Cell. Mol. Life Sci. 65 (2008) 777 – 797
1420-682X/08/050777-21
DOI 10.1007/s00018-007-7403-5


Cellular and Molecular Life Sciences

# Review

# Neuroactive steroids: State of the art and new perspectives

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Received 3 September 2007; received after revision 24 October 2007; accepted 29 October 2007
Online First 26 November 2007

**Abstract.** Neuroactive steroids include synthetic steroidal compounds and endogenous steroids, produced by endocrine glands (hormonal steroids) or the nervous tissue (neurosteroids), which regulate neural functions. These steroids bind to nuclear receptors or act through the activation of membrane-associated signaling pathways to modulate various important processes including the development of the nervous system, neural plasticity and the adaptive responses of neurons and glial cells under pathological conditions. Reviewed and updated in the present paper are the pleiotropic and protective abilities of neuroactive steroids. The fundamental evidence and knowledge gained constitute a profound background that offers interesting possibilities for developing effective strategies against several disorders of the nervous system.

**Keywords.** Steroidogenesis, neuroprotection, neurosteroids, myelin, brain, spinal cord, mitochondria, glia.

## Introduction

The nervous system is not only a target for endocrine effects exerted by hormonal steroids released by peripheral steroidogenic tissues, but is also controlled in a paracrine or autocrine manner by neurosteroids, which are steroids directly synthesized by neurons and glial cells [1]. In addition to endogenous steroids (hormonal steroids and neurosteroids) naturally produced in the body, several exogenous or synthetic steroids also have the ability to regulate the activity of the nervous system. It appears that a wide variety of steroids, acting through different mechanisms, exert a large array of biological effects on the nervous system. Consequently, the term neuroactive steroids [2] is used to designate all steroids that are able to regulate neural functions, including hormonal steroids, neurosteroids and synthetic steroids. Over the two past decades, neuroactive steroids have received a great amount of attention because of their capacity to control both homeostatic parameters and crucial pathophysiological mechanisms such as neurodegenerative processes and signaling pathways involved in neuronal cell death [3–6]. Thus, many recent works have raised a great hope for the therapeutic exploitation of neuroactive steroids to counteract neurodegenerative events. Reviewed and updated here are the most pertinent data on the effects and the mechanisms of action of neuroactive steroids in the central (CNS) and peripheral (PNS) nervous systems. This review also critically analyzed the possible clinical relevance of promising results obtained with neuroactive steroids in various experimental models reproducing different neuropathological symptoms similar to those observed in humans. We initially

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analyze the effects of hormonal steroids in the CNS and PNS and then review the actions and functional relevance of neurosteroids. Table 1 shows a summary of the neuroactive steroids mentioned in this paper with their chemical nomenclature.

**Table 1.** Neuroactive steroids mentioned in the text with their chemical nomenclature

| Endogenous steroids | Chemical name |
|---|---|
| 3α-Androstanediol | 5α-Androstane-3α,17β-diol |
| Androstenedione | 4-Androstene-3,17-dione |
| Corticosterone | 4-Pregnen-11β,21-diol-3,20-dione |
| Cortisol | 4-Pregnen-11β,17α,21-triol-3,20-dione |
| Dehydroepiandrosterone | 5-Androsten-3β-ol-17-one |
| 11-Deoxycorticosterone | 4-Pregnen-21-ol-3,20-dione |
| Dihydrodeoxycorticosterone | 1,4-Pregnadien-21-ol-3,20-dione |
| Dihydroprogesterone | 5α-Pregnan-3,20-dione |
| Dihydrotestosterone | 5α-Androstan-17β-ol-3-one |
| Estradiol | 1,3,5(10)-Estratrien-3,17β-diol |
| Estrone | 1,3,5(10)-Estratrien-3-ol-17-one |
| 17-hydroxy-pregnenolone | 5-Pregnen-3β,17-diol-20-one |
| 17-hydroxy-progesterone | 4-Pregnen-17α-ol-3,20-dione |
| Pregnenolone | 5-Pregnen-3β-ol-20-one |
| Progesterone | 4-Pregnen-3,20-dione |
| Testosterone | 4-Androsten-17β-ol-3-one |
| Tetrahydrodeoxycorticosterone (allotetrahydrodeoxycorticosterone) | 5α-Pregnan-3α,21-diol-20-one |
| Tetrahydroprogesterone (allopregnanolone) | 5α-Pregnan-3α-ol-20-one |
| **Synthetic steroids** | **Chemical name** |
| Dexamethasone | 1,4-Pregnadien-9α-fluoro-16α-methyl-11β,17α,21-triol-3,20-dione |

## Actions of hormonal steroids in the nervous system

**Actions of hormonal steroids in the CNS.** The classical action of hormonal steroids in the nervous system is *via* the activation of nuclear receptors. However, hormonal steroids are also able to activate different membrane or cytoplasmic signaling pathways (Fig. 1). Therefore, the hormonal action is the result of a combination of rapid signaling and transcriptional regulation [7, 8]. Gonadal and stress steroids affect neural development and the function of the nervous system in adult life. Gonadal steroids, acting on specific moments during fetal or early postnatal development, induce the generation of sexual dimorphisms in the nervous system. The main hormone implicated is testosterone, which is locally metabolized by neural tissue to estradiol or dihydrotestosterone (DHT) by the enzymes aromatase and 5α-reductase (5α-R), respectively (Fig. 2). Estradiol is a ligand of estrogen receptors (ER) and DHT a ligand of androgen receptor (AR) and both receptors are involved in the organizational effects of testosterone in the nervous system. The organizational effects of testosterone and its metabolites generate male-specific traits in specific regions of the brain and spinal cord, resulting in differences in the morphology, size and number of neurons and glial cells, the density of neuronal and glial processes in the neuropil and the number of synapses between males and females (see [9–12] for reviews). Gonadal steroids may promote sex differences in synaptic connectivity by regulating microtubule assembly in neuronal processes, one of the key events involved in neurite elongation. Microtubule-associated proteins are known to promote tubulin polymerization or microtubule stability during active process extension. Interestingly, the effects of estradiol on the growth of neurites *in vitro* are paralleled by an increase in the expression of microtubule-associated proteins, such as Tau or microtubule-associated protein 2 [13, 14]. In addition, estradiol and progesterone (PROG) regulate the expression of microtubule-associated protein 2 in the brain *in vivo* [15], and testosterone [16], estradiol [17, 18] and PROG [19] regulate Tau phosphorylation, which is essential for its association with axonal microtubules and the regulation of axonal growth. Estradiol also regulates the interaction of Tau with neurotransmitter receptors [20]. All these hormonal effects may be involved in the regulation of neuritic growth and synaptic plasticity.

Actions of gonadal steroids on glial cells may also be highly relevant for the sexual differentiation of neuronal connectivity [21]. The morphology, immunoreactivity, enzymatic activity and gene expression of astroglia are sexually dimorphic in several brain areas and can be modified by postnatal actions of gonadal steroids [21]. Furthermore, glial cells express receptors for gonadal steroids and participate in steroid metabolism [21]. These cells also participate in the synthesis of endogenous steroids by the nervous system (see below). Considering the close morphological and functional relationships between glial cells and neurons, it is obvious that hormonal effects on astroglia during the development of the nervous system may have important functional consequences. Several steps during the genesis of sexually dimorphic neuronal networks could conceivably be regulated by glial cells, including the proliferation, survival, migration and functional maturation of neurons. Hormonal modulation of glial cell morphology may be involved in the estab-

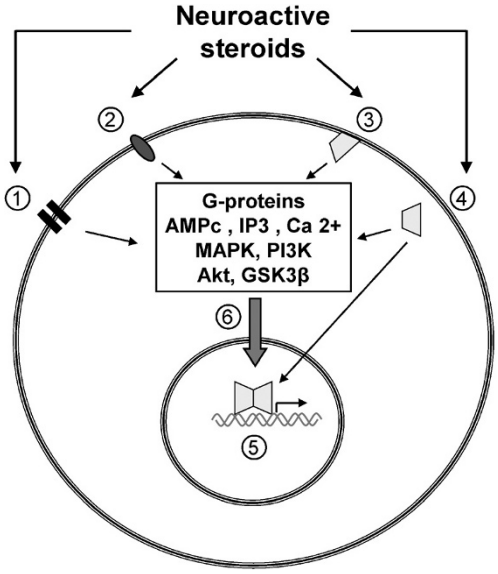


**Figure 1.** Different potential mechanisms of action of neuroactive steroids, including hormonal steroids, in the nervous system. Neuroactive steroids may bind to ion channels associated to neurotransmitter receptors (1), to putative steroid receptors in the plasma membrane (2), to classical nuclear steroid receptors associated to the plasma membrane (3) or to classical nuclear steroid receptors located in the cytoplasm (4). These membrane and cytoplasmic-associated actions of neuroactive steroids regulate intracellular signaling *via* the activation of G-proteins, the modulation of intracellular levels of AMPc, inositol 1,4,5-trisphoshate (IP3) and $Ca^{2+}$ and the activity of kinases such as mitogen-activated protein kinase (MAPK), phosphoinositide-3 kinase (PI3K), Akt or glycogen synthase kinase 3β (GSK3β). The activation of classical nuclear receptors by neuroactive steroids (4) results in their dimerization and binding to steroid responsive elements in the promoters of specific genes and the consequent regulation of transcription (5). In addition, membrane and cytoplasmic signaling modulated by neuroactive steroids will finally impact on transcriptional activity (6).

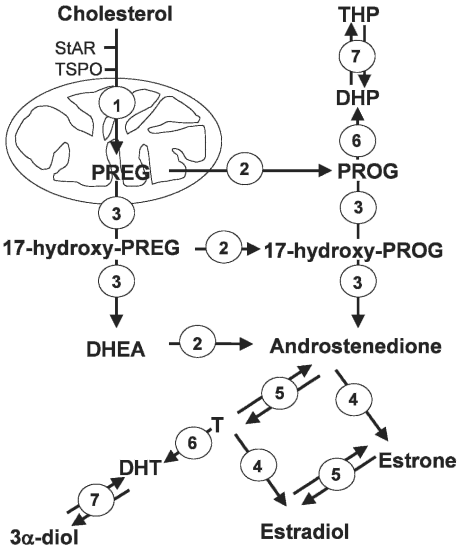


**Figure 2.** Biosynthesis of steroids in nervous system. The first enzymatic step of steroidogenesis is the synthesis of pregnenolone (PREG) from cholesterol by the enzyme P450 side chain cleavage (1). The transfer of cholesterol from the outer to the inner mitochondrial membrane is mediated by translocator protein-18kDa (TSPO) and steroidogenic acute regulatory protein (StAR). PREG is then converted to progesterone (PROG), by the enzyme 3β-hydroxysteroid dehydrogenase (2). PREG and PROG can be further converted, by the enzyme cytochrome P450c17 (3) into 17-hydroxy-PREG and dehydroepiandrosterone (DHEA) and into 17-hydroxy-PROG and androstenedione, respectively. The enzyme 3β-hydroxysteroid dehydrogenase (2) is also responsible of the conversion of 17-hydroxy-PREG into 17-hydroxy-PROG and DHEA into androstenedione. Androstenedione and testosterone (T) can be converted to estrone and estradiol by the enzyme aromatase (4), respectively. Androstenedione and T, estrone and estradiol are in equilibrium by the action of the enzyme17β-HSD (5). T and PROG are converted by the action of enzyme 5α-reductase (6) into dihydrotestosterone (DHT) and dihydroprogesterone (DHP) and subsequently by the enzyme 3α-hydroxysteroid oxido-reductase (7) into 3α-androstanediol (3α-diol) and tetrahydroprogesterone (THP), respectively.

lishment of the sexually dimorphic pattern of neuronal connectivity in various brain regions [22, 23].

Another important hormonal influence on the development of the CNS, which has long-lasting consequences in postnatal life, is that exerted by stress steroids. Stress and stress steroids exert organizational effects in the developing brain that persist throughout adulthood. Stress during pregnancy or during early postnatal period impairs the responsiveness of the hypothalamic-pituitary-adrenal axis in adult life and may lead to alterations in the developmental pattern of the brain that may represent a risk factor for the development of psychiatric and cognitive disorders. In humans, prenatal stress has been associated to aggression, hyperactivity, anxiety, attention-deficit disorders and cognitive problems in postnatal life [24–26]. In non-human primates prenatal stress results in reduced exploratory behavior, attention-deficits, neuromotor impairments and a variety of other behavioral disorders [27]. In rodents, prenatal or early postnatal stress causes cognitive impairments [28–32]. In addition, perinatally stressed rodents show symptoms of affective disorders such as enhanced emotional reactivity, anxiety, conditioned fear and depression-like behaviors. These alterations are associated with modifications in brain morphology and plasticity, such as an impaired neurogenesis and a reduced number of granule cells in the dentate gyrus of the hippocampus [29, 33–36], abnormal synaptic physiology in CA3, remodeling of excitatory mossy fiber innervation and impairment of long-term potentiation in CA3 and CA1 [31, 37].

The effects of prenatal stress on the postnatal function of brain may be in part mediated by the prenatal action of stress steroids. The brain is very sensitive to prenatal and early postnatal programming by glucocorticoids [38] and several studies assessing the prenatal effects of the synthetic glucocorticoid dexamethasone, which crosses the placenta, suggest that

these hormones may indeed mediate the prenatal actions of stress in the brain as in other organs. The brain of rats treated prenatally with dexamethasone show, in adult life, an altered sensitivity to stress steroids [39, 40]. Furthermore, the administration of dexamethasone to pregnant dams during the third week of gestation mimics the effect of prenatal stress on spatial learning [41]. In addition, administration of corticotropin-releasing hormone in the cerebral ventricles of immature rats results in deficits in the hippocampal function in adult life and these deficits have the same characteristics as those caused by early life stress [42].

Hormonal steroids also have different effects in the adult CNS. Gonadal steroids act on brain regions involved in the control of sex behavior and neuroendocrine regulation, modulating the release of neurotransmitter and the expression and function of neurotransmitter receptors and inducing plastic functional remodeling of synapses [43–45] and associated glial processes [22, 46]. In addition, cognitive brain regions are also affected by gonadal steroids and these hormones regulate the number of dendritic spines and synapses and the induction of long-term potentiation in the CA1 region of the rat hippocampus [47–55]. Gonadal steroids also regulate adult neurogenesis in the dentate gyrus [56–62]. These plastic actions of gonadal steroids in the hippocampus are associated to anti-depressive effects and the modulation of learning and memory processes [63–65] (Fig. 3).

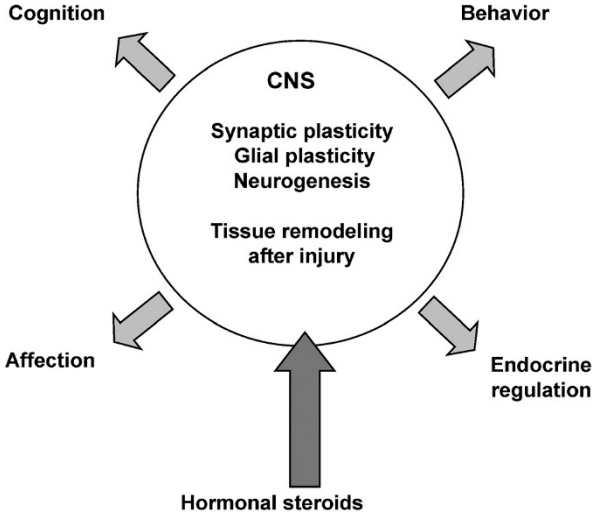


**Figure 3.** Steroid hormones regulate structural and functional plastic responses of the central nervous system (CNS) including synaptic and glial plasticity and adult hippocampal neurogenesis. In addition, steroid hormones regulate the response of brain tissue to injury and neurodegeneration, regulating gliosis, neuronal survival and axonal regeneration. These actions of steroid hormones are involved in the modulation of pituitary secretions, affection, behavior, learning and memory under physiological and pathological conditions.

Stress and stress steroids have different effects in the adult brain, regulating cognition and anxiety responses. These effects are also associated to morphological and functional modifications in several brain structures, such as changes in adult hippocampal neurogenesis [32, 35, 66–70] and in the dendritic morphology of the amygdala [71, 72], the prefrontal cortex [73–76] and the hippocampus [77–84]. Stress also affects long-term potentiation in the basolateral amygdala [85, 86] and hippocampus [87, 88] (Fig. 3). Another important aspect of the hormonal steroids in the CNS that merits consideration is their actions as regulators of neuronal survival under neurodegenerative conditions (Fig. 3). While chronic elevated levels of stress steroids may impair brain function, gonadal steroids exert different neuroprotective actions. Testosterone, estradiol and PROG have been shown to be neuroprotective in different experimental models of neuronal injury including hippocampal excitotoxicity, substantia nigra degeneration and experimental forebrain ischemia and are also protective against affective disorders [89–91]. However, there has not been an unambiguous translation of the data from animal models to human studies, and the results from therapy with estrogens and progestins or estrogen-only therapy on neurological and cognitive function in postmenopausal women are not yet conclusive. The use of selective nuclear receptor modulators and the pharmacological modulation of local brain steroidogenesis have been proposed as alternatives to hormonal therapy for neuroprotection [92].

**Actions of hormonal steroids in the PNS.** Hormonal steroids, affecting both glial cells and neurons of PNS, are involved in the regulation of different functions of peripheral nerves. For instance, both *in vivo* (in the rat sciatic nerve) and *in vitro* (in cultures of rat Schwann cells), the synthesis of two important myelin proteins, myelin protein zero (P0) and the peripheral myelin protein 22 (PMP22), is modulated by the treatment with the gonadal steroid PROG and its neuroactive metabolites (*i.e.*, dihydroprogesterone, DHP, and tetrahydroprogesterone, THP, also known as allopregnanolone). The expression of P0 in sciatic nerve of adult male rats and in rat Schwann cell culture is increased by the treatment with PROG, DHP or THP, whereas in case of PMP22, only THP is effective [93–95]. Other gonadal steroids may also affect the synthesis of these myelin proteins. For instance, in adult male rats, castration decreases the expression of P0 and of PMP22 in the sciatic nerve [96, 97]. These effects are counteracted by the treatment with neuroactive metabolites of testosterone, such as DHT or 3α-androstanediol (3α-diol) in case of P0 and by the treatment with 3α-diol in case of PMP22 [97]. A very

similar pattern of effects is also evident in cultures of rat Schwann cells. In this experimental model, DHT increases P0 mRNA levels [96], while the treatment with 3α-diol increases PMP22 mRNA levels [98]. Finally, a stimulatory effect of glucocorticoids has been also observed on the expression of P0 and PMP22 [99].

Recent observations have indicated that different signaling pathways depending on the myelin protein considered may be proposed. The expression of P0 seems to be under the control of classical steroid receptors, such as progesterone receptor (PR) and AR, while a role for a non-classical steroid receptor, like $GABA_A$ receptor may be hypothesized in case of PMP22 [95]. Indeed, as demonstrated in culture of rat Schwann cells, mifepristone (an antagonist of PR) is able to block the stimulatory effect exerted by PROG or its derivatives on P0 [100]. A role for this classical steroid receptor is also supported by *in vivo* observations. Treatment with mifepristone from the first day of life causes a decrease in the expression of P0 in rat sciatic nerve by day 20 [101].

The activation of PR suggests that the effect of PROG and its derivatives on P0 expression may be due to a classical steroid genomic effect. This hypothesis is supported by the finding that an important coactivator, steroid receptor coactivator-1 (SRC-1), participates in the regulation of P0 gene expression by DHP [102] and by the presence of putative progesterone responsive elements on P0 promoter [96].

A role for AR in controlling expression of P0 may also be hypothesized. Indeed, *in vivo* treatment with flutamide (an AR antagonist) decreases the synthesis of this myelin protein in rat sciatic nerve [97]. Interestingly, at variance to that observed in case of PR, where the effect on P0 expression is only evident during development [101], the effect exerted by flutamide is evident only in adult age. It is possible that PROG and its derivatives are necessary for inducing P0 synthesis during the first steps of the myelination process, while testosterone and its derivatives participate in the maintenance of this process.

As mentioned above, the expression of PMP22 seems to be under the control of $GABA_A$ receptor. Neuroactive steroids, which preferentially bind to $GABA_A$ receptor (*i.e.*, THP or 3α-diol), induce a stimulatory effect on PMP22 [94, 97], and this action is respectively blocked or mimicked by agonist or antagonist of this neurotransmitter receptor [100].

However, recent data also suggest that expression of P0 and PMP22 are not only merely under the control of classical (PR and AR) and non-classical steroid ($GABA_A$ receptor) receptors, respectively, but also that sex is another variable. Treatment with PROG or DHP induces a stimulatory effect on P0 mRNA levels in Schwann cell cultures from male rats but not in those from females. In contrast, treatment with THP increases gene expression of P0 in Schwann cells from female rats but not in cells from males. A similar sex difference was also evident for PMP22. The expression of this myelin protein is stimulated by PROG in cultures from males and by THP in cultures from females [103].

Further observations have also indicated that neuroactive steroids, such as PROG and its derivatives might coordinate Schwann cell myelinating program utilizing different intracellular pathways. Thus, in the Schwann cells they stimulate not only the expression of myelin proteins as mentioned above, but also the expression of transcription factors, which exert key roles in the myelination process. Indeed, data obtained in culture of rat Schwann cells have indicated that PROG and/or its derivatives stimulate the gene expression of Krox-20, Krox-24, Egr-3, FosB and Sox-10 [104–106].

PROG may also affect the myelination process through neurons. In co-cultures of Schwann cells and dorsal root ganglion neurons, PROG accelerates the time of initiation and enhances the rate of myelin synthesis [107]; this effect is correlated with an increase in expression of two genes, encoding a small Ras-like GTP-binding protein (Rap 1b) and phosphoribosyl diphosphate synthase-associated protein [107, 108].

Acting on glial cells and neurons of PNS, gonadal steroids may also affect other physiological parameters. A stimulatory effect of PROG on proliferation of Schwann cells has been detected *in vitro* [109]. In agreement with that observed for myelin proteins (see above) this effect also seems to be sex dependent. Thus, PROG increases Schwann cell proliferation in cultures of segments of rat sciatic nerve from females, but is ineffective in cultures from males [109]. Steroid coactivators are also able to affect cell proliferation. For instance, overexpression of SRC-1 or of steroid receptor RNA activator (SRA) in an immortalized line of Schwann cells (MSC80 cells) induces, respectively, a decrease or an increase of cell proliferation [95]. An effect of androgens on Schwann cell proliferation is also evident. The number of terminal Schwann cells unsheathing the synaptic junction between motor nerve endings and muscles decrease after castration and this effect is counteracted by testosterone replacement [110].

Recent observations have also identified in Schwann cells new target genes for glucorticoids (*e.g.*, aspartate aminotransferase and glutamine synthetase). These effects are through glucocorticoid receptors (GR), which are expressed in Schwann cells. GR signaling in Schwann cells has been recently evaluated, and it has

been shown that GR recruits different members of the p160 coactivator family (SRC-1a, SRC-1e and SRC-3) [111–113]. Interestingly, neither CREB-binding protein (CBP) nor its close homolog p300 are recruited by p160 proteins in the transcriptional complex, but beta-catenin replaces CBP in its binding to GR and acts as a coactivator of the receptor [114]. This suggests the presence of a non-classical GR-coactivator complex in the Schwann cells.

As mentioned before, gonadal steroids may also affect neurons. The blockade of PR during development results in a reduced axon diameter compared to myelin thickness and an increased neurofilament density [101]. Moreover, both PROG and estradiol, either alone or in combination, stimulate the expression of a potent vasodilatory peptide, such as the calcitonin gene-related peptide, in dorsal root ganglion neurons *via* nerve growth factor-mediated mechanisms [115, 116]. These gonadal steroids have also been demonstrated to exert membrane effect at the level of dorsal root ganglion neurons. Estradiol induces a rapid attenuation of ATP-induced intracellular calcium concentration *via* activation of a membrane-associated ERalpha [117, 118]. Recent observations have also demonstrated that in dorsal root ganglion neurons, PROG as well as THP inhibit the $GABA_A$-induced intracellular calcium transients in a rapid non-genomic manner [119]. This is an unusual effect because, as so far demonstrated in other neurons, the treatment with these neuroctive steroids usually induces a potentiation of the response to GABA agonist [120].

As recently observed in several experimental models, gonadal steroids and their neuroactive metabolites may be considered as protective agents in peripheral neuropathy [5, 95]. Examples are those due to aging process, to physical injury, to diabetes, and to inherited forms of peripheral neuropathy, which are a group of disorders collectively referred to Charcot-Marie-Tooth (CMT) disease. In case of aging, treatment with PROG or DHP increases the low levels of P0 present in the sciatic nerve of aged male rat, while THP significantly increases those of PMP22 [93, 94]. These neuroactive steroids also have clear effects on the number and shape of myelinated fibers as well as on the frequency of myelin abnormalities [121]. In particular, the treatment increases the number and the g ratio (the ratio between the axonal and the entire fiber diameter) of myelinated fibers of small caliber ($<5$ μm). Moreover, reductions in the frequency of axons with myelin infoldings and in the proportion of fibers with irregular shapes are observed [121].

Another experimental model in which the effect of gonadal steroids and their neuroactive metabolites has been evaluated is that of physical injury. PROG and DHP significantly increase the low mRNA levels of P0 in the distal portion of a cut of the sciatic nerve [98]. PROG, as well as its precursor pregnenolone (PREG), are also able to counteract the decrease of the amounts of myelin membranes induced by a cryolesion in the sciatic nerve of the mouse [122]. Moreover, biodegradable prostheses, such as chitosan, impregnated with PROG produce the best result for guided regeneration of facial nerve of rabbit [123]. This is due to an increase in the number of Schwann cell nuclei, of non-myelinated and myelinated nerve fibers (with increase also in their diameters), as well as in the g-ratio of myelinated nerve fibers.

In the model of physical injury, other hormonal steroids also exert interesting protective effects. Testosterone and its neuroactive derivative, DHT, accelerate regeneration and functional recovery of nerves in the models of rat sciatic and pudendal motoneurons, and in hamster facial motoneuron [124–128]. Dehydroepiandrosterone (DHEA) is protective after rat sciatic nerve transection, reducing the extent of denervation atrophy and inducing an earlier onset of axonal regeneration [129], and after crush injury of rat sciatic nerve, inducing a faster return to normal values of sciatic function index and increasing the number of myelinated fibers and fiber diameters [130]. As evaluated in mouse sciatic nerve transection, similar effects are exerted by estradiol [131].

Diabetic neuropathy is another experimental model in which protective effects of neuroactive steroids have been recently ascertained. In the experimental model of streptozotocin-treated rat, it has been shown that PROG and DHP are able to counteract the increase in the number of fibers with myelin infoldings induced by streptozotocin treatment in the sciatic nerve [132]. In the same experimental model, treatment with PROG, testosterone or their respectively derivatives also exert protective effects on other functional and biochemical parameters of PNS. Thus, PROG, DHP, THP, DHT and 3α-diol counteract the impairment of nerve conduction velocity and thermal threshold. Moreover, DHP, THP, testosterone and its derivatives restore skin innervation density and PROG, DHP and DHT improve $Na^+$,$K^+$-ATPase activity. Furthermore, mRNA levels of P0 present in sciatic nerve of diabetic rats are stimulated by the treatment with PROG, DHP or DHT, while those of PMP22 are stimulated by PROG treatment [133, 134]. An effect of DHEA in preventing vascular and neuronal dysfunction in the sciatic nerve of streptozotocin-rats has been observed [135]. The effects of hormonal steroids and their neuroactive metabolites here mentioned on diabetic neuropathy are not correlated to a possible control of glycemia. Indeed, none of the neuroactive steroids so far tested affects

the glucose levels in streptozotocin-induced diabetic rats [133–135].

PR has also been recently suggested as a pharmacological target for therapy of CMT1A, a disease caused by a 1.5-mB duplication of chromosome 17p11.2, containing the gene coding for PMP22 [136]. As demonstrated in an animal model overexpressing this myelin protein (PMP22-transgenic rats), treatment with onapristone (an antagonist of PR) ameliorated the neuropathic phenotype [137, 138].

Altogether, these observations clearly indicate that the treatment with hormonal steroids or their neuroactive metabolites seem to be a promising therapeutic approach against acquired and inherited peripheral neuropathy. However, it is clear that a sustained research and development effort should be devoted to the identification of steroid receptor (classical and non-classical) involved in such effects. This is extremely important for the design of specific synthetic ligands that are devoid of side effects. On the other hand, another interesting therapeutic strategy may comprise increasing the level of endogenous protective neuroactive steroids in the nervous system. This could be possible by the use of translocator protein 18kDa (TSPO, also known as peripheral benzodiazepine receptor)-stimulating ligands, since TSPO plays a pivotal role in the onset of neuroactive steroid synthesis (see below). Interestingly, recent reports have already revealed the occurrence of protective effects for certain TSPO ligands in experimental models of peripheral neuropathy [139, 140].

## Biosynthesis and actions of neurosteroids

**Discovery and definition of neurosteroids.** A major finding in the research on neuroactive steroids was the identification of the ability of neurons and glial cells to synthesize bioactive steroids, also called neurosteroids [1]. This important discovery stemmed from a series of pioneer works showing the persistence of substantial amounts of PREG, DHEA and their sulfated derivatives in the rodent brain after gonadectomy and adrenalectomy [141, 142]. However, the consolidation of the concept of neurosteroids has required several investigations performed in various laboratories using different animal species [1, 143–145]. These investigations, which significantly increased the basic knowledge on neurosteroids, have also allowed a strict definition of the term neurosteroid with specific identification criteria. The consensual definition that emerged considers these molecules as endogenous steroidal compounds synthesized in neurons or glial cells of the CNS and PNS. To be qualified as a neurosteroid, the candidate steroidal molecule must persist in substantial amounts in the nervous system after removal of the peripheral or traditional steroidogenic glands such as the adrenals and gonads [1]. Furthermore, it has been demonstrated that neurosteroids act as paracrine or autocrine factors, regulating the activity of classical nuclear steroid receptors and through the modulation of membrane receptors, including steroidal membrane receptors coupled to G proteins [146, 147], $GABA_A$ and T-type calcium channels [120, 148–150] or *via* NMDA [151, 152], P2X [153] and sigma [154, 155] receptors.

**Evidence for neurosteroid biosynthesis.** The demonstration of neurosteroid biosynthesis requires the observation of the expression and biological activity of key steroidogenic enzymes in nerve cells [1]. Neurosteroid formation has been evidenced in the nervous system by various molecular and biochemical studies, which have revealed in neurons and/or glial cells the expression of TSPO, the steroidogenic acute regulatory protein (StAR) and of several key steroid-synthesizing enzymes such as cytochrome P450 side chain cleavage (P450scc; CYP11A), 3β-hydroxysteroid dehydrogenase (3β-HSD), cytochrome P450c17 (P450c17; CYP17), 5α-reductase (5α-R), 3α-hydroxysteroid oxido-reductase (3α-HSOR), 17β-hydroxysteroid dehydrogenase (17β-HSD) and aromatase (CYP19) [1, 143–145].

**TSPO, StAR and cytochrome P450scc.** Cholesterol conversion to PREG, the first step in the biosynthesis of all classes of steroid hormones (Fig. 2), is catalyzed by the mitochondrial enzyme P450scc. However, there is a previous step that is rate limiting and hormonally regulated: the transfer of cholesterol from the outer to the inner mitochondrial membrane, where the P450scc enzyme is located. In this mechanism, two proteins located in the mitochondrial membranes, TSPO [156] and StAR [157], exert an active role. TSPO is expressed in the nervous system, predominantly in glial cells of CNS and PNS. Neural injury and different neuropathological conditions result in the induction of the expression of this molecule. TSPO basal expression is up-regulated in gliomas, in neurodegenerative disorders (*e.g.*, Alzheimer's disease), as well as in various forms of brain injury and inflammation induced by neurotoxins [158]. The induction of TSPO expression after injury in the CNS is mainly restricted to microglia and astrocytes [159]. In the PNS, the expression of TSPO in Schwann cells is increased after nerve lesion, and return to normal levels when regeneration is completed [160].

StAR is formed as a 37-kDa protein, which is rapidly transported into mitochondria where it is cleaved, generating a mature 30-kDa intramitochondrial StAR

protein that is inactive. StAR appears to be widely distributed throughout the brain, although different levels of expression have been detected between different brain areas. For instance, StAR expression seems to be restricted to very specific neuronal and astroglial populations in each brain area, although it is predominantly expressed by neurons [161]. In the PNS, StAR is expressed by Schwann cells [162].

Molecular cloning revealed the existence of a single gene encoding P450scc in the human and rat genome [163–165]. Immunoreactivity for P450scc was detected in the white matter throughout the rat brain and biochemical experiments have shown the conversion of cholesterol to PREG by primary culture of glial cells [166, 167]. However, these studies, which were focused only on the brain, did not investigate the presence and activity of P450scc in the spinal cord. Initial data suggesting P450scc gene expression in the spinal cord were provided by Mellon and coworkers who have mainly used developing rodent embryos [168]. These authors reported the expression of P450scc mRNA and immunoreactivity in sensory structures of the PNS, including the dorsal root ganglion, the trigeminal and facio-acoustic preganglia, and mentioned P450scc existence in the rodent embryo spinal cord ventral horn as data not shown. Expression of this enzyme has also been located in the glial component of peripheral nerves (*i.e.*, Schwann cells) [169].

The first anatomical and cellular distribution of P450scc in the adult rat spinal cord has been provided by Patte-Mensah and coworkers [170]. The highest density of P450scc immunolabeling was found in superficial layers laminae I and II of spinal cord dorsal horn, where sensory neurons are located [170]. Double-labeling experiments revealed that most of the P450scc-positive fibers in the dorsal horn were also immunoreactive for microtubule-associated protein-2, a specific marker for neuronal fibers [170]. Motor neurons of the ventral horn also expressed immunoreactivity for P450scc, suggesting a possible role of the enzyme or its steroid products in the control of motor activity [170]. Moreover, P450scc immunostaining was detected in ependymal glial cells bordering the central canal in the spinal cord, an observation which suggests a possible release of neurosteroids in the cerebrospinal fluid and their involvement in volume transmission mechanisms in the CNS [170]. Furthermore, P450scc-immunoreactive cell bodies were localized in various nociceptive nuclei of the adult rat brainstem, including the parabrachial, the raphe magnus and the dorsal raphe nuclei (Fig. 4). Biochemical experiments showing that homogenates from the adult rat spinal cord and brainstem are capable of converting cholesterol into PREG have demonstrated that P450scc-like immunoreactivity detected in the adult rat brain and spinal tissue corresponds to an active form of the enzyme [170].

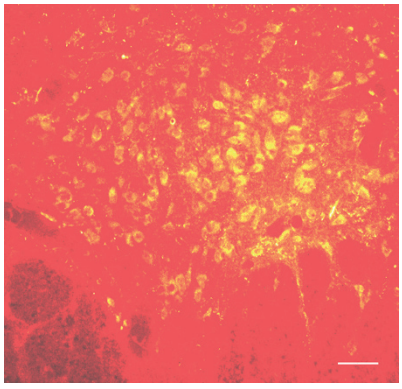

**Figure 4.** P450 side chain cleavage-immunoreactive cell bodies in the adult rat dorsal raphe nucleus. Scale bar, 100 μm.

**Cytochrome P450c17.** The enzymatic complex P450c17, also called 17α-hydroxylase/17,20 lyase, catalyzes a two-step reaction that converts PREG successively into 17-hydroxy-PREG and DHEA (Fig. 2). P450c17 is also responsible for the successive transformation of PROG into 17-hydroxy-PROG and androstenedione.

The P450c17 gene is expressed in human adrenals and gonads, whereas in rodents the enzyme has been detected in gonads but not in adrenals [171–173]. The production of DHEA in the rat brain, independently from peripheral endocrine glands, was suggested in early studies. However, for a long time the attempts to demonstrate the presence and bioactivity of P450c17 in the nervous system were unsuccessful [1]. Therefore, a series of investigations that analyzed the oxidative processes involved in steroidogenesis suggested the possibility of DHEA biosynthesis in the brain through a biochemical P450c17-independent pathway [174, 175]. The expression of mRNA encoding P450c17 was, however, finally demonstrated in the cerebellum and brain stem of young adult rats using a combination of RNase protection assays with PCR experiments [176, 177]. Subsequently, evidence has been provided for the expression and enzymatic activity of P450c17 in adult rat hippocampal neurons [178]. Molecular, immunohistochemical and neurochemical approaches have been used to investigate of P450c17 existence and biological activity in adult rodent spinal cord [179]. A significant amount of P450c17 mRNA was detected in all regions of the spinal cord using real-time PCR after reverse transcription. The development of a specific antiserum has allowed the detection by Western blot analysis of a specific band corresponding to P450c17 in total homogenates and microsomal fractions from the rat

spinal cord and testis. This antiserum also allowed the anatomical and cellular localization of P450c17 throughout the white and gray matters of the spinal cord using immunohistochemistry and confocal laser microscopy. P450c17 immunostaining was found in both neurons and glial cells. In the white matter the enzyme was mainly detected in astrocytes, while in the gray matter P450c17 was essentially found in neurons and oligodendrocytes [179]. The presence of P450c17 in both the dorsal and ventral horns of the spinal cord suggests its potential involvement in the modulation of sensory and motor functions [179]. Pulse-chase experiments showing that spinal cord slices converted [$^3$H]PREG into [$^3$H]DHEA indicate that P450c17-like immunoreactivity detected in the adult rat spinal cord corresponds to an active form of the enzyme [179].

P450c17 enzymatic activity in the spinal cord was further demonstrated with biochemical experiments using ketoconazole, a selective inhibitor of the enzyme. A significant decrease was observed in the conversion of [$^3$H]PREG to [$^3$H]DHEA by spinal cord slices when the pulse-chase experiments were performed in the presence of ketoconazole, a result which unambiguously confirms the existence of P450c17 activity in the adult rat spinal cord [179].

**3β-hydroxysteroid dehydrogenase.** The enzyme 3β-HSD catalyzes the conversion of $\Delta^5$ – 3β-hydroxysteroids (PREG, 17OH-PREG, DHEA) into $\Delta^4$ – 3-ketosteroids (PROG, 17OH-PROG, androstenedione) (Fig. 2).

Molecular cloning of the cDNA encoding 3β-HSD has revealed the existence of two isoforms of the enzyme in humans: 3β-HSD type I, which is essentially expressed in the placenta and 3β-HSD type II, which is mainly expressed in the adrenal gland and gonads [180, 181]. Four types of 3β-HSD have been characterized in rats [182] and six types in mice [183]. Apart from the adrenals and gonads, 3β-HSD has been localized in other tissues including the skin [184], mammary gland [181] and prostate [185].

The observation of PREG conversion into PROG in homogenates of rat amygdala and septum constitutes the first result suggesting the existence of 3β-HSD in the CNS [186]. The biological activity of 3β-HSD has also been detected in primary cultures of rodent oligodendrocytes and neurons [167]. The expression of 3β-HSD protein and/or mRNA has been reported in various brain regions of several classes of vertebrates including fish [187, 188], amphibians [189], birds [190] and mammals [191–194]. 3β-HSD was also localized in the PNS, particularly in dorsal root ganglion neurons and in Schwann cells of sciatic nerve [122, 195, 196]. The first demonstration of 3β-HSD mRNA in the spinal cord was performed in rat using RT-PCR [193]. A further study by Coirini et al. [197], using *in situ* hybridization, provided the anatomical and cellular distribution of 3β-HSD mRNA in the spinal cord. This study revealed that the dorsal horn laminae I–III exhibited the highest density of 3β-HSD mRNAs, which were also detected in layer X around the central canal, in the ventral horn and in the lateral and ventral funiculi. At the cellular level, 3β-HSD mRNAs were found mainly in sensory neurons of the dorsal horn and in motor neurons of the ventral horn throughout the cervical, thoracic, lumbar and sacral segments of the spinal cord [197]. Moreover, evidence for the existence of 3β-HSD protein and enzymatic activity in the spinal cord was provided by Western blot analysis and gas chromatography/mass spectrometry assays, which revealed that the concentrations of PREG and PROG were higher in the spinal cord than in plasma [197].

**5α-reductase.** The enzyme 5α-R is responsible for the transformation of testosterone and PROG into DHT and DHP, respectively (Fig. 2). In a similar manner, 5α-R converts 11-deoxycorticosterone to dihydrodeoxycorticosterone (DHDOC). Two isoforms of 5α-R, designated type 1 (5α-R1) and type 2 (5α-R2), have been cloned in humans and rats [198–200]. The genes encoding 5α-R1 and 5α-R2 are located on chromosome 5 and 2, respectively, and the two isoenzymes have different optimal pH and sensitivity to substrates [201, 202]. In humans, the 5α-R1gene is predominantly expressed in the skin, notably in the pubic skin and scalp [198, 203]. The 5α-R2 gene is mainly expressed in the prostate and gonads and its deletion provokes male pseudohermaphroditism [199, 204]. In rats, 5α-R1 and 5α-R2 cDNAs have been cloned from a prostate library but the two genes are transcribed in distinct cells: mRNAs encoding 5α-R1 are found in the basal epithelial cells, while 5α-R2 mRNAs are localized in stroma cells [198, 200].

The expression of 5α-R in the brain has been extensively studied [205–208]. It has been suggested that 5α-R1 essentially plays a catabolic and neuroprotective role whereas 5α-R2 participates in sexual differentiation of the CNS; however, the neurophysiological significance of these two isoenzymes remains a matter of speculation [209–212]. In contrast to that observed in the brain, the quantity of 5α-R2 mRNAs extracted from the whole adult rat spinal cord is higher than that of 5α-R1 [213]. Immunoreactivity for 5α-R1 and 5α-R2 has been detected in the white matter of the spinal cord, from the cervical to sacral regions. However, the intensity of 5α-R1 immunostaining was low and cell bodies as well as fibers containing this isoenzyme were observed mainly in

the white matter of the cervical and thoracic segments. 5α-R2 immunofluorescence, which was moderate in the white matter, was intense in the dorsal and ventral horns of the gray matter [214]. Double-labeling identification with specific markers for nerve cells revealed that the 5α-R1 immunostaining was mainly expressed in oligodendrocytes and astrocytes of the white matter, whereas 5α-R2-immunolabeling colocalized with neurons and glial cells in the gray and white matters [214]. The observation of a restricted localization of 5α-R1 to the spinal cord white matter is in agreement with its localization in myelinated structures of the female and male rat brain [215, 216].

**3α-hydroxysteroid oxido-reductase.** The enzyme 3α-HSOR also called 3α-hydroxysteroid dehydrogenase converts in a reversible manner DHT and DHP into the respective neuroactive steroids 3α-diol and THP (Fig. 2). Similarly, DHDOC is converted to tetrahydrodeoxycorticosterone (THDOC). 3α-HSOR is a member of the aldo-keto reductase superfamily which includes aldehyde reductase, aldo reductase and dihydrodiol dehydrogenase [217, 218]. There are four human 3α-HSOR isozymes, but, to date, only one isoform has been cloned in rats [219, 220].

3α-HSOR enzymatic activity and its encoding mRNA have been detected in the brain [208, 221–223]. In the spinal cord, intense immunoreactivity for 3α-HSOR has been detected in the white and gray matters throughout the cervical, thoracic, lumbar and sacral regions. However, the highest density of 3α-HSOR-immunostaining is found in sensory areas of the dorsal horn [214]. This study also revealed that 45 % of 3α-HSOR immunofluorescence was localized in oligodendrocytes, 35 % in neurons and 20 % in astrocytes. A comparative analysis of 5α-R1, 5α-R2 and 3α-HSOR-positive elements in the spinal cord reveals three different situations: (i) cell bodies and fibers that express both 3α-HSOR and 5α-R; (ii) cell bodies that express either 5α-R1 or 5α-R2 and (iii) cell bodies that express 3α-HSOR only [214]. Consequently, it appears that certain glial cells and neurons of the spinal cord contain both 5α-R and 3α-HSOR enzymatic proteins, which could catalyze the biochemical reductions required for the biosynthesis of 5α/3α-reduced steroids that control, through allosteric modulation of $GABA_A$ receptors, neurobiological mechanisms including nociception, pain and locomotion [148, 214, 224–227]. The production of 5α/3α-reduced steroids may involve the cooperation of neurons, astrocytes and oligodendrocytes, which contain only one of the two enzymes, *i.e.*, 3α-HSOR or 5α-R. This cooperation may occur during physiological and pathological situations [228]. In addition, 5α-R1 or 5α-R2 may convert PROG or testosterone from peripheral sources into DHP or DHT, metabolites that act *via* genomic receptors, the existence of which has been demonstrated in spinal tissues [229–231]. In a similar manner, 3α-HSOR may convert, in the spinal cord, DHP, DHT or DHDOC into THP, 3α-diol or THDOC, respectively, for the modulation of $GABA_A$ receptors [148, 232–234]. The fact that the rat spinal tissue homogenates are capable of converting [$^3$H]cholesterol into various metabolites including THP clearly indicates that 5α-R1, 5α-R2 and 3α-HSOR detected in the spinal cord correspond to active forms of these enzymes [170, 214]. The capability to convert PROG and testosterone into their 5α- (by 5α-reductase) and 5α/3α-reduced metabolites (by 3α-HSOR) is not restricted to CNS but is also present in peripheral nerves and in Schwann cells [235, 236].

**Aromatase.** The conversion of androgens into estrogens (*i.e.*, androstenedione and testosterone into estrone and estradiol, respectively) is catalyzed by aromatase (Fig. 2). Aromatase activity occurs in various tissues including the placenta [237], ovary [238], testis [239] and adipocytes [240]. Molecular cloning of aromatase cDNAs revealed the existence of a single enzyme in most species including trout [241], chicken [242], rat [243], mouse [244], bovine [245] and human [246]. A remarkable exception has been reported in pig, which possesses two distinct isoforms of aromatases [247, 248].

The presence of aromatase activity in the CNS has been suggested from biochemical studies demonstrating androstenedione conversion into estrone in the rat brain [249, 250]. Immunocytochemical studies have shown that aromatase is expressed in neurons but not in glial cells [251]. 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 [252, 253]. 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 [254, 255]. It should be noted, however, that during ontogenesis aromatase-positive neurons have been visualized in the preoptic area, the ventromedian nucleus and the arcuate nucleus at embryonic day 13 (E13), E16 and E19, respectively. In these regions, the number of aromatase-positive neurons increases during gestation, peaks before birth, and

decreases or vanishes during the two first postnatal weeks [256]. These data reveal the existence of spatio-temporal variations in the level of transcription of aromatase gene during development. Since estrogens stimulate the expression of AR and increase the duration of AR occupation in the rat brain [257], it is conceivable that estrogens and androgens may exert a coordinate action in the control of aromatase gene expression in the CNS. Recently, aromatase expression has been evidenced in the human temporal cortex [258]. The broad range of potential modulators of aromatase gene in the cerebral cortex, as well as the widespread distribution of the protein in specific neuronal and glial subpopulations, suggests that the local estrogen formation may play an important role in human cortical function [258].

**Functional relevance of the local synthesis of steroids in the nervous system.** Based on behavioral responses evoked in animals by synthetic steroid injections, several studies suggested neurosteroid involvement in the control of various important neurophysiological processes. Even though these studies were useful in identifying potential roles of exogenously administered neurosteroids, they were unable to provide direct evidence for the importance of local steroid synthesis in the control of the nervous system activity. Endogenous neurosteroid involvement in the regulation of a neurobiological process becomes strongly probable when neurosteroidogenesis occurs in neural pathways controlling this mechanism. The functional role of endogenous neurosteroids has been evidenced by recent investigations that established a direct link between the local synthesis of neurosteroids in the nervous system and the achievement of a specific neurophysiological mechanism. Thanks to a recent series of studies, remarkable progress has been made in the determination of the biological effects of endogenously synthesized neurosteroids.

A first series of investigations has focused on the significance of local estrogen synthesis on spine synapse formation as well as on the synthesis of synaptic proteins [259–261]. The effects of low gonad-derived serum estrogen concentrations were distinguished from those of intermediate concentrations provided by hippocampal cells and also from pharmacological doses of synthetic estrogens. To study the effects of hippocampal-derived estradiol, hippocampal estrogen synthesis was inhibited by treatment of hippocampal cell cultures with letrozole, an aromatase inhibitor [259]. This study revealed a significant decrease in the density of spine synapses and in the number of presynaptic boutons. Quantitative immunohistochemical analysis showed a down-regulation of spinophilin (a marker of dendritic spines) and synaptophysin (a protein of presynaptic vesicles) in response to letrozole [259]. Furthermore, siRNA against StAR was also used to reveal a down-regulation of spines, synapses and synaptic proteins in the absence of steroidogenesis in the hippocampus [261]. Endogenous androgens and estrogens produced in the hippocampal formation have also been identified as paracrine modulators of synaptic plasticity [262]. In a first step, the authors of these investigations demonstrated that estrogens and androgens are locally synthesized in the hippocampal neurons by a steroidogenic pathway involving P450scc, P450c17, 3β-HSD, 17β-HSD and aromatase [178]. Afterwards, they observed that hippocampal neuronal-derived estradiol acts rapidly on neurons, enhancing long-term depression in CA1, CA3 and dentate gyrus [262].

Neuroprotective effects of THP have been demonstrated by Griffin and coworkers [263] using the naturally occurring mutant mouse model of Niemann-Pick type C (NP-C) disease (a fatal autosomal recessive childhood neurodegenerative disease characterized by defective trafficking of intracellular cholesterol and lysosomal accumulation of unesterified cholesterol gangliosides and other lipids). They found that NP-C mouse brain contains less neurosteroids than the wild-type brain and has an age-related decrease in the ability to synthesize DHP and THP [263]. Furthermore, the neonatal administration of THP delays the onset of neurological symptoms, increases Purkinje and granule cell survival, reduces cortical ganglioside accumulation and double the lifespan of NP-C mice. Taken together, the results provided by Griffin and coworkers [263] clearly demonstrate that early compensation of the brain deficit in endogenous THP synthesis by the administration of exogenous THP is an effective treatment to rescue NP-C mice from certain neurological disorders. In agreement with this observation, it has also been shown that reduced progesterone metabolites including DHP and THP locally produced in the brain protect rat hippocampal neurons from kainic acid excitotoxicity *in vivo* [264, 265].

Other studies have also contributed significantly to the demonstration of neuroprotective effects of endogenous neurosteroids in the brain. These studies, which showed that PREG and DHEA protect hippocampal hilar neurons against kainic acid-induced excitotoxicity in dose-dependent manner, have also demonstrated that aromatase inhibitor letrozole blocked the neuroprotective effects of PREG and DHEA [266]. Together, these findings suggest that estradiol formation by aromatase mediates neuroprotective effects of PREG and DHEA against excitotoxicity-induced neuronal cell death in the hippocampus [267]. Interestingly, aromatase expres-

sion is induced in reactive glia after brain injury and exerts an endogenous neuroprotective role [267].

The possible relationship between pain modulation and neurosteroid production has been also recently evaluated [224, 225, 268]. Using the model of the sciatic nerve tied loosely by ligatures, it was observed that the mononeuropathic pain significantly increased the level of P450scc mRNA in the rat spinal cord [268]. Reversed-phase HPLC analysis was coupled with flow scintillation detection to show that the amount of newly synthesized [$^3$H]PREG from [$^3$H]cholesterol was 80 % higher in tissue homogenates from the spinal cord of neuropathic rats compared to controls. In addition, HPLC purification of spinal cord and blood extracts, combined with radioimmunological detection of PREG and THP, revealed that endogenous concentrations of these neurosteroids are significantly increased in spinal cord sensory networks of neuropathic rats, while their plasma levels do not change [224, 225, 268]. The up-regulation of P450scc gene expression and bioactivity in the spinal cord during neuropathic pain strongly suggests that neurosteroidogenesis may be an endogenous mechanism triggered in adequate networks to facilitate adaptation of the body to the painful state. In support of this suggestion, it has been shown that PREG, which is hyper-secreted under pain state, promotes cytoskeleton development by stimulating tubulin polymerization and microtubule formation [269, 270]. In addition, since the regulation of neuronal plasticity linked to the pathogenesis of chronic pain [271] is pivotal for efficient therapy, hyper-release of PREG in sensory neural networks constitutes a promising finding that can be exploited for the development of novel strategies against pain. Moreover, the data also revealed that hyper-production of PREG in the spinal cord under painful conditions results in an increase in the endogenous amount of THP, a potent stimulator of $GABA_A$ receptors, which are crucial in the regulation of pain sensations [268]. Therefore, the increased THP concentration in the spinal cord in a painful situation may be an adaptive mechanism activated in neuropathic rats to reduce their sensitivity to noxious stimuli from the ligated nerve. The biochemical pathways leading to the synthesis of THP from PREG can also generate PROG, which has been shown to decrease sensitivity to pain by increasing levels of endorphins and opioid receptors in the CNS [272, 273]. Trophic effects of PROG have been characterized during brain development: PROG promotes dendrite growth, synaptogenesis and dendritic spine formation in developing Purkinje cells [188, 274]. Several studies have also demonstrated that PROG and its metabolites DHP and THP, which stimulate the process of myelination in the PNS and CNS, are potent neuroactive steroids in the protection of nerve cells against degeneration [95, 122, 145, 226, 227, 275]. Since the occurrence of apoptotic cell death has been demonstrated in the dorsal horn during peripheral neuropathic pain [276–278], the activation in spinal tissues of the production of neuroprotective neurosteroids, including PREG, PROG and its derivatives, may represent an adaptation of the body to chronic pain state.

Biochemical changes related to steroid synthesis have also been evidenced in the spinal cord of streptozotocin-diabetic rats. In particular, pulse-chase experiments were combined with reversed-phase HPLC analysis and flow scintillation detection to show that the amount of [$^3$H]PROG newly synthesized from [$^3$H]PREG in the spinal cord of diabetic rats was 200 % higher than in the controls, whereas the level of [$^3$H]THP produced from [$^3$H]PREG in spinal tissues of streptozotocin-treated rats was 30 % lower than in buffer-treated animal [279]. Liquid chromatography-tandem mass spectrometry has also been used to demonstrate that the levels of various endogenous neurosteroids are affected in the CNS of streptozotocin-diabetic rats [236]. Altogether, these results suggest endogenous neurosteroid involvement in the regulation of neuropathic mechanisms induced by long-term diabetes.

## Concluding remarks

Hormonal steroids exert a variety of developmental and regulatory actions in the nervous system and are essential for adapting neural activity to modifications in the internal environment of the organisms for an adequate homeostatic response. In addition, steroids produced locally in the nervous system, or neurosteroids, participate in the modulation of neural development and function, acting as endogenous autocrine or paracrine modulators. Hormonal steroids and neurosteroids are therefore important regulators of brain and spinal cord physiology. However, pathological modifications in the levels of hormonal steroids, such as in conditions of chronic stress, may result in cognitive impairment, affective disorders and altered brain plasticity. In contrast, some hormonal steroids, synthetic steroids and neurosteroids exert neuroprotective and regenerative effects and regulate the response of central and peripheral neural tissue to injury and neurodegeneration. Neuroactive steroids or the modification of their local synthesis or of their local action may represent promising therapeutic alternatives for the treatment of disorders of the CNS and PNS.

*Acknowledgments.* The financial supports of PRIN (2005060584_004) and FIRST from University of Milan, Italy to R.C.M., Ministerio de Educacion y Ciencia, Spain (SAF2005–00272) and the European Union (EWA project: LSHM-CT-2005–518245) to L.M.G-S. are gratefully acknowledged. The authors also thank the Centre National de la Recherche Scientifique (CNRS, France), Université Louis Pasteur (Strasbourg, France) and Association Ti'toine (Elbeuf, France) for their support.

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