacz et al., 1996). Proteolytic cleavage of DBI generates several bioactive peptides including the triakontatetraneuropeptide (TTN) DBI[17-50], the octadecaneuropeptide (ODN) DBI[33-50], and the triakontaseptaneuropeptide DBI[39-75] (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 Δ^5P (Papadopoulos et al.,

1992). These results strongly suggest that endozepines 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/Δ^5-Δ^4 isomerase (3β-HSD), which catalyzes the conversion of Δ^5-3β-hydroxysteroids into Δ^4-3-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; Abbaszade 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 Weidenfield et al. (1980) who showed that homogenates of rat amygdala and septum are capable of converting Δ^5P 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 isotypes, 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 Δ^4-3-ketosteroids (progesterone and 17-hydroxyprogesterone) in the frog brain and the capability of frog hypothalamic explants to catalyze the conversion of tritiated pregnenolone ([^{3}H]Δ^5P) into progesterone demonstrate that the 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 olfactive 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 isoenzymes 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 imunoreactivities (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 endozepine TTN causes a dose-dependent stimulation of the conversion of Δ^5P 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 Lenfant, 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.

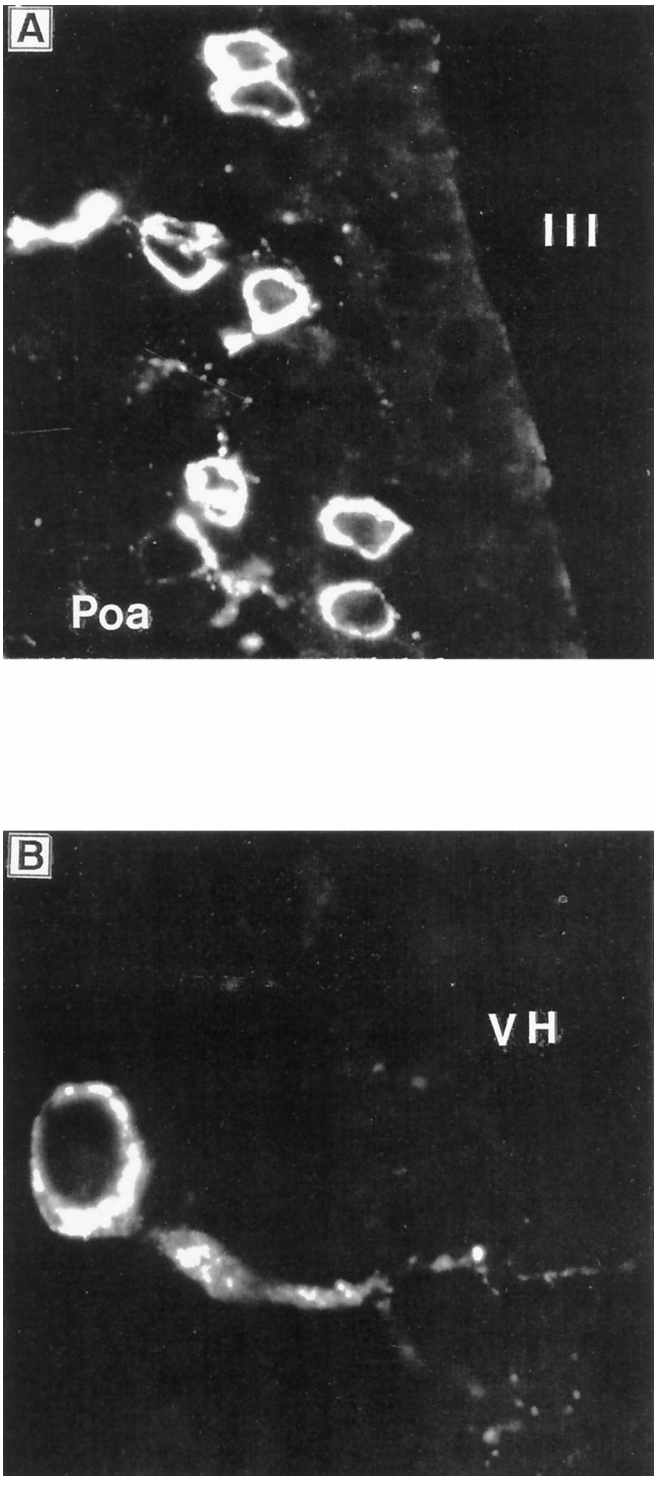

FIG. 2. Confocal laser scanning microscope photomicrographs of 3β-HSD-immunoreactive neurons in the frog diencephalon. A, frontal section through the anterior preoptic area (Poa) showing 3β-HSD-positive cell bodies close to the third ventricle (III). Original magnification, 700×. B, high magnification of a 3β-HSD-immunoreactive neuron in the ventral hypothalamic nucleus (VH). Original magnification, 1300×.

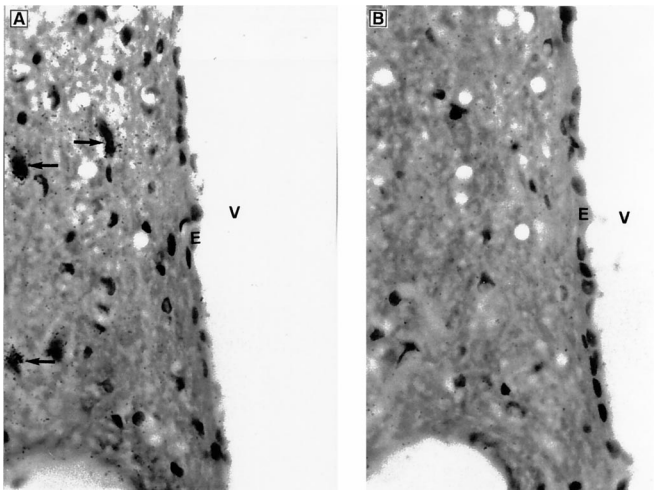

FIG. 3. Autoradiographic localization of 3β-HSD mRNAs by in situ hybridization on frontal sections of the rat brain at the level of the nucleus prepositus hypoglossi. A, dense accumulation of silver grains is observed in a subpopulation of neurons (arrows) located in the vicinity of the fourth ventricle (V). Ependymal cells (E) do not express the *3β-HSD* gene. Original magnification, 560×. B, pretreatment of the brain section with RNase abolished autoradiographic labeling. Original magnification, 560×. Reprinted from Dupont et al. (1994) with permission from Academic Press, Inc.

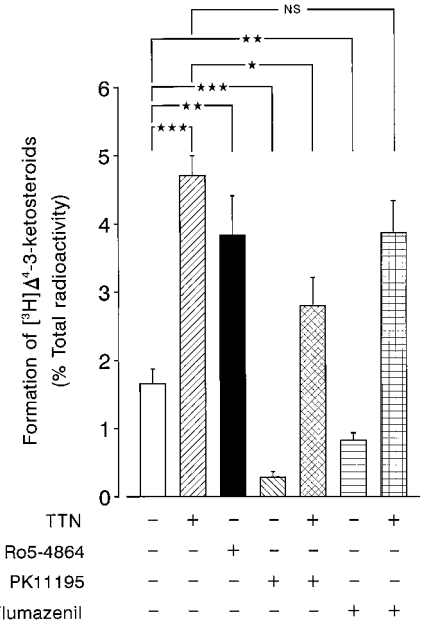

FIG. 4. Effect of the triakontatetraneuropeptide TTN and other benzodiazepine receptor ligands on the conversion of [^{3}H]Δ^5P into Δ^4-3-ketoneurosteroids. Frog hypothalamic explants were incubated for 2 h with 24 μCi/ml of [^{3}H]Δ^5P and the radioactive newly synthesized Δ^4-3-ketosteroids were separated by HPLC analysis. The endozepine TTN and the PBR agonist Ro-5-4864 both induced a marked increase of neurosteroid biosynthesis. The PBR antagonist PK11195 (10^{-4} M) reduced the basal synthesis of neurosteroids and significantly attenuated the effect of TTN. The central-type benzodiazepine receptor antagonist flumazenil (10^{-5} M) also reduced the basal synthesis of neurosteroids but did not affect the stimulatory action of TTN on Δ^4-3-ketoneurosteroid formation. ⋆$P < .05$; ⋆⋆, $P < .01$; ⋆⋆⋆, $P < .001$; NS, not statistically different.

V. Cytochrome P-450c$_{17}$

The enzymatic system 17α-hydroxylase/17,20 lyase (cytochrome P-450c$_{17}$) is responsible for the transformation of C$_{21}$ steroids (Δ^5P, progesterone) into C$_{19}$ 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-450c$_{17}$ (P-450c$_{17}$ or P-450$_{17\alpha}$), 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 (Corpéchot et al., 1981) suggested the existence of P-450c$_{17}$ 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 Robel, 1990; Le Goascogne et al., 1991; Mellon and Deschepper, 1993). In 1994, it was demonstrated that frog hypothalamic explants are capable of converting [^{3}H]Δ^5P into [^{3}H]17α-hydroxyprogesterone (Mensah-Nyagan et al., 1994). This observation provided the first evidence for the presence of P-450c$_{17}$ in the CNS. Subsequently, P-450c$_{17}$ mRNAs have been detected by RT-PCR in the brain of rat embryos (Compagnone et al., 1995b). P-450c$_{17}$-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-450c$_{17}$* gene is only expressed in the PNS of rat and mouse, whereas other studies have described the presence of P-450$_{17\alpha}$ mRNAs in various brain regions of adult rodents (Strömstedt and Waterman, 1995).

The regulation of *P-450c$_{17}$* 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-Behling 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-450c$_{17}$* 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 [^{3}H]Δ^5P into [^{3}H]17α-hydroxyprogesterone, indicating that endozepines can increase P-450c$_{17}$ 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 Moghrabi, 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 the Ke 6 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 the Ke 6 protein 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 was 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 has also been determined in the frog brain using antibodies against human placental type I 17β-HSD (Mensah-Nyagan et al., 1996a,b). In the CNS of both mammals and amphibians, type I 17β-HSD is exclusively expressed in glial cells (Pelletier et al., 1995; Mensah-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

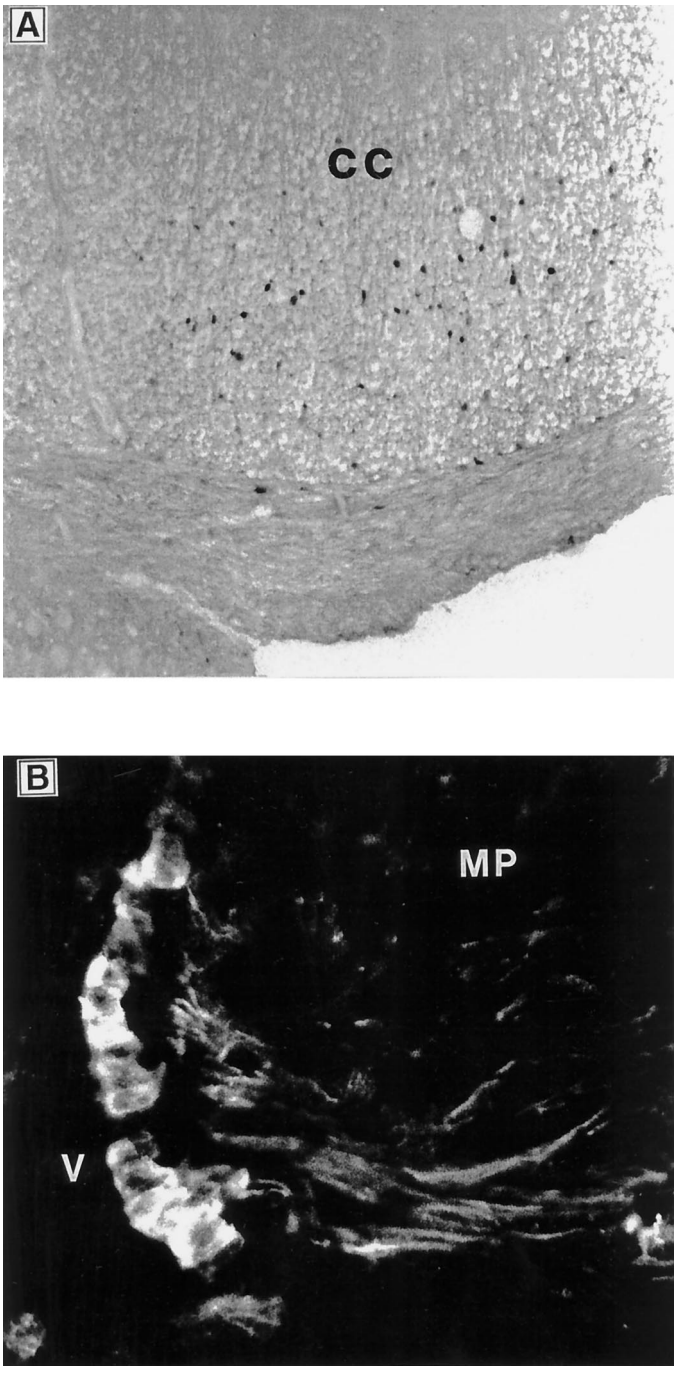

FIG. 5. Immunocytochemical localization of 17β-HSD in the CNS of rat and frog. A, 17β-HSD-positive glial cells in the rat cerebellar cortex (CC). Reprinted from Pelletier et al. (1995) with kind permission from Elsevier Science-NL (Sara Burgerhartstraat 25, 1055 KV Amsterdam, the Netherlands). Original magnification, 80×. B, 17β-HSD-immunoreactive ependymal cells bordering the lateral ventricle (V) in the frog telencephalon. MP, medial pallium. Original magnification, 500×.

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 unkown. 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 [^{3}H]testosterone and [^{3}H]5α-DHT from [^{3}H]Δ^5P 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 [^{3}H]Δ^5P or [^{3}H]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 C_4-C_5 double bond and a ketone group at the C_3 position. This enzyme catalyzes the transfer of two hydrogens from NADPH causing the reduction of the C_4-C_5 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 dihydroprogester-

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 *5α-RI* 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 *5α-RII* 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 *5α-RII* 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, 5α-RI and 5α-RII 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; Martini 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 tanycytes 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 individuals 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 $GABA_A$ 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 C_{19} 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 adipocytes (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 neurons (Shinoda et al., 1989a,b; 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 (E_{13}), E_{16}, and E_{19}, 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-Dohmae 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 (Baillien 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., 1990a,b, 1991a,b; Sanghera et al., 1991; Jakab et al., 1993, 1994; Beyer et al., 1994a,c; Foidart et al., 1995), where a number of neuropeptides controlling sexual behavior are present (Wehrenberg et al., 1989; DeVries, 1990; Kalra et al., 1990; Kawata et al., 1991; Albers et al., 1992; Andersen et al., 1992; King and Millar, 1992; Sherwood et al., 1993; Winslow et al., 1993; Moore et al., 1994), suggests that some of these neuropeptides could be involved in the regulation of aromatase gene transcription and/or activity in the CNS.

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 (Δ^5PS) and dehydroepiandrosterone sulfate (DHEAS) modulate the functions of $GABA_A$ receptors (Majewska, 1992), NMDA receptors (Wu et al., 1991; Weaver et al., 1997), σ receptors (Monnet et al., 1995), and voltage-gated calcium channels (Ffrench-Müllen and Spence, 1991; Ffrench-Müllen et al., 1994). The fact that the inhibitory action of Δ^5PS on calcium channel currents in pyramidal neurons is abolished after substitution of the sulfate moiety by an acetate (Ffrench-Müllen 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, Δ^5P 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 sulfatases 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 Δ^5P, DHEA, and their sulfated esters (Δ^5PS and DHEAS) have been detected in the brain of castrated and adrenalectomized rats, suggesting the presence of sulfotransferase and sulfatase activities in the CNS (Corpéchot 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 Δ^5PS and DHEAS (Fig. 6), as demonstrated by pulse-chase experiments using [^{35}S]PAP and [^{3}H]Δ^5P or [^{3}H]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 (cortisol, 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 an 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

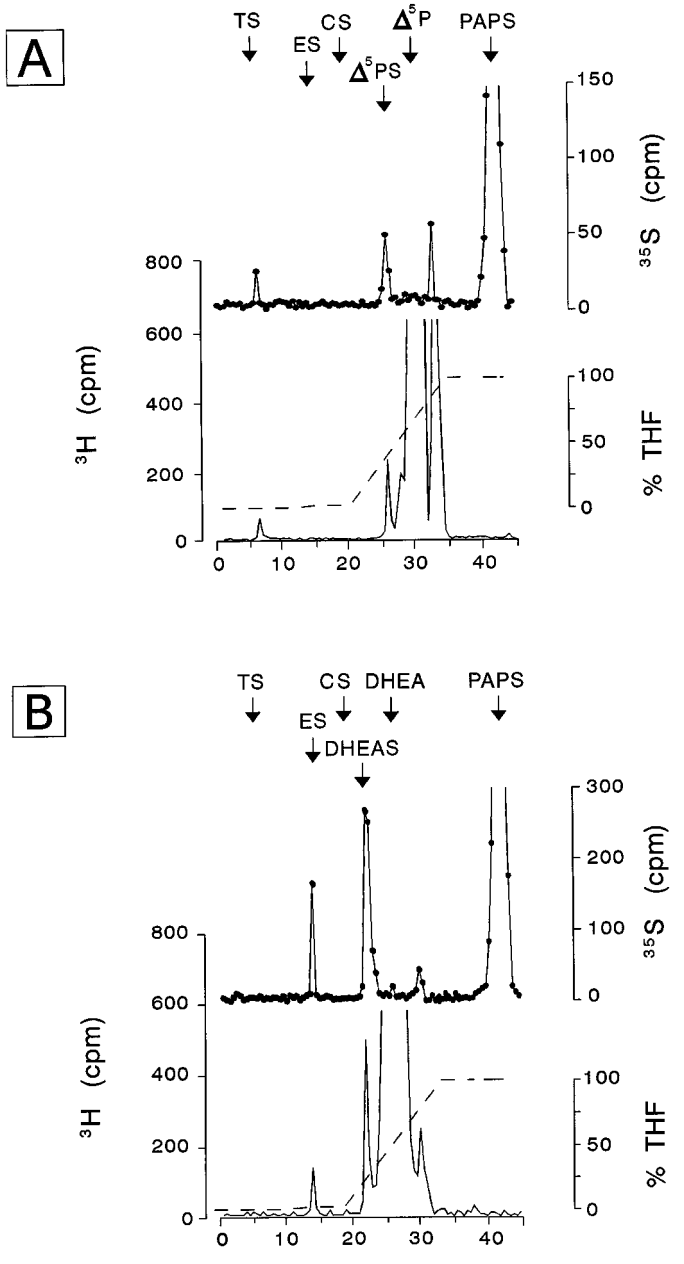

FIG. 6. Biosynthesis of Δ^5PS (A) and DHEAS (B) in the frog hypothalamus in vitro. Frog hypothalamic homogenates were incubated for 3 h with [^{3}H]Δ^5P and [^{35}S]PAPS (A) or with [^{3}H]DHEA and [^{35}S]PAPS (B). The steroids were extracted in a mixture of water and dichloromethane (v/v) and the aqueous phase, containing sulfated steroids, was analyzed by HPLC using a hexane/tetrahydrofuran (THF) gradient. The ordinates indicate the radioactivity (^{3}H and ^{35}S) measured in the HPLC fractions. The dashed linerepresents the gradient of secondary solvent (% THF). The arrows indicate the elution position of standard steroids. Reprinted from Beaujean et al. (1999) with permission from the *Journal of Neurochemistry*, Lippincott-Raven Publishers.

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 experiments 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 monooxygenase activity as well as the 11β-hydroxylation of [4-^{14}C]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 dihydrodiol 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 cDNAs 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 characterized 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 corresponding gene has now been fully characterized (Lin and Penning, 1995; Penning et al., 1996).

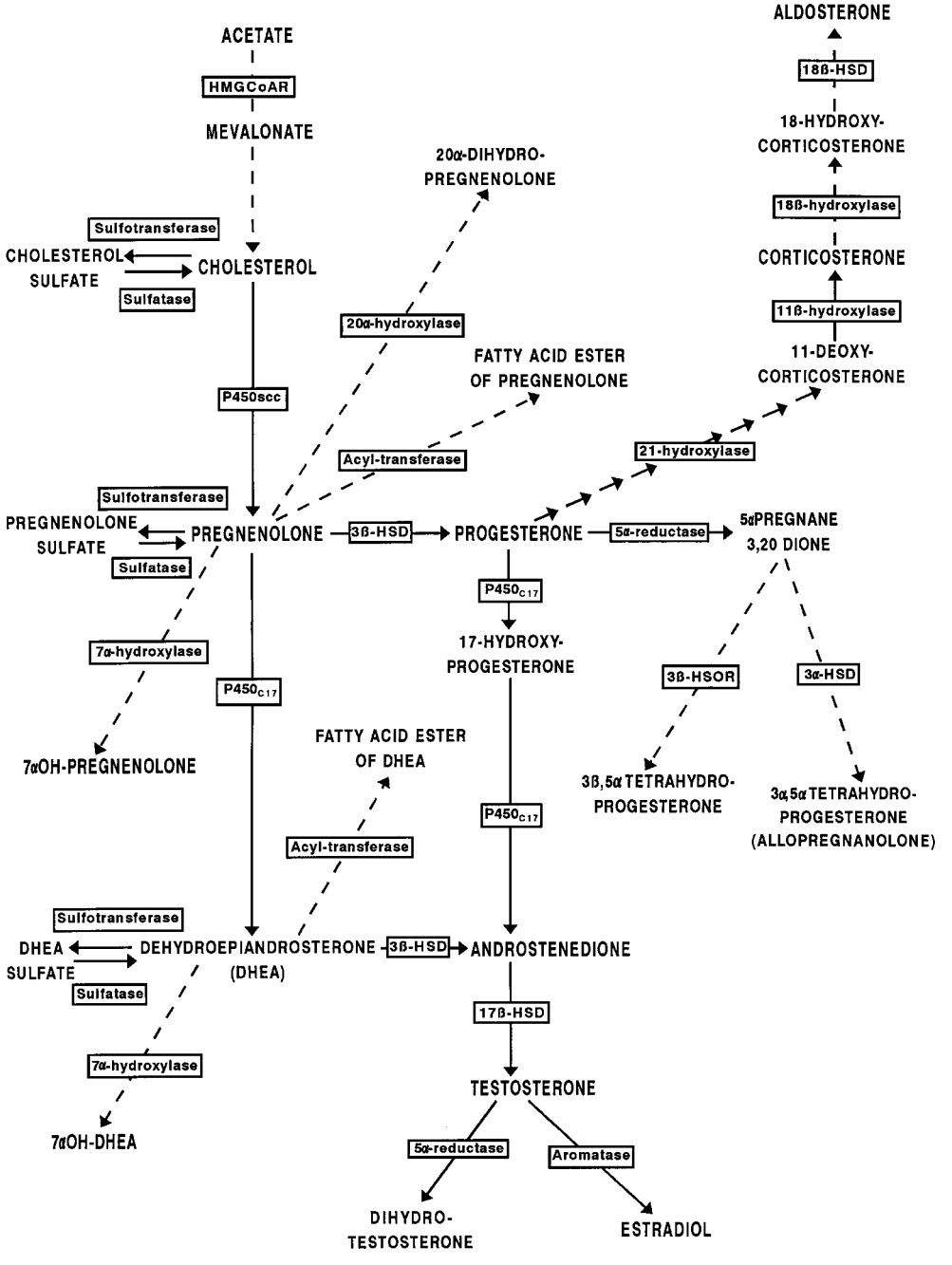

FIG. 7. Current knowledge concerning the biochemical pathways of neurosteroidogenesis. Solid arrows indicate that the biochemical reaction has been formally demonstrated, i.e., both the biological activity of the enzyme in neural tissue and the localization of the enzyme by immunohistochemistry or its 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. Δ^5-3β-Hydroxysteroid Acyltransferase

The enzyme Δ^5-3β-hydroxysteroid acyltransferase (acyltransferase) catalyzes the saponification reaction which converts Δ^5-3β-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 hypothalamus, amygdala, or olfactory bulb explants with [^{3}H]Δ^5P or [^{3}H]DHEA yields to the formation of fatty acid esters of [^{3}H]Δ^5P and [^{3}H]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 C_7 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 Δ^5P and DHEA into 7α-hydroxy-Δ^5P 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 Δ^5P that are respectively converted by this enzyme into 7α-hydroxy-DHEA and 7α-hydroxy-Δ^5P (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-450c$_{11\beta}$ 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 hypothalamus, hippocampus, amygdala, and cerebellum. These authors have also shown that hippocampic, hypothalamic and cerebellar explants are capable of converting [^{3}H]11-deoxycorticosterone into [^{3}H]corticosterone, [^{3}H]18-hydroxycorticosterone, and [^{3}H]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-450scc, 3β-HSD, cytochrome P-450c$_{17}$, 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 Δ^5PS 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, Δ^5P, 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-450scc, 3β-HSD, cytochrome P-450c$_{17}$, 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 isozymes 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 endozepine family.

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REFERENCES

Abbaszade IG, Arensburg J, Park CH, Kasa-Vubu JZ, Orly J and Payne AH (1997) Isolation of a new mouse 3β-hydroxysteroid dehydrogenase isoform, 3β-HSD VI, expressed during early pregnancy. *Endocrinology* **138:**1392–1399.

Abdelgadir SE, Resko JA, Ojeda SR, Lephart ED, McPhaul MJ and Roselli CE (1994) Androgens regulate aromatase cytochrome P-450 messenger ribonucleic acid in rat brain. *Endocrinology* **135:**395–401.

Abe-Dohmae S, Tanaka R and Harada N (1994) Cell-type and region-specific expression of aromatase mRNA in cultured brain cells. *Mol Brain Res* **24:**153–158.

Adamski J, Husen B, Marks F and Jungblut PW (1992) Purification and properties of estradiol 17β-dehydrogenase extracted from cytoplasmic vesicles of porcine endometrial cells. *Biochem J* **288:**375–381.

Adamski J, Normand T, Leenders F, Monte D, Begue A, Stehelin D, Jungblut PW and de Launoit Y (1995) Molecular cloning of a novel, widely expressed human 80 kDa 17β-hydroxysteroid dehydrogenase IV. *Biochem J* **311:**437–443.

Agarwal AK, Monder C, Eckstein B and White PC (1989) Cloning and expression of rat cDNA encoding corticosteroid 11β-dehydrogenase. *J Biol Chem* **264:**18939–18943.

Agarwal AK, Mune T, Monder C and White PC (1994) NAD$^+$-dependent isoform of 11β-hydroxysteroid dehydrogenase: cloning and characterization of cDNA from sheep kidney. *J Biol Chem* **269:**25959–25962.

Akwa Y, Morfin RF, Robel P and Baulieu EE (1992) Neurosteroid metabolism: 7α-hydroxylation of dehydroepiandrosterone and pregnenolone by rat brain microsomes. *Biochem J* **288:**959–964.

Albers HE, Hennessey AC and Whitman DC (1992) Vasopressin and the regulation of hamster social behavior. *Ann NY Acad Sci* **652:**227–242.

Albiston AL, Obeyesekere VR, Smith RE and Krozowski KS (1994) Cloning and tissue distribution of the human 11β-hydroxysteroid dehydrogenase type 2 enzyme. *Mol Cell Endocrinol* **105:**R11–R17.

Alho H, Varga V and Krueger KE (1994) Expression of mitochondrial benzodiazepine receptor and its putative endogenous ligand diazepam binding inhibitor in cultured primary astrocytes and C-6 cells: Relation to cell growth. *Cell Growth & Differ* **5:**1005–1014.

Andersen AC, Tonon MC, Pelletier G, Conlon JM, Fasolo A and Vaudry H (1992) Neuropeptides in the amphibian brain. *Int Rev Cytol* **138:**89–210.

Andersson S (1995) 17β-hydroxysteroid dehydrogenase: isozymes and mutations. *J Endocrinol* **146:**241–251.

Andersson S, Berman DM, Jenkins EP and Russell DW (1991) Deletion of steroid 5α-R 2 gene in male pseudohermaphroditism. *Nature (London)* **354:**159–161.

Andersson S and Moghrabi N (1997) Physiology and molecular genetics of 17β-hydroxysteroid dehydrogenases. *Steroids* **62:**143–147.

Andersson S and Russell DW (1990) Structural and biochemical properties of cloned and expressed human and rat steroid 5α-reductases. *Proc Natl Acad Sci USA* **87:**3640–3644.

Arnold AP and Gorski RA (1984) Gonadal steroid induction of structural sex differences in the central nervous system. *Annu Rev Neurosci* **7:**413–442.

Aziz N, Brown D, Lee WS and Naray-Fejes-Toth A (1996) Aberrant 11β-hydroxysteroid dehydrogenase-1 activity in the *cpk* mouse: implications for regulation by the Ke 6 gene. *Endocrinology* **137:**5581–5588.

Baillien M and Balthazart J (1997) A direct dopaminergic control of aromatase activity in the quail preoptic area. *J Steroid Biochem Mol Biol* **63:**99–113.
Ballabio A and Shapiro LJ (1995) Steroid sulfatase deficiency and X-linked ichthyosis, in *The Metabolic and Molecular Bases of Inherited Disease* (Schriver CR, Beaudet AL, Sly WS and Valle D eds) pp 2999–3022, McGraw-Hill, New York.
Balthazart J, Foidart A and Harada N (1990a) Immunocytochemical localization of aromatase in the brain. *Brain Res* **514:**327–333.
Balthazart J, Foidart A, Surlemont C and Harada N (1991a) Distribution of aromatase-immunoreactive cells in the mouse forebrain. *Cell Tissue Res* **263:**71–79.
Balthazart J, Foidart A, Surlemont C and Harada N (1991b) Neuroanatomical specificity in the co-localization of aromatase and estrogen receptors. *J Neurobiol* **22:**143–157.
Balthazart J, Foidart A, Surlemont C, Harada N and Naftolin F (1992) Neuroanatomical specificity in the autoregulation of aromatase-immunoreactive neurons by androgens and estrogens: an immunocytochemical study. *Brain Res* **574:**280–290.
Balthazart J, Foidart A, Surlemont C, Vockel A and Harada N (1990b) Distribution of aromatase in the brain of the Japanese quail, ring dove, and zebra finch: an immunocytochemical study. *J Comp Neurol* **301:**276–288.
Balthazart J, Gahr M and Surlemont C (1989) Distribution of estrogen receptors in the brain Japanese quail: An immunocytochemical study. *Brain Res* **501:**205–214.
Bartsch W, Klein H, Schiemann U, Bauer HW and Voigt KD (1990) Enzymes of androgen formation and degradation in the human prostate. *Ann NY Acad Sci* **595:**53–66.
Bauer HC and Bauer H (1989) Micromethod for the determination of 3β-HSD activity in cultured cells. *J Steroid Biochem* **33:**643–646.
Baulieu EE (1981) Steroid hormones in the brain: Several mechanisms?, in *Steroid Hormone Regulation of the Brain* (Fuxe K, Gutafsson JA and Wetterberg L eds) pp 3–14, Pergamon Press, Oxford.
Baulieu EE and Robel P (1990) Neurosteroids: A new brain function? *J Steroid Biochem Mol Biol* **37:**395–403.
Baulieu EE and Robel P (1996) Dehydroepiandrosterone and dehydroepiandrosterone sulfate as neuroactive neurosteroids. *J Endocrinol* **150:**S221–S239.
Baulieu EE and Robel P (1998) Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) as neuroactive neurosteroids. *Proc Natl Acad Sci USA* **95:**4089–4091.
Baulieu EE, Robel P, Vatier O, Haug M, Le Goascogne C and Bourreau E (1987) Neurosteroids: Pregnenolone and dehydroepiandrosterone in the brain, in *Receptor-Receptor Interactions* (Fuxe K and Agnati LF eds) vol 48**,** pp 89–104, MacMillan Press, Basingstoke.
Baulieu EE, Schumacher M, Koenig H, Jung-Testas I and Akwa Y (1996) Progesterone as a neurosteroid: actions within the nervous system. *Cell Mol Neurobiol* **16:**143–153.
Beaujean D, Mensah-Nyagan AG, Do-Régo JL, Luu-The V, Pelletier G and Vaudry H (1999) Immunocytochemical localization and biological activity of hydroxysteroid sulfotransferase in the frog brain. *J Neurochem* **72:**848–857.
Bélanger B, Bélanger A, Labrie F, Dupont A, Cusan L and Monfette G (1989) Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: Unique importance of extratesticular androgens in men. *J Steroid Biochem* **32:**695–698.
Benavides JJ, Guilloux F, Allan DE, Uzan A, Mizoule J, Renault C, Guérémy C and Le Fur G (1984) Opposite effects of antagonist, PK11195, of the peripheral benzodiazepine binding sites on audiogenic seizure in DBA/2J mice. *Life Sci* **34:**2613–2620.
Berman DM and Russell DW (1993) Cell-type-specific expression of rat 5α-reductase isozymes. *Proc Natl Acad Sci USA* **90:**9359–9363.
Beyer C, Green SJ, Baker PJ, Huskisson NS and Hutchison JB (1994a) Aromatase-immunoreactivity is localized in neurons in the developing mouse hypothalamus and cortex. *Brain Res* **638:**203–210.
Beyer C, Green SJ and Hutchison JB (1994b) Androgens influence sexual differentiation of embryonic mouse hypothalamic aromatase neurons *in vitro*. *Endocrinology* **135:**1220–1226.
Beyer C, Tramonte R and Hutchison JB (1994c) Aromatase-immunoreactive neurons in the adult female chicken brain detected using a specific antibody. *Brain Res Bull* **33:**583–588.
Biswas MG and Russell (1997) Expression cloning and characterization of oxidative 17β- and 3α-hydroxysteroid dehydrogenases from rat and human prostate. *J Biol Chem* **272:**15959–15966.
Blomquist CH (1995) Kinetic analysis of enzymatic activities: prediction of multiple forms of 17β-hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* **55:**515–524.
Bohren KM, Bullock B, Wermuth B and Gabbay KH (1989) The aldoketo reductase superfamily. cDNAs and deduced amino acid sequences of human aldehyde and aldose reductases. *J Biol Chem* **264:**9547–9551.
Bonsall RW, Rees HD and Michael RP (1989) Identification of radioactivity in cell nuclei from brain, pituitary gland and genital tract of male rhesus monkeys after the administration of [^{3}H]testosterone. *J Steroid Biochem* **32:**599–608.
Bonsall RW, Zumpe D and Michael RP (1990) Comparison of the nuclear uptake of [^{3}H]-testosterone and its metabolites by brains of male and female macaque fetuses at 122 days of gestation. *Neuroendocrinology* **51:**474–480.
Brodgen RN and Goa KL (1991) Flumazenil: A reappraisal of its pharmacological properties and therapeutic efficacity as benzodiazepine antagonist. *Drugs* **42:**1061–1089.
Brown AS, Hall PF, Shoyab M and Papadopoulos V (1992) Endozepine/diazepam binding inhibitor in adrenocortical and Leydig cell lines: Absence of hormonal regulation. *Mol Cell Endocrinol* **83:**1–9.
Brown RW, Chapman KE, Edwards CRW and Seckl JR (1993) Human placental 11β-hydroxysteroid dehydrogenase: Partial purification of and evidence for a distinct NAD-dependent isoform. *Endocrinology* **132:**2641–2621.
Brown RW, Kotolevtsev Y, Leckie C, Lindsay RS, Lyons V, Murad P, Mullins JJ, Chapman KE, Edwards CRW and Seckl JR (1996) Isolation and cloning of human placental 11β-hydroxysteroid dehydrogenase-2 cDNA. *Biochem J* **313:**1007–1017.
Burgos-Trinidad M, Youngblood GL, Maroto MR, Scheller A, Robins DM and Payne AH (1997) Repression of cAMP-induced expression of the mouse P-450 17α-hydroxylase/C17–20 lyase gene (Cyp17) by androgens. *Mol Endocrinol* **11:**87–96.
Callard CV, Petro Z and Ryan KJ (1980) Aromatization of androgen to estrogen by cultured turtle brain. *Brain Res* **202:**117–130.
Casey ML, MacDonald PC and Andersson S (1994) 17β-hydroxysteroid dehydrogenase type 2: Chromosomal assignment and progestin regulation of gene expression in human endometrium. *J Clin Invest* **94:**2135–2141.
Celotti F, Melcangi R and Martini L (1992) The 5alpha-reductase in the brain: Molecular aspects and relation to brain function. *Front Neuroendocrinol* **13:**163–215.
Celotti F, Melcangi R, Negri-Cesi P, Ballabio M and Martini L (1987) Differential distribution of the 5α-reductase in the central nervous system of the rat and the mouse: Are the white matter structures of the brain target tissues for testosterone action? *J Steroid Biochem* **26:**125–129.
Celotti F, Negri-Cesi P, Limonta P and Melcangi C (1983) Is the 5alpha-reductase of the hypothalamus and of the anterior pituitary neurally regulated? Effects of hypothalamic deafferentations and of centrally acting drugs. *J Steroid Biochem* **19:**229–234.
Cheng CY, Flasch MV and Hornsby PJ (1992) Expression of 17 alpha-hydroxylase and 3 beta-hydroxysteroid dehydrogenase in fetal human adrenocortical cells transfected with SV40 antigen. *J Mol Endocrinol* **9:**7–17.
Clark AS, MacLusky NJ and Goldman-Rakic PS (1988) Androgen binding and metabolism in the cerebral cortex of the developing rhesus monkey. *Endocrinology* **123:**932–940.
Clemens JW, Lala DS, Parker KL and Richards JS (1994) Steroidogenic factor-1 binding and transcriptional activity of the cholesterol side-chain cleavage promoter in rat granulosa cells. *Endocrinology* **134:**1499–1508.
Cole TJ (1995) Cloning of a mouse 11 beta-hydroxysteroid dehydrogenase type 2: Tissue specific expression and localization in distal convoluted tubules and collecting ducts of the kidney. *Endocrinology* **136:**4693–4696.
Compagnone NA, Bulfone A, Rubenstein JLR and Mellon S (1995a) Expression of the steroidogenic enzyme cytochrome P450scc in the central and peripheral nervous systems during rodent embryogenesis. *Endocrinology* **136:**2689–2696.
Compagnone NA, Bulfone A, Rubenstein JLR and Mellon S (1995b) Steroidogenic enzyme P450c17 is expressed in the embryonic central nervous system. *Endocrinology* **136:**5212–5223.
Compagnone NA, Salido E, Shapiro LJ and Mellon SH (1997) Expression of steroid sulfatase during embryogenesis. *Endocrinology* **138:**4768–4773.
Conley AJ, Corbin CJ, Smith T, Hinshelwood M, Liu Z and Simpson E (1997) Porcine aromatases: Studies on tissue-specific, functionally distinct isozymes from a single gene? *J Steroid Biochem Mol Biol* **61:**407–413.
Corbin CJ, Graham-Lorence S, McPhaul M, Mason JL, Mendelson CR and Simpson ER (1988) Isolation of a full length cDNA insert encoding human aromatase system cytochrome P-450 and its expression in non-steroidogenic cells. *Proc Natl Acad Sci USA* **85:**8948–8952.
Corbin CJ, Khalil MW and Conley AJ (1995) Functional ovarian and placental isoforms of porcine aromatase. *Mol Cell Endocrinol* **113:**29–37.
Corpéchot C, Robel P, Axelsön M, Sjövall J and Baulieu EE (1981) Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. *Proc Natl Acad Sci USA* **78:**4704–4707.
Corpéchot C, Synguelakis M, Talha S, Axelsön M, Sjövall J, Vihko R, Baulieu EE and Robel P (1983) Pregnenolone and its ester sulfate in the rat brain. *Brain Res* **270:**119–123.
Costa E, Auta J, Guidotti A, Korneyev A and Romeo E (1994) The pharmacology of neurosteroidogenesis. *J Steroid Biochem Mol Biol* **49:**385–389.
De Vries GJ (1990) Sex differences in neurotransmitter systems. *J Neuroendocrinol* **2:**1–13.
Do-Régo JL, Mensah-Nyagan AG, Feuilloley M, Ferrara P, Pelletier G and Vaudry H (1998) The endozepine triakontatetraneuropeptide diazepam-binding inhibitor [17-50] stimulates neurosteroid biosynthesis in the frog hypothalamus. *Neuroscience* **83:**555–570.
Duan WR, Linzer DIH and Gibori G (1996) Cloning and characterization of an ovarian-specific protein that associates with the short form of the prolactin receptor. *J Biol Chem* **271:**15602–15607.
Duan WR, Parmer TG, Albarracin CT, Zhong L and Gibori G (1997) PRAP, a prolactin receptor associated protein: Its gene expression and regulation in the corpus luteum. *Endocrinology* **138:**3216–3221.
Dumont M, Luu-The V, Dupont E, Pelletier G and Labrie F (1992) Characterization, expression and immunohistochemical localization of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in human skin. *J Invest Dermatol* **99:**415–427.
Duperrex H, Kenouch S, Gaeggeler HP, Seckl JR, Edwards CRW, Farman N and Rossier BC (1993) Rat liver 11β-hydroxysteroid dehydrogenase cDNA encodes oxoreductase activity in a mineralocorticoid-responsive toad bladder cell line. *Endocrinology* **132:**612–619.
Dupont E, Luu-The V, Labrie F and Pelletier G (1990a) Ontogeny of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) in human adrenal gland performed by immunocytochemistry. *Mol Cell Endocrinol* **74:**R7–R10.
Dupont E, Luu-The V, Labrie F and Pelletier G (1990b) Light microscopic immunocytochemical localization of 3β-hydroxy-5-ene-steroid dehydrogenase/Δ^5-Δ^4-isomerase in the gonads and adrenal glands of the guinea pig. *Endocrinology* **126:**2906–2909.
Dupont E, Simard J, Luu-The V, Labrie F and Pelletier G (1994) Localization of 3β-hydroxysteroid dehydrogenase in rat brain as studied by *in situ* hybridization. *Mol Cell Neurosci* **5:**119–123.
Dupont E, Zhao HF, Rhéaume E, Simard J, Luu-The V, Labrie F and Pelletier G (1990c) Localization of 3β-hydroxy-5-ene steroid dehydrogenase/Δ^5-Δ^4 isomerase in the rat gonads and adrenal glands by immunocytochemistry and *in situ* hybridization. *Endocrinology* **127:**1394–1403.
Edwards CRM, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, de Kloet

ER and Monder C (1988) Localisation of 11β-hydroxysteroid dehydrogenase- tissue specific protector of the mineralocorticoid receptor. *Lancet* **2:**986–989.
Ehrhart-Bornstein M, Bornstein SR, Trzeciak WH, Usadel H, Güse-Behling H, Waterman MR and Scherbaum WA (1991) Adrenaline stimulates cholesterol side chain cleavage cytochrome P450 mRNA accumulation in bovine adrenocortical cells. *J Endocrinol* **131:**R5–R8.
Erdmann B, Gerst H, Bülow H, Lenz D, Bähr V and Bernhardt R (1995) Zone-specific localization of cytochrome P45011B1 in human adrenal tissue by PCR-derived riboprobes. *Histochem Cell Biol* **104:**301–307.
Erdmann B, Gerst H, Lippoldt A, Bülow H, Ganten D, Fuxe K and Bernhardt R (1996) Expression of cytochrome P45011B1 mRNA in the brain of normal and hypertensive transgenic rats. *Brain Res* **733:**73–82.
Ferrero P, Santi MR, Conti-Tronconi B, Costa E and Guidotti A (1986) Study of an octadecaneuropeptide derived from diazepam binding inhibitor (DBI): Biological activity and presence in rat brain. *Proc Natl Acad Sci USA* **83:**827–831.
Ffrench-Müllen JMH, Danks P and Spence KT (1994) Neurosteroids modulate calcium currents in hippocampal CA1 neurons via a pertussis toxin-sensitive G-protein-coupled mechanism. *J Neurosci* **14:**1963–1977.
Ffrench-Müllen JMH and Spence KT (1991) Neurosteroids block Ca^{2+} channel currents in freshly isolated hippocampal CA1 neurons. *Eur J Pharmacol* **202:**269–272.
Foidart A, Harada N and Balthazart J (1995) Aromatase-immunoreactive cells are present in the mouse brain areas that are known to express high levels of aromatase activity. *Cell Tissue Res* **280:**561–574.
Fomitcheva J, Baker ME, Anderson E, Lee GY and Aziz N (1998) Characterization of Ke 6, a new 17β-hydroxysteroid dehydrogenase, and its expression in gonadal tissues. *J Biol Chem* **273:**22664–22671.
Fournet-Dulguerov N, MacLusky NJ, Leranth CZ, Todd R, Mendelson CR, Simpson ER and Naftolin F (1987) Immunocytochemical localization of aromatase cytochrome P450 and estradiol in the syncytiotrophoblast of the human placenta. *J Clin Endocrinol Metab* **65:**757–764.
Fritz IB, Griswold MD, Louis BF and Dorrington JH (1976) Similarity of responses of cultured Sertoli cells to cholera toxin and FSH. *Mol Cell Endocrinol* **5:**294–298.
Geissler WM, Davis DL, Wu L, Bradshaw KD, Sushma P, Mendonca BB, Elliston KO, Wilson JD, Russell DW and Andersson S (1994) Male pseudohermaphroditism caused by mutations of testicular 17β-hydroxysteroid dehydrogenase 3. *Nature (London)* **7:**34–39.
Ghersevich S, Nokelainen P, Poutanen M, Orova M, Autio-Harmainen H, Rajaniemi H and Vihko R (1994) Rat 17β-hydroxysteroid dehydrogenase type 1: Primary structure and regulation of enzyme expression in rat ovary by diethylstilbestrol and gonadotropins *in vivo*. *Endocrinology* **135:**1477–1487.
Gomez-Sanchez CE, Zhou MY, Cozza EN, Morita H, Foecking, MF and Gomez-Sanchez EP (1997) Aldosterone biosynthesis in the rat brain. *Endocrinology* **138:**3369–3373.
Grazzini E, Guillon G, Mouillac B and Zingg HH (1998) Inhibition of oxytocin receptor function by direct binding of progesterone. *Nature (London)* **392:**509–512.
Green PS, Bishop J and Simpkins JW (1997) 17α-Estradiol exerts neuroprotective effects on SK-N-SH cells. *J Neurosci* **17:**511–515.
Grosser BI and Axelrod LR (1968) Conversion of cortisol to cortisol acetate, cortisone acetate and cortisone by the developing primate brain. *Steroids* **11:**827–836.
Guarneri P, Papadopoulos V, Pan B and Costa E (1992) Regulation of pregnenolone synthesis in C6B glioma cells by 4′-chlorodiazepam. *Proc Natl Acad Sci USA* **89:**5118–5122.
Guennoun R, Fiddes RJ, Gouézou M, Lombes M and Baulieu EE (1995) A key enzyme in the biosynthesis of neurosteroids, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase (3β-HSD), is expressed in rat brain. *Mol Brain Res* **30:**287–300.
Guennoun R, Schumacher M, Robert F, Delespierre B, Gouézou M, Eychenne B, Akwa Y, Robel P and Baulieu EE (1997) Neurosteroids: Expression of functional 3β-hydroxysteroid dehydrogenase by rat sensory neurons and Schwann cells. *Eur J Neurosci* **9:**2236–2247.
Guérin SL, Leclerc S, Verreault H, Labrie F and Luu-The V (1995) Overlapping cis-acting elements located in the first intron of the gene for type I 3β-hydroxysteroid dehydrogenase modulates its transcriptional activity. *Mol Endocrinol* **9:**1583–1597.
Güse-Behling H, Ehrhart-Bornstein M, Bornstein SR, Waterman MR, Scherbaum WA and Adler G (1992) Regulation of adrenal steroidogenesis by adrenaline: Expression of cytochrome P450 genes. *J Endocrinol* **135:**229–237.
Haidan A, Bornstein SR, Glasow A, Uhlmann K, Lübke C and Ehrhart-Bornstein M (1998) Basal steroidogenic activity of adrenocortical cells is increased 10-fold by coculture with chromaffin cells. *Endocrinology* **139:**772–780.
Harada N (1988) Cloning of a comparative cDNA encoding human aromatase: immunochemical identification and sequence analysis. *Biochem Biophys Res Commun* **156:**725–732.
Harada N, Yamada K, Foidart A and Balthazart J (1992) Regulation of aromatase cytochrome P-450 (estrogen synthetase) transcripts in the quail brain by testosterone. *Mol Brain Res* **15:**19–26.
Harada N, Yamada K, Saitoh K, Kibe N, Dohmae S and Takagi Y (1990) Structural characterization of the human estrogen synthetase (aromatase) gene. *Biochem Biophys Res Commun* **166:**365–372.
Hatakeyama H, Miyamori I, Fujita T, Takeda R and Yamamoto H (1994) Vascular androsterone. Biosynthesis and a link to angiotensin II-induced hypertrophy of vascular smooth muscle cells. *J Biol Chem* **269:**24316–24320.
Hickey GT, Krasnow JS, Beattie WG and Richards JS (1990) Aromatase cytochrome P450 in rat ovarian granulosa cells before and after luteinization: Adenosine 3,5-monophosphate-dependent and independent regulation. Cloning and sequencing of rat aromatase cDNA and 5 genomic DNA. *Mol Endocrinol* **4:**3–12.
Hinshelwood MM, Corbin CJ, Tsang PCW and Simpson ER (1993) Isolation and characterization of a cDNA insert encoding bovine aromatase cytochrome P450. *Endocrinology* **133:**1971–1977.
Ho MM and Vinson GP (1993) 11β-hydroxylase gene expression in the rat adrenal cortex. *J Endocrinol* **139:**301–306.
Ikeda Y, Shen WH, Ingraham HA and Parker KL (1994) Developmental expression of mouse steroidogenic factor-1, an essential regulator of the steroid hydroxylases. *Mol Endocrinol* **8:**654–662.
Inano H and Tamaoki BI (1971) Regulation of testosterone biosynthesis in rat testis by 7α-hydroxylated C19-steroids. *Biochim Biophys Acta* **239:**482–493.
Iwamori M, Moser HW and Kishimoto Y (1976) Steroid sulfate in brain: Comparison of sulfohydroxylase activities for various steroid sulfates in normal and pathological brains, including the various forms of metachromatic leukodystrophy. *J Neurochem* **27:**1389–1395.
Jakab RL, Harada N and Naftolin F (1994) Aromatase -(estrogen synthetase)-immunoreactive neurons in the rat septal area. A light and electron microscopic study. *Brain Res* **664:**85–93.
Jakab RL, Horvath TL, Leranth C, Harada N and Naftolin F (1993) Aromatase immunoreactivity in the rat brain: Gonadectomy-sensitive neurons and unresponsive "limbic ring" of the lateral septum-bed nucleus-amygdala complex. *J Steroid Biochem Mol Biol* **44:**481–498.
Jantus-Lewintre E, Orava M and Vihko R (1994) Regulation of 17β-hydroxysteroid dehydrogenase type 1 by epidermal growth factor and transforming growth factor alpha in choriocarcinoma cells. *Endocrinology* **135:**2624–2629.
Jelinek DF, Andersson S, Slaughter CA and Russel DW (1990) Cloning and regulation of cholesterol 7 alpha-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis. *J Biol Chem* **265:**8190–8197.
Jenkins EP, Andersson S, Imperato-McGinley J, Wilson JD and Russell DW (1992) Genetic and pharmacological evidence for more than one human steroid 5α-reductase. *J Clin Invest* **89:**293–300.
Jo DH, Abdallah MA, Young J, Baulieu EE and Robel P (1989) Pregnenolone, dehydroepiandrosterone and their sulfate and fatty acid esters in the rat brain. *Steroids* **54:**287–297.
Jones KJ (1993) Gonadal steroids and neuronal regeneration. A therapeutic role. *Adv Neurol* **59:**227–297.
Jung-Testas I, Hu ZY, Baulieu EE and Robel P (1989) Neurosteroids: Biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. *Endocrinology* **125:**2083–2091.
Kabbadj K, El-Etr M, Baulieu EE and Robel P (1993) Pregnenolone metabolism in rodent embryonic neurons and astrocytes. *Glia* **7:**170–175.
Kalra SP, Sahu A, Kalra PS and Crowley WR (1990) Hypothalamic neuropeptide Y: A circuit in the regulation of gonadotropin secretion and feeding behavior. *Ann NY Acad Sci* **611:**273–283.
Kawata M, McCabe JT, Chung SK, Dutt A, Yuri K, Hirakawa M, Kumamoto K, Hirayama Y and Pfaff DW (1991) The effect of progesterone on oxytocin messenger RNA in hypothalamic neurons of estrogen-treated female rats studied with quantitative *in situ* hydridization histochemistry. *Biomed Res* **12:**405–415.
Khanna M, Qin KN and Cheng KC (1995a) Distribution of 3 alpha-hydroxysteroid dehydrogenase in rat brain and molecular cloning of multiple cDNAs encoding structurally related proteins in humans. *J Steroid Biochem Mol Biol* **53:**41–46.
Khanna M, Qin KN, Wang RW and Cheng KC (1995b) Substrate specificity, gene structure, and tissue-specific distribution of multiple human 3α-hydroxysteroid dehydrogenases. *J Biol Chem* **270:**20162–20168.
Kimonides VG, Khatibi NH, Svendsen CN, Sofroniew MV and Herbert J (1998) Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. *Proc Natl Acad Sci USA* **95:**1852–1857.
King JA and Millar RP (1992) Evolution of gonadotropin-releasing hormones. *Trends Endocrinol Metab* **9:**339–346.
Kishimoto Y and Sostek R (1972) Activity of sterol-sulphate sulphohydroxylase in rat brain: Characterization, localization and change with age. *J Neurochem* **19:**123–130.
Klangkalya B and Chan A (1988) Structure-activity relationships of steroid hormones on muscarinic receptor binding. *J Steroid Biochem* **29:**111–118.
Knapstein P, David A, Wu C, Archer DF, Flickinger GL and Touchstone JC (1968) Metabolism of free and sulfoconjugated DHEA in brain tissue *in vivo* and *in vitro*. *Steroids* **11:**885–896.
Korneyev A, Pan BS, Polo A, Romeo E and Costa E (1993) Stimulation of brain pregnenolone synthesis by mitochondrial diazepam-binding inhibitor receptor ligands *in vivo*. *J Neurochem* **61:**1515–1524.
Krieger NR and Scott RG (1984) 3α-Hydroxysteroid oxidoreductase in rat brain. *J Neurochem* **42:**887–890.
Krieger NR and Scott RG (1989) Nonneuronal localization for steroid converting enzyme 3α-hydroxysteroid oxidoreductase in olfactory tubercle of rat brain. *J Neurochem* **52:**1866–1870.
Krueger KE and Papadopoulos V (1990) Peripheral-type benzodiazepine receptors mediate translocation of cholesterol from outer to inner mitochondrial membrane in adrenocortical cells. *J Biol Chem* **265:**15015–15022.
Labrie F, Luu-The V, Lin SX, Labrie C, Simard J, Breton R and Bélanger A (1997) The key role of 17β-hydroxysteroid dehydrogenases in sex steroid biology. *Steroids* **62:**148–158.
Labrie F, Simard J, Luu-The V, Pelletier G and Bélanger A (1992) Cloning, expression and regulation of tissue-specific expression of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase, in *Cellular and Molecular Biology of the Adrenal Cortex* (Saez JM, Brownie AC, Capponi EM, Chambaz E and Mantero F eds) vol 222, pp 89–109, London, INSERM/J Libbey.
Labrie F, Simard J, Luu-The V, Pelletier G, Belghmi K and Bélanger A (1994) Structure, regulation and role of 3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase and aromatase enzymes in the formation of sex steroids in classical and peripheral intracrine tissues. *Bailière's Clin Endocrinol Metab* **8:**451–474.
Lachance Y, Luu-The V, Verreault H, Dumont M, Rhéaume E, Leblanc G and Labrie F (1991) Structure of the human type II 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase (3β-HSD) gene: adrenal and gonadal specificity. *DNA Cell Biol* **10:**701–711.

Lakshmi V, Sakai RR, McEwen BS and Monder C (1991) Regional distribution of 11β-hydroxysteroid dehydrogenase in rat brain. *Endocrinology* **128:**1741–1748.
Lamacz M, Tonon MC, Smih-Rouet F, Patte C, Gasque P, Fontaine M and Vaudry H (1996) The endogenous benzodiazepine receptor ligand ODN increases cytosolic calcium in cultured rat astrocytes. *Mol Brain Res* **37:**290–296.
Leenders F, Adamski J, Husen B, Thole HH and Jungblut PW (1994a) Molecular cloning and amino acid sequence of the porcine 17β-estradiol dehydrogenase. *Eur J Biochem* **222:**221–227.
Leenders F, Husen B, Thole HH and Adamski J (1994b) The sequence of porcine 80 kDa 17β-hydroxysteroid dehydrogenase reveals similarities to the short chain alcohol dehydrogenase family, to actin binding motifs and to sterol carrier protein 2. *Mol Cell Endocrinol* **104:**127–131.
Le Goascogne C, Robel P, Gouézou M, Sananès N, Baulieu EE and Waterman M (1987) Neurosteroids: cytochrome P450scc in rat brain. *Science (Wash DC)* **237:** 1212–1215.
Le Goascogne C, Sananès N, Eychenne B, Gouézou M, Baulieu EE and Robel P (1995) Androgen biosynthesis in the stomach: expression of cytochrome P450 17α-hydroxylase/17,20-lyase messenger ribonucleic acid and protein, and metabolism of pregnenolone and progesterone by parietal cells of the rat gastric mucosa. *Endocrinology* **136:**1744–1752.
Le Goascogne C, Sananès N, Gouézou M, Takemori S, Kominami S, Baulieu EE and Robel P (1991) Immunoreactive cytochrome P-450$_{17\alpha}$ in rat and guinea pig gonads, adrenal glands and brain. *J Reprod Fertil* **93:**609–622.
Lephart ED (1993) Brain 5α-reductase: Cellular, enzymatic, and molecular perspectives and implications for biological function. *Mol Cell Neurosci* **4:**473–484.
Lephart ED (1996) A review of brain aromatase cytochrome P450. *Brain Res Rev* **22:**1–26.
Lephart ED, Herbst MA and McPhaul MJ (1995) Characterization of aromatase cytochrome P-450 mRNA in rat perinatal brain, ovary, and a Leydig tumor cell line: Evidence for the existence of brain specific aromatase transcripts. *Endocrine* **3:**25–31.
Lephart ED, Simpson ER and Ojeda SR (1992) Effect of cyclic AMP and androgens on *in vitro* brain aromatase enzyme activity during development in the rat. *J Neuroendocrinol* **4:**29–36.
Lephart ED, Simpson ER and Trzeciak WH (1991) Rat adrenal 5α-reductase mRNA content and enzyme activity are sex hormone dependent. *J Mol Endocrinol* **6:**163–170.
Levallet J and Carreau S (1997) Expression *in vitro* du gène de l'aromatase dans les cellules testiculaires du rat. *C R Acad Sci (Paris)* **320:**123–129.
Li XM, Salido EC, Kitada K, Serikawa T, Ten PH and Shapiro LJ (1996) Cloning of the rat steroid sulfatase gene (Sts), a non-pseudoautosomal X-linked gene that undergoes X inactivation. *Mamm Genome* **7:**420–424.
Lihrmann I, Plaquevent JC, Tostivint H, Raijmakers R, Tonon MC, Conlon JM and Vaudry H (1994) Frog diazepam-binding inhibitor: peptide sequence, cDNA cloning, and expression in the brain. *Proc Natl Acad Sci USA* **91:**6899–6903.
Lin HK and Penning TM (1995) Cloning, sequencing, and functional analysis of the 5′-flanking region of the rat 3α-hydroxysteroid/dihydrodiol dehydrogenase gene. *Cancer Res* **55:**4105–4113.
Low SC, Chapman KE, Edwards CRW and Seckl JR (1994) Liver-type 11β-hydroxysteroid dehydrogenase cDNA encodes reductase not dehydrogenase activity in intact mammalian COS-7 cells. *J Mol Endocrinol* **13:**167–174.
Luu-The V, Lachance Y, Labrie C, Leblanc G, Thomas JL, Strickler RC and Labrie F (1989) Full length cDNA structure and deduced amino acid sequence of human 3β-hydroxy-5-ene steroid dehydrogenase. *Mol Endocrinol* **3:**1310–1312.
Luu-The V, Sugimoto Y, Puy L, Labrie Y, Lopez SI, Singh M and Labrie F (1994) Characterization, expression, and immunohistochemical localization of 5α-reductase in human skin. *J Invest Dermatol* **102:**221–226.
Majewska MD (1992) Neurosteroids: endogenous bimodal modulators of the GABA$_A$ receptor. Mechanism of action and physiological significance. *Prog Neurobiol* **38:** 379–395.
Malagon M, Vaudry H, Vallarino M, Gracia-Navarro F and Tonon MC (1992) Distribution and characterization of endozepine-like immunoreactivity in the central nervous system of the frog *Rana ridibunda*. *Peptides* **13:**99–107.
Malagon M, Vaudry H, Van Strien F, Pelletier G, Gracia-Navarro F and Tonon MC (1993) Ontogeny of diazepam-binding inhibitor-related peptides (endozepines) in the rat brain. *Neuroscience* **57:**777–786.
Martel C, Melner MH, Gagné D, Simard J and Labrie F (1994) Widespread tissue distribution of steroid sulfatase, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD), 17β-HSD, 5α-reductase and aromatase in the rhesus monkey. *Mol Cell Endocrinol* **104:**103–111.
Martel C, Rhéaume E, Takahashi M, Trudel C, Couet J, Luu-The V, Simard J and Labrie F (1992) Distribution of 17β-hydroxysteroid dehydrogenase gene expression and activity in rat and in human tissues. *J Steroid Biochem Mol Biol* **41:**597–603.
Martini L, Celotti F and Melcangi RC (1996) Testosterone and progesterone metabolism in the central nervous system: cellular localization and mechanism of control of the enzymes involved. *Cell Mol Neurobiol* **16:**271–282.
Mason JI (1993) The 3β-hydroxysteroid dehydrogenase gene family of enzymes. *Trends Endocrinol Metab* **4:**199–203.
Mason JI, Keeney DS, Bird IM, Rainey WE, Morohashi KI, Leers-Sucheta S and Melner MH (1997) The regulation of 3β-hydroxysteroid dehydrogenase expression. *Steroids* **62:**164–168.
McAllister JM and Hornsby PJ (1988) Dual regulation of 3 beta-hydroxysteroid dehydrogenase, 17 alpha-hydroxylase, and dehydroepiandrosterone sulfotransferase by adenosine 3′,5′-monophosphate and activators of protein kinase C in cultured human adrenocortical cells. *Endocrinology* **122:**2012–2018.
McEwen BS (1994) Endocrine effects on the brain and their relationship to behavior, in *Basic Neurochemistry* (Siegel GJ, Agranoff BW, Albers RW and Molinoff B eds) pp 1003–1023, Raven Press, New York.
McNatty KP, Baird DT, Bolton A, Chambers P, Corker CS and MacLean H (1976) Concentration of estrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. *J Endocrinol* **71:**77–85.
McPhaul MJ, Noble JF, Simpson ER, Mendelson CR and Wilson D (1988) The expression of a functional cDNA encoding the chicken cytochrome P450arom (aromatase) that catalyzes the formation of estrogen from androgen. *J Biol Chem* **263:**16358–16363.
Means GD, Mahendroo MS, Corbin CJ, Mathis MJ, Powell FE, Mendelson CR and Simpson ER (1989) Structural analysis of the gene encoding human aromatase cytochrome P-450, the enzyme responsible for estrogen biosynthesis. *J Biol Chem* **264:**19385–19391.
Melcangi RC, Celotti P, Castano P and Martini L (1993) Differential localization of the 5α-reductase and the 3α-hydroxysteroid dehydrogenase in neuronal and glial cultures. *Endocrinology* **132:**1252–1259.
Melcangi RC, Celotti F and Martini L (1994) Progesterone 5α-reduction in neuronal and in different types of glial cell cultures: type 1 and 2 astrocytes and oligodendrocytes. *Brain Res* **639:**202–206.
Mellon S and Deschepper CF (1993) Neurosteroid biosynthesis: genes for adrenal steroidogenic enzymes are expressed in the brain. *Brain Res* **629:**283–292.
Mellon-Nussbaum S and Hochberg RB (1980) Biosynthesis of lipoidal derivatives of pregnenolone and dehydroisoandrosterone by the adrenal. *J Biol Chem* **255:**5566–5572.
Mensah-Nyagan AG, Feuilloley M, Do-Régo JL, Marcual A, Lange C, Pelletier G and Vaudry H (1996a) *In vivo* and *in vitro* evidence for the biosynthesis of testosterone in the telencephalon of the female frog. *J Neurochem* **67:**413–422.
Mensah-Nyagan AG, Feuilloley M, Do-Régo JL, Marcual A, Lange C, Tonon MC, Pelletier G and Vaudry H (1996b) Localization of 17β-hydroxysteroid dehydrogenase and characterization of testosterone in the brain of the male frog. *Proc Natl Acad Sci USA* **93:**1423–1428.
Mensah-Nyagan AG, Feuilloley M, Dupont E, Do-Régo JL, Leboulenger F, Pelletier G and Vaudry H (1994) Immunocytochemical localization and biological activity of 3β-hydroxysteroid dehydrogenase in the central nervous system of the frog. *J Neurosci* **14:**7306–7318.
Miller WL (1988) Molecular biology of steroid hormone synthesis. *Endocr Rev* **9:**295–318.
Mitani F, Ogishima T, Miyatomo H and Ishimura Y (1995) Localization of P450$_{aldo}$ and P450$_{11\beta}$ in normal and regenerating rat adrenal cortex. *Endocr Res* **21:**413–423.
Miyabo S, Kishida S and Hisada T (1973) Metabolism and conjugation of cortisol by various dog tissue *in vitro*. *J Steroid Biochem* **4:**567–576.
Moisan MP, Edwards CRW and Seckl JR (1992) Differential promoter usage by the rat 11β-hydroxysteroid dehydrogenase gene. *Mol Endocrinol* **6:**1082–1087.
Moisan MP, Seckl JR and Edwards CRW (1990) 11β-Hydroxysteroid dehydrogenase bioactivity and messenger RNA expression in rat forebrain: Localization in hypothalamus, hippocampus and cortex. *Endocrinology* **127:**1450–1455.
Monder C, Stewart PM, Lakshmi V, Valentino R, Burt D and Edwards CRW (1989) Licorice inhibits corticosteroid 11β-dehydrogenase of rat kidney and liver: *In vivo* and *in vitro* studies. *Endocrinology* **125:**1046–1053.
Monnet FP, Mahe V, Robel P and Baulieu EE (1995) Neurosteroids via s receptors, modulate the [^{3}H]norepinephrine release evoked by *N*-methyl-D-aspartate in the rat hippocampus. *Proc Natl Acad Sci USA* **92:**3774–3778.
Moore CC, Brentano ST and Miller WL (1990) Human P450scc gene transcription is induced by cyclic AMP and repressed by 12-*O*-tetradecanoylphorbol-13-acetate and A23187 through independent *cis* elements. *Mol Cell Biol* **10:**6013–6023.
Moore FL, Lowry CA and Rose JD (1994) Steroid-neuropeptide interactions that control reproductive behaviors in an amphibian. *Psychoneuroendocrinology* **19:** 581–592.
Mowszowicz I, Berthauit I, Mestayer C, Wright F, Kutten F and Mauvais-Jarvis P (1995) 5-Alpha-reductases: Physiology and pathology. *Ann Endocrinol Paris* **56:** 555–559.
Naftolin F, Ryan KJ, Davies IJ, Reddy VV, Flore F, Petro Z, Kuhn M, White RJ, Takaota Y and Wolin L (1975) The formation of estrogens by central neuroendocrine tissues. *Recent Prog Horm Res* **31:**295–319.
Naftolin F, Ryan KJ and Petro Z (1972) Aromatization of androstenedione by the anterior hypothalamus of adult male and female rats. *Endocrinology* **90:**295–298.
Nakajin S, Shively J, Yuan P and Hall P (1981) Microsomal cytochrome P450 from neonatal pig testis: Two enzymatic activities (17alpha hydroxylase and C17, 20 lyase) associated with one protein. *Biochemistry* **20:**4037–4042.
Namiki M, Kitamura M, Buczko E and Dufau ML (1988) Rat testis P450$_{17\alpha}$ cDNA: The deduced amino acid sequence, expression and secondary structural configuration. *Biochem Biophys Res Commun* **157:**705–712.
Naseeruddin SA and Hornsby PJ (1990) Regulation of 11 beta- and 17 alpha-hydroxylases in cultured bovine adrenocortical cells: 3′,5′-cyclic adenosine monophosphate, insulin-like growth factor-I, and activators of protein kinase C. *Endocrinology* **127:**1673–1681.
Negri-Cesi P, Colciago A and Celotti F (1996a) The role of aromatase in the brain, in *The Brain: Source and Target for Sex Steroid Hormones* (Gennazzani AR, Petraglia AF and Purdy RH eds) pp 135–149, The Parthenon Publishing Group, London.
Negri-Cesi P, Poletti A and Celotti F (1996b) Metabolism of steroids in the brain: a new insight into the role of 5α-reductase and aromatase in brain differentiation and functions. *J Steroid Biochem Mol Biol* **58:**455–466.
Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez FJ, Coon MJ, Gunsalus IC, Gotoh O, Okuda K and Nebert DW (1993) The P450 superfamily: Update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA Cell Biol* **12:**1–51.
Nokelainen P, Peltoketo H, Vihko R and Vihko P (1998) Expression cloning of a novel estrogenic mouse 17β-hydroxysteroid dehydrogenase/17-ketosteroid reductase (m17HSD7), previously described as a prolactin receptor-associated protein (PRAP) in rat. *Mol Endocrinol* **12:**1048–1059.
Normand T, Husen B, Leenders F, Pelczar H, Baret JL, Begue A, Flourens AC, Adamski J and de Launoit Y (1995) Molecular characterization of mouse 17β-hydroxysteroid dehydrogenase IV. *J Steroid Biochem Mol Biol* **55:**541–548.

Normington K and Russell DW (1992) Tissue distribution and kinetic characteristics of rat steroid 5α-reductase isozymes. *J Biol Chem* **267:**19548–19554.
Noshiro M, Nishimoto M, Morohashi KI and Okuda K (1989) Molecular cloning of cDNA for cholesterol 7α-hydroxylase from rat liver microsomes. *FEBS Lett* **257:** 97–100.
Noshiro M and Okuda JY (1990) Molecular cloning and sequence analysis of cDNA encoding human cholesterol 7 alpha-hydroxylase. *FEBS Lett* **268:**137–140.
Ogishima T, Suzuki H, Hata J, Mitani F and Ishimura Y (1992) Zone-specific expression of aldosterone synthase cytochrome P-450 and cytochrome P-45011β in rat adrenal cortex: histochemical basis for the functional zonation. *Endocrinology* **130:**2971–2977.
Orchinik M, Murray TF, Franklin PH and Moore FL (1992) Guanyl nucleotides modulate binding to steroid receptors in neuronal membranes. *Proc Natl Acad Sci USA* **89:**3830–3834.
Ozaki HS, Iwahashi K, Tsubaki M, Fukui Y, Ichikawa Y and Takeuchi Y (1991) Cytochrome P45011β in rat brain. *J Neurosci Res* **28:**518–524.
Paden CM and Roselli CE (1987) Modulation of aromatase activity by testosterone in transplants of fetal rat hypothalamus-preoptic area. *Dev Brain Res* **33:**127–133.
Panzica GC, Viglietti-Panzica C and Balthazart J (1996) The sexually dimorphic medial preoptic nucleus of quail: A key brain area mediating steroid action on male sexual behavior. *Front Neuroendocrinol* **17:**51–125.
Papadopoulos V (1993) Peripheral-type benzodiazepine/diazepam-binding inhibitor receptor: Biological role in steroidogenic cell function. *Endocr Rev* **14:**222–240.
Papadopoulos V and Guarneri P (1994) Regulation of C6 glioma cell steroidogenesis by adenosine 3′,5′-cyclic monophosphate. *Glia* **10:**75–78.
Papadopoulos V, Guarneri P, Krueger KE, Guidotti A and Costa E (1992) Pregnenolone biosynthesis in C6–2B glioma cell mitochondria: Regulation by a mitochondrial diazepam-binding inhibitor receptor. *Proc Natl Acad Sci USA* **89:**5113–5117.
Papadopoulos V, Mukhin AG, Costa E and Krueger KE (1990) The peripheral-type benzodiazepine receptor is functionally linked to Leydig cell steroidogenesis. *J Biol Chem* **265:**3772–3779.
Park IH, Han BK and Jo DH (1997) Distribution and characterization of neurosteroid sulfatase from the bovine brain. *J Steroid Biochem Mol Biol* **62:**315–320.
Patchev VK, Hassan AHS, Holsboer F and Almeida OFX (1996) The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. *Neuropsychopharmacology* **15:**533–540.
Patte C, Gandolfo P, Leprince J, Thoumas JL, Fontaine M, Vaudry H and Tonon MC (1999) GABA inhibits endozepine release from cultured rat astrocytes. *Glia* **25:** 404–411.
Patte C, Vaudry H, Desrues L, Gandolfo P, Strijdveen I, Lamacz M and Tonon MC (1995) The endozepine ODN stimulates polyphosphoinositide metabolism in rat astrocytes. *FEBS Lett* **362:**106–110.
Pawlowski JE, Huizinga M and Penning TM (1991) Cloning and sequencing of the cDNA for rat liver 3α-hydroxysteroid/dihydrodiol dehydrogenase. *J Biol Chem* **266:**8820–8825.
Pelletier G, Liao N, Follea N and Govidan MV (1988) Mapping of estrogen receptor-producing cells in the rat by *in situ* hybridization. *Neurosci Lett* **94:**23–28.
Pelletier G, Luu-The V and Labrie F (1994) Immunocytochemical localization of 5α-reductase in the rat brain. *Mol Cell Neurosci* **5:**394–399.
Pelletier G, Luu-The V and Labrie F (1995) Immunocytochemical localization of type I 17β-hydroxysteroid dehydrogenase in the rat brain. *Brain Res* **704:**233–239.
Peltoketo H, Isomaa V, Poutanen M and Vihko R (1996) Expression and regulation of 17β-hydroxysteroid dehydrogenase type 1. *J Endocrinol* **150:**S21–S30.
Penning TM (1997) Molecular endocrinology of hydroxysteroid dehydrogenases. *Endocr Rev* **18:**281–305.
Penning TM, Pawlowski JE, Schlegel BP, Jez JM, Lin HK, Bennett MJ and Lewis M (1996) Mammalian 3α-hydroxysteroid dehydrogenases. *Steroids* **61:**508–523.
Phillips MD, Lakshmi V and Monder C (1989) Corticosteroid 11β-dehydrogenase in rat testis. *Endocrinology* **125:**209–216.
Piao YS, Peltoketo H, Oikarinen J and Vihko R (1995) Coordination of transcription of the human 17β-hydroxysteroid dehydrogenase type 1 gene (EDH17B2) by a cell-specific enhancer and a silencer: Identification of a retinoic acid response element. *Mol Endocrinol* **9:**1633–1644.
Puranen T, Poutanen M, Ghosh D, Vihko P and Vihko R (1997) Characterization of structural and functional properties of human 17β-hydroxysteroid dehydrogenase type 1 using recombinant enzymes and site-directed mutagenesis. *Mol Endocrinol* **11:**77–86.
Qin KN, New MI and Cheng KC (1993) Molecular cloning of multiple cDNAs encoding human enzymes structurally related to 3α-hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* **46:**673–679.
Rajan V, Chapman KE, Lyons V, Jamieson P, Mullins JJ, Edwards CRW and Seckl JR (1995) Cloning sequencing and tissue-distribution of mouse 11β-hydroxysteroid dehydrogenase-1 cDNA. *J Steroid Biochem Mol Biol* **52:**141–147.
Rajkowski KM, Robel P and Baulieu EE (1997) Hydroxysteroid sulfotransferase activity in the rat brain and liver as a function of age and sex. *Steroids* **62:**427–436.
Ramirez VD and Zheng J (1996) Membrane sex-steroid receptors in the brain. *Front Neuroendocrinol* **17:**402–439.
Reddy VVR (1979) Estrogen metabolism in neural tissues of rabbits: 17β-hydroxysteroid dehydrogenase oxidoreductase activity. *Steroids* **34:**207–215.
Resko JA, Stadelman HL and Norman RL (1979) 17β-hydroxysteroid dehydrogenase activity in the pituitary gland and neural tissue of rhesus monkey. *J Steroid Biochem* **11:**1429–1434.
Rhéaume E, Lachance Y, Zhao HF, De Launoit Y, Trudel C, Luu-The V, Simard J and Labrie F (1991) Structure and expression of a new cDNA encoding the almost exclusive 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase in human adrenals and gonads. *Mol Endocrinol* **5:**1147–1157.
Rhéaume E, Tonon MC, Smih F, Simard F, Desy L, Vaudry H and Pelletier G (1990) Localization of the endogenous benzodiazepine ligand octadecaneuropeptide in the rat testis. *Endocrinology* **127:**1986–1994.
Robel P and Baulieu EE (1985) Neuro-steroids: 3β-Hydroxy-Δ^5-derivates in the rodent brain. *Neurochem Int* **7:**953–958.
Robel P and Baulieu EE (1994) Neurosteroids: Biosynthesis and function. *Trends Endocrinol Metab* **5:**1–8.
Robel P, Bourreau E, Corpéchot C, Dang DC, Halberg F, Clarke C, Haug M, Sclegel ML, Synguelakis M, Vourc'h C and Baulieu EE (1987) Neurosteroids: 3β-Hydroxy-Δ^5-derivatives in rat and monkey brain. *J Steroid Biochem* **27:**649–655.
Robel P, Corpéchot C, Clarke C, Groyer A, Synguelakis M, Vourc'h C and Baulieu EE (1986) Neurosteroids: 3β-Hydroxy-Δ^5-derivatives in the rat brain, in *Neuroendocrine Molecular Biology* (Fink AJ, Harmar AJ and Mc Kerns KW eds) pp 367–377, Plenum Press, New York.
Robel P, Young J, Corpéchot C, Mayo W, Perche F, Haug M, Simon H and Baulieu EE (1995) Biosynthesis and assay of neurosteroids in rats and mice: functional correlates. *J Steroid Biochem Mol Biol* **53:**355–360.
Roland BL, Li KXZ and Funder JW (1995) Hybridization histochemical localization of 11β-hydroxysteroid dehydrogenase type 2 in rat brain. *Endocrinology* **136:**4697–4700.
Rose KA, Stapleton G, Dott K, Kieny MP, Best R, Schwarz M, Russel DW, Bjorkhem I, Seckl J and Lathe R (1997) Cyp7β, a novel brain cytochrome P450, catalyses the synthesis of neurosteroids 7α-hydroxy dehydroepiandrosterone and 7α-hydroxy pregnenolone. *Proc Natl Acad Sci USA* **94:**4925–4930.
Roselli CE, Abdelgadir SE, Jorgensen E and Resko JA (1996a) Sex differences in androgen-regulated cytochrome P450 aromatase mRNA in the rat brain. *Endocrine* **5:**59–65.
Roselli CE, Abdelgadir SE and Resko JA (1997) Regulation of aromatase gene expression in the adult rat brain. *Brain Res Bull* **44:**351–357.
Roselli CE and Fasasi TE (1992) Estradiol increases the duration of nuclear androgen receptor occupation in the preoptic area of male rat treated with dihydrotestosterone. *J Steroid Biochem Mol Biol* **42:**161–168.
Roselli CE, Horton LE and Resko JA (1985) Distribution and regulation of aromatase activity in the rat hypothalamus and limbic system. *Endocrinology* **117:**2471–2477.
Roselli CE, Klosterman SA and Fasasi TA (1996b) Sex differences in androgen responsiveness in the rat brain: Regional differences in the induction of aromatase activity. *Neuroendocrinology* **64:**139–145.
Roselli CE and Resko JA (1993) Aromatase activity in the rat brain: hormonal regulation and sex differences. *J Steroid Biochem Mol Biol* **44:**499–508.
Roselli CE, Stadelman H, Horton LE and Resko JA (1987) Regulation of androgen metabolism and luteinizing hormone-releasing content in discreet hypothalamic and limbic areas of male rhesus macaques. *Endocrinology* **120:**97–106.
Rouet-Smih F, Tonon MC, Pelletier G and Vaudry H (1992) Characterization of endozepine-related peptides in the central nervous system and in peripheral tissues of the rat. *Peptides* **13:**1219–1225.
Rusvai E and Naray-Fejes-Toth A (1993) A new isoform of 11β-hydroxysteroid dehydrogenase in aldosterone target cells. *J Biol Chem* **268:**10717–10720.
Saitoh H, Hirato K, Yanaihara T and Nakayama T (1982) A study of 5α-reductase in human fetal brain. *Endocrinol Jpn* **29:**461–467.
Salido EC, Li XM, Yen PH, Martin N, Mohandas TK and Shapiro LJ (1996) Cloning and expression of the mouse pseudoautosomal steroid sulphatase gene (Sts). *Nat Genet* **13:**83–86.
Sanghera MK, Simpson ER, McPhaul MJ, Kozlowski G, Conley AJ and Lephart ED (1991) Immunocytochemical distribution of aromatase cytochrome P450 in the rat brain using peptide-generated polyclonal antibodies. *Endocrinology* **129:**2834–2844.
Sapolsky RM (1996) Why is the stress bad for your brain? *Science (Wash DC)* **273:**749–750.
Schumacher M, Robel P and Baulieu EE (1996) Development and regeneration of the nervous system: A role for neurosteroids. *Dev Neurosci* **18:**6–21.
Seckl JR (1997) 11β-hydroxysteroid dehydrogenase in the brain: A novel regulator of glucocorticoid action. *Front Neuroendocrinol* **18:**49–99.
Seckl JR, Brown RW, Rajan V, Low SC and Edwards CRW (1993) 11β-Hydroxysteroid dehydrogenase and corticosteroid actions in the brain. *J Endocrinol* **137:**9.
Sherwood NM, Lovejoy DA and Coe IR (1993) Origin of mammalian gonadotropin-releasing hormones. *Endocr Rev* **14:**241–254.
Shinoda K, Shiotani Y and Osawa Y (1989a) "Necklace olfactory glomeruli" form unique components of the rat primary olfactory system. *J Comp Neurol* **284:**362–373.
Shinoda K, Yagi H, Fujita H, Osawa Y and Shiotani Y (1989b) Screening of aromatase containing-neurons in rat forebrain: An immunohistochemical study with antibody against human placental antigen X-P2 (hPAX-P2). *J Comp Neurol* **290:** 502–515.
Silvestre JS, Robert V, Heymes C, Aupetit-Faisant B, Mouas C, Moalic JM, Swynghedauw B and Delcayre C (1998) Myocardial production of aldosterone and corticosterone in the rat. Physiological regulation. *J Biol Chem* **273:**4883–4891.
Simard J, Durocher F, Mebarki F, Sanchez R, Labrie Y, Couet J, Trudel C, Rhéaume E, Morel Y, Luu-The V and Labrie F (1996) Molecular biology and genetics of the 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase gene family. *J Endocrinol* **150:** S189–S207.
Simpson ER, Merrill JC, Hollub AJ, Graham-Lorence S and Mendelson CR (1989) Regulation of estrogen biosynthesis by human adipose cells. *Endocr Rev* **10:**136–148.
Slobodyansky E, Antkiewicz-Michaluk L and Martin B (1994) Purification of a novel DBI processing product, DBI_{39-75}, and characterization of its binding site in rat brain. *Regul Pept* **50:**29–35.
Slobodyansky E, Kurriger G and Kultas-Ilinsky K (1992) Diazepam binding inhibitor processing in the rhesus monkey brain: an immunocytochemical study. *J Chem Neuroanat* **5:**169–180.
Stapleton G, Steel, M, Richardson M, Mason JO, Rose KA, Morris RGM and Lathe R (1995) A novel cytochrome P450 expressed primarily in brain. *J Biol Chem* **270:**29739–29745.

Strömstedt M and Waterman MR (1995) Messenger RNAs encoding steroidogenic enzymes are expressed in rodent brain. *Mol Brain Res* **34:**75–88.
Strott C (1996) Steroid sulfotransferases. *Endocr Rev* **17:**670–697.
Tanaka M, Telecky TM, Fukada S, Adachi S, Chen S and Nagahama Y (1992) Cloning and sequence analysis of the cDNA encoding P450 aromatase (P450arom) from a rainbow trout (*Oncorhynchus mykiss*) ovary; relationship between the amount of P450arom mRNA and the production of oestradiol-17β in the ovary. *J Mol Endocrinol* **8:**53–61.
Tannin GM, Agarwal AK, Monder C, New MI and White PC (1991) The human gene for 11β-hydroxysteroid dehydrogenase. *J Biol Chem* **266:**16653–16658.
Terashima M, Toda K, Kawamoto T, Kuribayashi I, Ogawa Y, Maeda T and Shizuta Y (1991) Isolation of a full-length cDNA encoding mouse aromatase P450. *Arch Biochem Biophys* **285:**231–237.
Thigpen AE, Silver RI, Guileyardo JM, Casey ML McConnell JD and Russell DW (1993) Tissue distribution and ontogeny of steroid 5α-reductase isozyme expression. *J Clin Invest* **92:**903–910.
Toda K, Terashima M, Kawamoto T, Sumimoto H, Yokoyama Y, Kuribayashi I, Mitsuuchi Y, Maeda T, Yamamoto Y, Sagara Y, Ikeda H and Shizuta Y (1990) Structural and functional characterization of human aromatase P450 gene. *Eur J Biochem* **193:**559–565.
Tong Y, Toranzo D and Pelletier G (1991) Localization of diazepam-binding inhibitor (DBI) mRNA in the rat brain by high resolution *in situ* hybridization. *Neuropeptides* **20:**433–443.
Tonon MC, Smih-Rouet F, Lamacz M, Louiset E, Pelletier G and Vaudry H (1994) Endozepines: endogenous ligands for benzodiazepine receptors. *Med/Sci* **10:**433–443.
Toranzo D, Tong Y, Tonon MC, Vaudry H and Pelletier G (1994) Localization of diazepam-binding inhibitor and peripheral type benzodiazepine binding sites in the rat ovary. *Anat Embryol* **979:**383–388.
Torran-Allerand CD, Miranda RC, Hochberg RB and MacLusky NJ (1992) Cellular variations in estrogen receptor mRNA translation in the developing brain: Evidence from combined [^{125}I]estrogen autoradiography and non-isotopic *in situ* hybridization histochemistry. *Brain Res* **576:**25–41.
Tremblay Y and Beaudouin C (1993) Regulation of 3 beta-hydroxysteroid dehydrogenase and 17 beta-hydroxysteroid dehydrogenase messenger ribonucleic acid levels by cylic adenosine 3′, 5′-monophosphate and phorbol myristate acetate in human choriocarcinoma cells. *Mol Endocrinol* **7:**355–364.
Tsuruo Y, Ishimura K, Fujita H and Osawa Y (1994) Immunocytochemical localization of aromatase-containing neurons in the rat brain during pre- and postnatal development. *Cell Tissue Res* **278:**29–39.
Tsuruo Y, Miyamoto T, Yokoi H, Kitagawa K, Futaki S and Ishimura K (1996) Immunohistochemical presence of 5α-reductase rat type 1-containing cells in rat brain. *Brain Res* **722:**207–211.
Tsutsui K and Yamazaki T (1995) Avian neurosteroids. 1. Pregnenolone biosynthesis in the quail brain. *Brain Res* **678:**1–9.
Ukena K, Usui M, Kohchi C and Tsutsui K (1998) Cyotchrome P450 side-chain cleavage enzyme in the cerebellar purkinje neuron and its neonatal change in rats. *Endocrinology* **139:**137–147.
Uno H, Tarara R, Else JG, Suleman MA and Sapolsky R (1989) Hippocampal damage associated with prolonged and fatal stress in primates. *J Neurosci* **9:**1705–1711.
Usui M, Yamazaki T, Kominami S and Tsutsui K (1995) Avian neurosteroids. 2. Localization of a cytochrome P450scc-like substance in the quail brain. *Brain Res* **678:**10–20.
Valera S, Ballivet M and Bertrand D (1992) Progesterone modulates a neuronal nicotinic acetylcholine receptor. *Proc Natl Acad Sci USA* **89:**9949–9953.
Valladares LE and Payne AH (1979) Induction of testicular aromatization by luteinizing hormone in mature rats. *Endocrinology* **105:**431–436.
Vanson A, Arnold AP and Schlinger BA (1996) 3β-hydroxysteroid dehydrogenase/isomerase and aromatase activity in primary cultures of developing zebra finch telencephalon: dehydroepiandrosterone as substrate for synthesis of androstenedione and estrogens. *Gen Comp Endocrinol* **102:**342–350.
Vourc'h C, Eychenne B, Jo DH, Raulin J, Lapous D, Baulieu EE and Robel P (1992) Δ^5-3β-hydroxysteroid acyl transferase activity in the rat brain. *Steroids* **57:**210–214.
Warner M, Strömstedt M, Moller L and Gustafsson G (1989) Distribution and regulation of 5α-androstane-3β, 17β-diol hydroxylase in the rat central nervous system. *Endocrinology* **124:**2699–2706.
Watanabe N, Inoue H and Fujii-Kuriyama Y (1994) Regulatory mechanisms of cAMP-dependent and cell-specific expression of human steroidogenic cytochrome P450scc (CYP11A1) gene. *Eur J Biochem* **222:**825–834.
Weaver CE Jr, Marek P, Park-Chung M, Tam SW and Farb DK (1997) Neuroprotective activity of a new class of steroidal inhibitors of the *N*-methyl-D-aspartate receptor. *Proc Natl Acad Sci USA* **94:**10450–10454.
Webb EC (1992) *Enzyme Nomenclature* (Webb EC ed), pp 299–303, Academic Press, New York.
Wehrenberg WB, Corder R and Gaillard RC (1989) A physiological role for neuropeptide Y in regulating the estrogen/progesterone induced luteinizing hormone surge in ovariectomized rats. *Neuroendocrinology* **49:**680–682.
Weidenfield J, Sziegel RA and Chowers I (1980) *In vitro* conversion of pregnenolone to progesterone by discrete brain areas of the male rat. *J Steroid Biochem* **13:**961–963.
Wilson JD (1975) Metabolism of testicular androgens, in *Handbook of Physiology* (Greep RO and Astwood EB eds) pp 491–508, American Physiological Society, Washington, DC.
Winslow JT, Hastings N, Carter CS, Harbaugh CR and Insel TR (1993) A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature (London)* **365:**545–548.
Wu FS, Gibbs TT and Farb DH (1991) Pregnenolone sulfate: a positive allosteric modulator at the *N*-methyl-D-aspartate receptor. *Mol Pharmacol* **40:**333–336.
Yabu M, Senda T, Nonaka Y, Matsukawa N, Okamoto M and Fujita H (1991) Localization of the gene transcripts of 11β-hydroxylase and aldosterone synthase in the rat adrenal cortex by *in situ* hybridization. *Histochemistry* **96:**391–394.
Yang K, Smith CL, Dales D, Hammond GL and Challis JR (1992) Cloning of an ovine 11 beta-hydroxysteroid dehydrogenase complementary deoxyribonucleic acid: Tissue and temporal distribution of its messenger ribonucleic acid during fetal and neonatal development. *Endocrinology* **131:**2120–2126.
Yu WH (1989) Survival of motoneurons following axotomy is enhanced by lactation or by progesterone treatment. *Brain Res* **491:**379–382.
Yuan H, Bowlby DA, Brown TJ, Hochberg RB and MacLusky NJ (1995) Distribution of occupied and unoccupied estrogen receptors in the rat brain: Effects of physiological gonadal steroid exposure. *Endocrinology* **136:**96–105.
Zavala F and Lenfant M (1987) Peripheral benzodiazepines enhance the respiratory burst of macrophage-like P 388D_1 cells stimulated by arachidonic acid. *Int J Immunopharmacol* **9:**269–274.
Zhang P, Rodriguez H and Mellon S (1995) Transcriptional regulation of P450scc gene expression in neural and steroidogenic cells: implication for regulation for neurosteroidogenesis. *Mol Endocrinol* **9:**1571–1582.
Zhao HF, Labrie C, Simard J, de Launoit Y, Trudel C, Martel C, Rhéaume E, Dupont E, Luu-The V, Pelletier G and Labrie L (1991) Characterization of 3β-hydroxysteroid dehydrogenase Δ^5-Δ^4 isomerase cDNA and differential tissue-specific expression of the corresponding mRNAs in steroidogenic and peripheral tissues. *J Biol Chem* **266:**583–593.
Zhao HF, Rhéaume E, Trudel C, Couet J, Labrie F and Simard J (1990) Structure and sexual dimorphic expression of a liver-specific rat 3β-hydroxysteroid dehydrogenase/isomerase. *Endocrinology* **127:**3237–3239.
Zhou MY, Gomez-Sanchez EP, Cosby D and Gomez-Sanchez CE (1995) Cloning of a NAD^+-dependent 11β-hydroxysteroid dehydrogenase from rat kidney. *Endocr Soc Abstr* **77:**413.