Original Paper

Developmental Neuroscience

Dev Neurosci 1996;18:6–21

Michael Schumacher[a]
Paul Robel[a]
Etienne-Emile Baulieu[a, b]

[a] Lab. Hormones, INSERM U33, Bicêtre, and
[b] Collège de France, Paris, France

Development and Regeneration of the Nervous System: A Role for Neurosteroids

Key Words
Astrocytes
Development
Memory
Myelin
Neurosteroids
Oligodendrocytes
Pregnenolone
Progesterone
Regeneration
Schwann cells

Abstract
Several steroids, termed 'neurosteroids', are synthesized from cholesterol within both the central and peripheral nervous systems. These include pregnenolone and its sulfate ester, progesterone and its 5α-reduced metabolites. Dehydroepiandrosterone, mainly in its sulfated form, also remains present in the brain long after removal of the steroidogenic endocrine glands. Its biosynthesis in brain remains an open possibility, but the pathways involved are unknown. Little information is available concerning the role of neurosteroids during the maturation of the nervous system, although they are already synthesized by glial cells and by some populations of neurons during embryonic life. Cell culture experiments suggest that neurosteroids may increase the survival and differentiation of both neurons and glial cells. In the adult nervous system, neurosteroids modulate neurotransmission by acting directly on the neuronal membrane and also produce structural changes in neurons and in astrocytes. Studies of neurosteroid levels are currently conducted to examine their possible role during aging. We have recently reported that progesterone, synthesized by Schwann cells, promotes the formation of new myelin sheaths after lesion of the mouse sciatic nerve. Thus, neurosteroids may also play an important role during regeneration of the nervous system.

Effects of Gonadal Steroids on the Developing and Adult Nervous System

The nervous system is particularly sensitive to steroids during late fetal and early postnatal life. At this stage of development, many nerve and glial cells are still undifferentiated, and gonadal steroids influence their survival and maturation [Arnold and Gorski, 1984; McEwen, 1991]. The effects of gonadal steroids on the developing nervous system are not restricted to regions which will become primarily involved in reproduction, such as the hypothalamus. Estrogens, for example, also influence the differentiation of the hippocampus and cerebral cortex, which play an important role in memory and cognition. During early development, these two brain areas temporarily express estradiol receptors and the aromatase enzyme, which converts androgens to estrogens [Naftolin et al., 1975; Brown et al., 1989;

KARGER
E-Mail karger@karger.ch
Fax +41 61 306 12 34
http://www.karger.ch

0378–5866/96/0182–0006 $ 10.00/0

Dr. Michael Schumacher
Lab. Hormones, INSERM U33
80, rue du Général-Leclerc
F–94276 Bicêtre (France)

Received:
October 10, 1995
Accepted:
October 27, 1995

MacLusky et al., 1994]. Likewise, androgens, besides their trophic effects on motoneurons of the spinal nucleus of the bulbocavernosus (SNB), which innervate muscles of the penis [Forger et al., 1992; Lubischer and Arnold, 1995], also promote survival and differentiation of other populations of spinal and cranial motoneurons [De Nicola, 1993]. In addition to their neurotrophic effects, gonadal steroids also affect glial maturation. In mixed neuron-glial cultures prepared from embryonic rat hypothalami, estradiol induced the differentiation of GFAP-immunoreactive astrocytes from a flattened morphology to bipolar, radial and stellate shapes [Torres-Aleman et al., 1992]. In primary glial cultures established from newborn rat forebrains, estradiol has been reported to increase the proliferation of astrocytes and the expression of GPAP. In contrast, progesterone (PROG) inhibited their proliferation [Jung-Testas et al., 1992].

Gonadal steroids continue to influence neuronal and glial activity and plasticity during adulthood. It was generally believed that their effects on the adult nervous system are restricted to the reversible activation of reproductive behavior and of neuroendocrine mechanisms. This picture has completely changed over the recent years. We know now that the so-called 'sex steroids' modulate the main neurotransmitter systems and, as a consequence, influence neuronal activity within large parts of the nervous system, where they exert a variety of effects that are not necessarily related to reproduction [Bock and Goode, 1995]. Studies of the effects of estrogen on the expression of choline acetyltransferase within the basal forebrain were among the first that indicated nonreproductive actions of gonadal steroids [Luine, 1985; McEwen et al., 1995] and these effects have recently been associated with memory performances [Singh et al., 1994]. Particularly well documented are the effects of PROG or its reduced metabolites on dopamine release from striatal neurons [Dluzen and Ramirez, 1989], on type A γ-aminobutyric acid ($GABA_A$) receptor [Majewska, 1992], on the nicotinic acetylcholine receptor [Valera et al., 1992] and on σ-receptors [Monnet et al., 1995] (see below).

The trophic effects which gonadal steroids exert on neurons and glial cells also continue on into adulthood. Thus, in adult rats, estrogens regulate synaptogenesis in the CA1 region of the hippocampus [McEwen et al., 1995] and promote neuroastroglial plasticity in the arcuate nucleus of the hypothalamus [Garcia-Segura et al., 1994, 1995]. Testosterone increases the number of synaptic contacts in a group of motoneurons of the adult spinal cord [Matsumoto et al., 1988]. Indeed, many motoneurons within the adult nervous system contain androgen receptors [Sar and Stumpf, 1977; Simerly, 1993], and testosterone has been considered as a specific trophic factor for these neurons [Jones, 1993]. From these observations, it follows that steroids may be therapeutically useful in activating the reparative response of neurons and glial cells to injury.

This very brief survey illustrates that gonadal steroids continue to produce plastic changes within the adult nervous system and that their effects extend far beyond their actions on reproductive functions. There is another significant finding which has cast a new light on the complex relationships between steroids and the nervous system. Some steroids, termed 'neurosteroids', are synthesized within the brain and peripheral nerves by glial cells and by some populations of neurons [Baulieu, 1991; Robel and Baulieu, 1994; Schumacher and Baulieu, 1995]. The nervous system thus has its own source of steroids.

The Concept of Neurosteroids

The term 'neurosteroids' has been applied to steroids which either *are* or *can be* synthesized de novo from cholesterol in the nervous system [Baulieu, 1981, 1991], i.e., all the enzymes required for their synthesis are present, even though a greater or lesser proportion of a 'neurosteroid' present in the nervous system may actually originate from the metabolism of precursors arising from the circulation. Indeed, as will be discussed below, gonads, adrenal glands and steroidogenic cells of the nervous system all contribute to the pool of steroids present in the brain and in peripheral nerves.

The term 'neurosteroid' should not include all the steroid metabolites that are formed from circulating hormones within the nervous system, however. It should not include androgens and estrogens because they disappear from the brain after gonadectomy (GDX) and adrenalectomy (ADX), and because the cytochrome $P450_{17\alpha}$, which converts pregnenolone (PREG) to dehydroepiandrosterone (DHEA) and PROG to androstenedione, the obligatory precursors of androgens and estrogens – does not seem to be expressed in the nervous system [Mellon, 1994]. Recently, however, $P450_{17\alpha}$ mRNA has been detected at low levels by RT-PCR in the rodent nervous system during embryogenesis [Compagnone and Mellon, 1995]. Glucocorticosteroids too are probably not neurosteroids because their brain levels become

undetectable after removal of the steroidogenic endocrine glands. However, the activity of a 21-hydroxylase and the mRNA of the $P450_{11\beta}$ involved in corticosterone biosynthesis have recently been detected in the brain [Mellon and Deschepper, 1993].

Biosynthesis of Neurosteroids

Which steroids can be synthesized from cholesterol within the nervous system? The first step in steroidogenesis is the conversion of cholesterol to PREG by the side-chain cleavage enzyme cytochrome P450scc in the inner mitochondrial membrane, a conversion characteristic of all steroidogenic cells (fig. 1). The first indication that PREG may also be synthesized within the nervous system came from the unexpected observation that its levels were much higher in the brain than in blood [Corpéchot et al., 1983; Robel et al., 1995]. This finding could not be explained by the cerebral retention of the steroid, as PREG persisted in the brain days after GDX +ADX in spite of a very rapid cerebral clearance [Baulieu, 1991; Robel and Baulieu, 1984]. However, these experiments did not rule out the possibility that PREG in the brain of castrated and adrenalectomized animals may have come from peripheral depots, for example from the progressive release from fat stores.

That the brain has the capacity to synthesize PREG from cholesterol was then demonstrated by different methodological approaches. It was shown by immunocytochemistry that cytochrome P450scc is expressed throughout the brain and in particular in the white matter [Le Goascogne et al., 1987]. The biosynthesis of PREG was then demonstrated by incubating mitochondria of newborn rat oligodendrocytes with [^{3}H]cholesterol, and also in mixed glial cultures with [^{3}H]mevalonate, a precursor of cholesterol which easily enters cells [Hu et al., 1987; Jung-Testas et al., 1989]. More recently, it has been shown by RT-PCR and in situ hybridization that the P450scc is expressed at low levels in cultured glial cells and throughout the adult brain [Mellon and Deschepper, 1993; Sanne and Krueger, 1995]. In agreement with the predominant localization of P450scc immunoreactivity in white matter, the enzyme is mainly present in cultured oligodendrocytes, which are the myelinating glial cells of the central nervous system (CNS) [Jung-Testas et al., 1989]. However, P450scc immunoreactivity and mRNA have also been detected in type I astrocytes [Mellon, 1994]. So far, the biosynthesis of PREG has not been demonstrated in neurons, but may occur in the rat embryo where P450scc message and protein have been localized in sensory neurons [Compagnone et al., 1995].

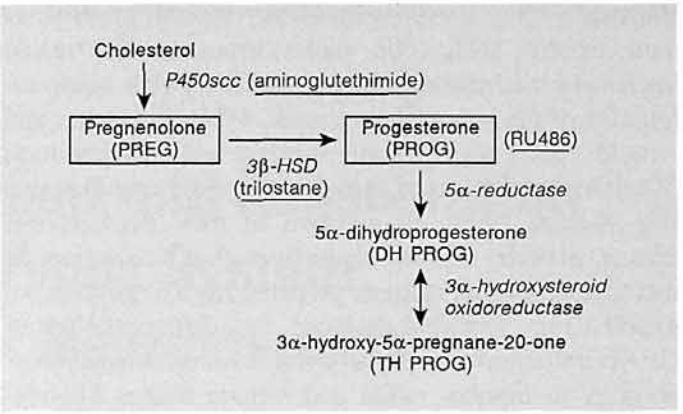

Fig. 1. Main biosynthetic pathways of neurosteroids. Enzymes are shown in italics. P450scc=side chain cleavage cytochrome P450, 3β-HSD=Δ5-3β-hydroxysteroid dehydrogenase isomerase. Aminoglutethimide inhibits the P450scc and trilostane the 3β-HSD. RU486 is an antagonist of PROG at the receptor level.

The synthesis of PREG in the nervous system is not restricted to the CNS. This was first suggested by the high levels of the steroid found in the human sciatic nerve [Morfin et al., 1992]. Also, the concentration of PREG is about ten times higher in the sciatic nerves of adult male rats than in plasma and is not reduced 5 days after GDX+ADX, strongly suggesting a local synthesis independent of glandular sources [Akwa et al., 1993b]. Schwann cells, prepared from newborn rat sciatic nerves, were shown to be a source of PREG. When incubated in the presence of [^{3}H]-25-OH-cholesterol, a cholesterol analogue which easily enters the cells, low amounts of [^{3}H]PREG were formed. The formation of PREG was only observed when Schwann cells were cultured in the presence of forskolin and a micromolar concentration of insulin, which may activate the IGF-I receptors present on the Schwann cell membrane [Schumacher et al., 1993]. Interestingly, cAMP and IGF-I also both stimulate the expression of cytochrome P450scc in classical steroidogenic cells [Oonk et al., 1989; Magoffin et al., 1990]. Thus, the synthesis of neurosteroids in glia may be modulated by growth factors and intracellular messengers.

PREG can be converted by the 3β-hydroxysteroid dehydrogenase Δ5/4-isomerase (3β-HSD) to PROG, which, in turn, can be metabolized successively to 5α-dihydroprogesterone (5α-DH PROG = 5α-pregnane-3,20-dione) by the 5α-reductase(s) and to 3α,5α-tetrahy-

droprogesterone (3α,5α-TH PROG = 3α-hydroxy-5α-pregnan-20-one = allopregnanolone) by the 3α-hydroxysteroid oxidoreductase(s) (3α-HOR, also frequently called 3α-hydroxysteroid dehydrogenase or 3α-HSD) (fig. 1). Neither PROG nor 5α-DH PROG disappeared from brain, contrary to plasma, in male and female rats as late as 2 weeks after combined GDX + ADX [Corpéchot et al., 1993]. The observation that trilostane, an inhibitor of the 3β-HSD, decreased PROG and increased PREG in the brains of GDX + ADX rats is consistent with the synthesis of PROG within the brain and shows that the formation of PROG is an important metabolic pathway for PREG in nervous tissues [Young et al., 1994; Robel et al., 1995].

The mRNA for 3β-HSD has been detected by RT-PCR and in situ hybridization in the brain and spinal cord of adult male rats, and three of the four known isoenzymes of the 3β-HSD (type I, II and IV) were identified [Guennoun et al., 1995a; Sanne and Krueger, 19955]. Mixed cultures of CNS glial cells and cultures of type I astrocytes isolated from embryonic or neonatal rat brains have been shown to convert [^{3}H]PREG to [^{3}H]PROG and its reduced metabolites [Jung-Testas et al., 1989; Kabbadj et al., 1993; Akwa et al., 1993a]. Whether oligodendrocytes have the capability to synthesize PROG from PREG remains to be established, but this would be consistent with the high expression of 3β-HSD mRNA in white matter [Sanne and Krueger, 1995]. Myelin-forming oligodendrocytes have been shown to possess a high 5α-reductase activity and it has been proposed that this enzyme may be incorporated into myelin sheaths [Celotti et al., 1992].

The 3α-HOR converts 5α-DH PROG to 3α,5α-TH PROG, also called allopregnanolone, well known as an allosteric modulator of the $GABA_A$ receptor (see below). In one study, 3α,5α-TH PROG could not be detected in the brains of adult male rats 2 weeks after GDX + ADX, although PREG, PROG and 5α-DH PROG remained present [Young et al., 1994]. However, it has also been reported that 3α,5α-TH PROG can still be measured as late as 3 weeks after GDX + ADX of adult males and that levels of 3α,5α-TH PROG differ between brain areas, its concentrations being highest in the olfactory bulb and low in the hippocampus [Cheney et al., 1995]. The latter observations match the finding that protein levels of the 3α-HOR, as determined by Western immunoblotting, and the activity of the enzyme, are elevated in the olfactory bulb and only moderate in the hippocampus [Khanna et al., 1995]. The 3α-HOR is thus expressed in a region-specific fashion within the brain.

PROG and its reduced metabolites can also be synthesized from PREG by Schwann cells of peripheral nerves. In the sciatic nerve of adult male rats and mice, the concentration of PROG is about ten times higher (~10 ng/g) than in plasma, and remains elevated after removal of the steroidogenic endocrine glands [Koenig et al., 1995]. Schwann cells, isolated from rat dorsal root ganglia (DRG) explants, convert [^{3}H]PREG to [^{3}H]PROG and its reduced metabolites 5αDH-PROG and 3α,5αTH-PROG. The presence of the 3β-HSD in the cultured Schwann cells has been confirmed by immunocytochemistry [Koenig et al., 1995].

Other metabolites, of unkown biological significance, can be formed from PREG and PROG, namely 7α-hydroxylated and 20α-reduced metabolites [Akwa et al., 1992; Robel and Baulieu, 1994]. PREG is also found as sulfate ester (PREG S) in the brain but the formation of sulfate esters in the nervous system, although very likely, has not been documented so far.

Dehydroepiandrosterone: A Neurosteroid?

As described above, cytochrome $P450_{17\alpha}$, which converts PREG to DHEA, does not seem to be expressed in the brain [Mellon and Deschepper, 1993]. However, DHEA, mainly in its sulfated form (DHEA S) remains present in the brain long after removal of the steroidogenic endocrine glands. It was in fact the first steroid shown to accumulate in the brain independently of peripheral sources [Corpéchot et al., 1981; Baulieu, 1991]. Other studies have confirmed that the concentrations of DHEA remain virtually unchanged in the rat brain after GDX + ADX [Korneyev et al., 1993]. Thus, DHEA will be referred to as a neurosteroid, although the precursor(s) and enzyme(s) responsible for its biosynthesis are unknown.

Regulation of Neurosteroid Biosynthesis

Little is known concerning the regulation of neurosteroid synthesis and metabolism. In both gonads and adrenal glands, the regulation of steroid production can be mediated by changes in the expression of steroidogenic enzymes. Transcriptional regulation of the P450scc gene is mediated by several factors, including the steroidogenic factor-1 (SF-1), an orphan nuclear receptor [Clemens et al., 1994]. SF-1 is also expressed in embryonic mouse forebrain [Ikeda et al., 1994] and

could thus play a role in the regulation of neurosteroid biosynthesis during early development. However, the neuroanatomical distribution of SF-1 message, as determined by in situ hybridization, does not match the one described for P450scc [Compagnone et al., 1995]. Moreover, SF-1 is absent from C6 glioma cells, which convert cholesterol to PREG [Papadopoulos et al., 1992]. It is thus possible that P450scc expression in the brain may be regulated by factors different from those known to be present in steroidogenic endocrine glands.

Two factors, which stimulate steroidogenesis, have been recently identified. Steroidogenesis-stimulating protein (STP), isolated from Sertoli cells, and steroidogenic acute regulatory protein (StAR), isolated from adrenal cortex, respectively stimulate the formation of PROG and PREG in steroidogenic cells [Boujrad et al., 1995; Sugawara et al., 1995]. Whether these two factors are also expressed in nervous tissues remains to be determined.

The increase in PREG synthesis by activation of the peripheral-type benzodiazepine receptor (PBR) has been particularly well documented in glial cells. These receptors differ from central benzodiazepine sites by their lack of coupling to $GABA_A$ receptors and by their ligand specificity [Papadopoulos et al., 1995]. As described above, steroidogenesis begins with the metabolism of cholesterol to PREG by the inner mitochondrial membrane P450scc enzyme. Detailed studies have shown that the rate of PREG formation depends on the rate of cholesterol transport from intracellular stores to the inner mitochondrial membrane and it has been proposed that the PBR, a multimeric complex, may form a pore through which the cholesterol molecules could be translocated into the mitochondria [Papadopoulos, 1993]. Agonists of the PBR, such as Ro 5-4864, stimulate the biosynthesis of PREG in C6 glioma cells by increasing the transport of cholesterol into the mitochondria [Guarneri et al., 1992; Papadopoulos et al., 1992]. A role of the PBR in neurosteroid synthesis has also been demonstrated in vivo: after intravenous infusion of PBR ligands into GDX + ADX male rats, concentrations of brain PREG dramatically increased within minutes (70–100%) [Korneyev et al., 1993].

Cyclic AMP (cAMP) is an important activator of steroidogenesis in endocrine glands, where its intracellular levels are raised by gonadotropins, and responsive elements for cAMP have been identified in the promoter region of the main steroidogenic enzymes [Waterman et al., 1992; Omura and Morohashi, 1995]. It is likely that cAMP also regulates the synthesis of neurosteroids in glial cells, because they express a large variety of receptors on their membrane, for example β-adrenergic and adenosine receptors, the activation of which increases the formation of intracellular cAMP [Murphy et al., 1991; Giaume et al., 1991]. It has been suggested that the addition of dibutyryl cAMP to cultures of rat glial cells may increase the formation of [^{3}H]20-OH PREG from [^{3}H]mevalonolactone [Jung-Testas et al., 1989] and cAMP has been shown to stimulate PREG formation from mevalonolactone in isolated rat retinae [Guarneri et al., 1994] and in C6 glioma cells [Papadopoulos and Guarneri, 1994]. In these cells, the β-adrenergic receptor agonist isoproterenol also stimulated cholesterol side-chain cleavage, and its effect could be blocked by Rp-cAMP, an antagonist of the cAMP-dependent protein kinase. Analogues of cAMP also significantly increase the activity of the 5α-reductase in cultured glial cells [Melcangi et al., 1995].

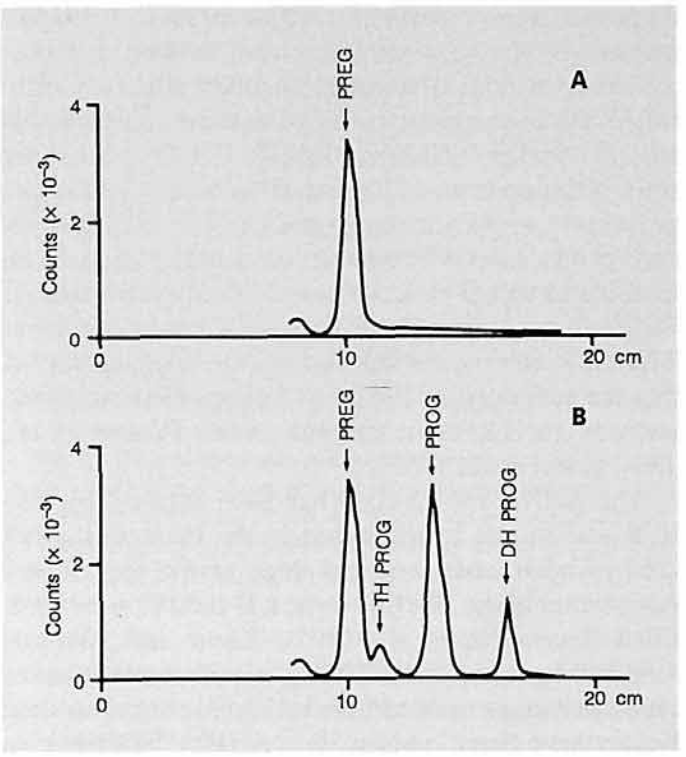

Fig. 2. Thin layer chromatograms showing the metabolites of [^{3}H]PREG formed by rat Schwann cells in culture: [^{3}H]PREG (100 μ*M*) is not metabolized by pure cultures of rat Schwann cells prepared from neonatal rat sciatic nerves (**A**) (M. Schumacher, A.N. Do-Thi, H. Koenig and E.E. Baulieu, unpublished work), but is converted by Schwann cells grown in the presence of sensory neurons for 4 weeks (**B**). From Koenig et al. [1995].

An interesting question: how do cell-to-cell interactions influence the synthesis of neurosteroids? A recent study suggests a role for autocrine factors in the regulation of neurosteroid synthesis by astrocytes. These glial cells preferentially convert [^{3}H]PREG to [^{3}H]PROG at low cell density and to 7α-OH PREG in dense cultures [Akwa et al., 1993a]. The synthesis of neurosteroids in glial cells is also influenced by diffusible neuronal factors, which still have to be identified. Thus, in coculture experiments, the formation of 5α-DH PROG and 3α,5α-TH PROG by astrocytes was found to be slightly increased by a diffusible neuronal factor [Melcangi et al., 1995]. Progesterone and its 5α-reduced metabolites are also produced by Schwann cells, but only in response to a neuronal factor. Thus, Schwann cells isolated from neonatal rat sciatic nerves do not convert [^{3}H]PREG to [^{3}H]PROG. In contrast, Schwann cells which have been cocultured for 4 weeks with sensory neurons, produce significant amounts of [^{3}H]PROG and its reduced metabolites 5αDH-PROG and 3α,5αTH-PROG when incubated with [^{3}H]PREG (fig. 2) [Schumacher and Baulieu, 1995]. By using a coculture system in which sensory neurons and Schwann cells were separated by a microporous membrane, we have shown that neurons induce the synthesis of PROG in Schwann cells by diffusible molecules [Schumacher et al., 1995].

Synthesis of Neurosteroids by Neurons

An increasing number of studies shows that certain neurons, in addition to glial cells, also synthesize neurosteroids. The presence of the cytochrome P450scc in selected neurons of the rat brain was first suggested by immunocytochemistry [Le Goascogne et al., 1987]. Recently, P450scc-positive neurons have been identified in the rat retina [Guarneri et al., 1994] and possibly in sensory neurons of the mouse embryo [Compagnone et al., 1995]. Rat cerebellar granule neurons in culture were found to express P450scc mRNA at levels comparable to that found in glial cells [Sanne and Krueger, 1995].

Neurons appear also to be able to convert PREG to PROG. A detailed study using both in situ hybridization and immunocytochemistry has shown that the 3β-HSD is expressed in neurons of the rat olfactory bulb, striatum, cortex, thalamus, hypothalamus, septum, hippocampus and cerebellum. PCR-amplified cDNA from cerebellum indicated the presence of the type I isoform, which is also expressed in the gonads and adrenal glands [Guennoun et al., 1995a]. Like the P450scc message, 3β-HSD mRNA is present in cultured granule neurons of the cerebellum [Sanne and Krueger, 1995]. Our recent findings show that the 3β-HSD is also expressed by DRG neurons of embryonic and adult rats and that these neurons can convert [^{3}H]PREG to [^{3}H]PROG and to [^{3}H]5α-DH PROG [Guennoun et al., 1995b].

A surprising observation has been made by studying the distribution of 3β-HSD immunoreactivity in the brain of the frog *Rana ridibunda* using an antiserum raised against the human placental enzyme. Immunoreactivity was exclusively expressed in neurons of the hypothalamus and in numerous fiber tracts, but could not be detected in glial cells [Mensah-Nyagan et al., 1995]. Whether this applies to other species of lower vertebrates remains to be established.

Cell culture experiments have shown that glial cells convert 5α-DH PROG to 3α,5α-TH PROG, but that neurons convert it to 3β,5α-TH PROG [Robel et al., 1991]. That is, the 3α-HOR appears to be mainly present in glial cells [Martini et al., 1993; Melcangi et al., 1994] and a nonneuronal localization of the 3α-HOR has been demonstrated in vivo: when kainic acid, a toxin that destroys neurons but leaves glia intact, was injected into the rat olfactory bulbs, the activity of the enzyme was undiminished [Krieger and Scott, 1989].

Interactions with Circulating Steroids

Steroids present in the nervous system may be synthesized locally by glial cells and neurons or they may originate from the endocrine glands. Steroids like PROG easily cross the blood-brain barrier [Pardridge, 1994], but to our knowledge, no information is available concerning the permeability of the blood-nerve barrier to steroids. Circulating PROG has indeed been shown to largely contribute to brain levels of PROG, 5α-DH PROG and 3α,5α-TH PROG. In the female rat brain, concentrations of these progestagens are particularly high during pregnancy, they are reduced by GDX + ADX and are positively correlated with the plasma concentrations of PROG. Injections of PROG (500 μg daily during 8 days) caused a nearly 40-fold increase of brain PROG. However, neither PROG nor 5α-DH PROG completely disappeared from the brain, contrary to plasma, after combined GDX + ADX, consistent with their synthesis in the brain [Corpéchot et al., 1993].

Another recent study has reported that GDX + ADX significantly reduced the brain levels of PROG, DH-PROG and TH-PROG and that the intravenous adminis-

tration of PREG S elicited a large increase in their levels [Cheney et al., 1995]. These data show that part of the brain PROG may be derived from circulating precursors and raises several important questions: Do steroids derived from the circulation have the same effects on nervous functions as steroids which are synthesized locally? How do circulating and brain-derived steroids interact in regulating neuron and glial functions? For example, some brain functions are activated by the sequential actions of estradiol and of PROG. It is thus possible that PROG, synthesized within the brain, may potentiate the actions of circulating estrogen. All these questions need to be answered by specific experiments.

Mechanisms of Action of Steroids in the Nervous System

Research over the past decade has revealed diverse modes of action of steroids [Baulieu and Robel, 1995]. They can activate the transcription of sensitive genes after binding to specific intracellular receptors [Truss and Beato, 1993], or they can modulate neurotransmission by acting on the neuronal membrane, presumably directly on receptors for neurotransmitters. Surprisingly, most of the effects of neurosteroids described so far pertain to the latter mechanism of action. It is beyond the scope of this review to give a complete survey of the diverse membrane effects of neurosteroids [for recent reviews, see Chadwick and Widdows, 1990; Schumacher, 1990; Orchinik and McEwen, 1993; Brann et al., 1995]. Only a few important examples will be cited. A now classical example is the modulation of the $GABA_A$ receptor by 3α,5α-TH PROG. This neurosteroid has been shown to facilitate GABA action at nanomolar concentrations and to open the Cl^- channel at micromolar concentrations [Majewska, 1992; Paul and Purdy, 1992]. These actions may explain the anesthetic, hypnotic and anxiolytic effects of some progestagens and make 3α,5α-TH PROG one of the most potent modulators of neuronal activity. Transfection studies have shown that the effects of 3α,5α-TH PROG on the $GABA_A$ receptor are dependent on its subunit composition [Lan et al., 1991; Shingai et al., 1991]. In contrast, PREG S and DHEA S appear to be excitatory neurosteroids because they antagonize $GABA_A$ receptor-mediated Cl^- uptake into synaptosomes and Cl^- conductance in cultured neurons [Majewska et al., 1992].

Another target for neurosteroids is the nicotinic acetylcholine receptor. PROG inhibits the response of this receptor in a voltage-insensitive manner, most likely by binding to an allosteric site [Valera et al., 1992; Léna and Changeux, 1993]. Progesterone has also been found to inhibit *d*-[^{3}H]SKF-10,047 binding to sigma (σ) receptors [Su et al., 1988]. More recently, it has been shown that PROG, PREG S and DHEA S modulate, via σ1-receptors, the N-methyl-*D*-aspartate (NMDA)-evoked [^{3}H]norepinephrine release from preloaded hippocampal slices. In this system, DHEA S acted as an agonist, PREG S as an inverse agonist and PROG as an antagonist [Monnet et al., 1995]. Several studies have reported that PREG S may be a positive allosteric modulator of the NMDA receptor and potentiate the NMDA-mediated increase in intracellular Ca^{2+} [Irwin et al., 1992; Fahey et al., 1995]. However, the physiological significance of these findings remains uncertain because of the high micromolar concentrations of steroid used in these studies ($\geq$100 μ*M*). The list of neurotransmitter systems which are sensitive to neurosteroids is still increasing. Yet, the physiological significance of the widespread effects of neurosteroids on neurotransmission remains to be elucidated.

In addition, steroids produced within the nervous system may activate protein synthesis by the mediation of classical genomic actions. Thus, PROG may potentiate myelination in peripheral nerves most likely by binding to intracellular receptors in Schwann cells (see below). Some neurosteroids may activate gene expression after their metabolic conversion. Thus, 3α,5α-TH PROG can be converted back to 5α-DH PROG, which can bind to the intracellular receptor for PROG and activate gene transcription [Rupprecht et al., 1993].

Behavioral and Neuroendocrine Effect

Neurosteroids influence memory processes, at least partially by modulating $GABA_A$ neurotransmission. Infusion of PREG S, which inhibits GABAergic neurotransmission, into the nucleus basalis magnocellularis (NBM) of the rat enhanced memory performance in a two-trial recognition task, whereas 3α,5α-TH PROG, which potentiates GABAergic neurotransmission, disrupted memory performance when injected before the acquisition trial [Mayo et al., 1993; Robel et al., 1995]. The intracerebroventricular administration of DHEA, PREG and their sulfates, which inhibit the $GABA_A$ receptor, has also been shown to enhance memory performance in a foot-shock avoidance test in mice, PREG and PREG S being the most potent [Flood et al., 1992].

PREG S also improves acquisition and retention of a food search task [Isaacson et al., 1994].

In particular test situations, neurosteroids also inhibit aggressive behavior. Thus, DHEA and its analog 3β-methyl-androst-5-en-17-one inhibited the aggressive behavior of female mice and castrated males towards lactating female intruders [Haug et al., 1989], presumably by decreasing the formation of PREG S within the brain. In fact, the degree of inhibition of aggressive behavior was related to the decrease in brain concentrations of PREG S [Young et al., 1991; Robel et al., 1995].

A recent study in our laboratory has shown that 3α,5α-TH PROG stimulates the release of gonadotropin-releasing hormone (GnRH) from immortalized rat GnRH neurons (GT1-1 cells) by modulating the $GABA_A$ receptor. Thus, 3α,5α-TH PROG increases muscimol-induced secretion of GnRH from GT1-1 cells which express both the α1- and β3-subunits of the $GABA_A$ receptor. The neurosteroid also increased GnRH secretion when added alone to the culture medium. Interestingly, GT1-1 cells are able to convert [^{3}H]PROG to [^{3}H]3α,5α-TH PROG, suggesting an autocrine mechanism of action of 3α,5α-TH PROG [El-Etr et al., 1995].

Effects on Neurons

Because of their multiple effects on various neurotransmitter systems, it is not surprising that neurosteroids are important for the growth and survival of nerve cells. However, neurosteroids may also provide a neurotrophic support and play a significant role in neuronal regeneration. Thus, adding 10 n*M* DHEA or DHEA S to the culture medium greatly enhances the survival and differentiation of neurons prepared from embryonic mouse brain [Bologa et al., 1987]. The role of PREG and PROG during regeneration of the nervous system has been documented by several in vivo studies. Treating adult rats with PROG increases the survival of motoneurons following axotomy [Yu, 1989] and reduces cerebral edema, secondary neuronal degeneration and behavioral impairment that accompany contusion of the rat frontal cortex [Roof et al., 1994]. These beneficial effects of PROG were first suggested by the observations that males have significantly more edema than females after cortical contusion and that edema is almost absent in pseudopregnant female rats, which have high levels of PROG [Roof et al., 1993]. This is a significant finding because the presence or absence of edema determines the chance of recovery after traumatic brain damage.

An important role has been attributed to PREG during the recovery from spinal cord injury. After compressive injury of the rat spinal cord, immediate treatment with subcutaneous pellets of PREG reduced histopathological changes of the nervous tissue, spared the tissue from secondary injury and increased the recovery of motor functions [Guth et al., 1994]. Whether these are direct effects of PREG or whether they are mediated by metabolites of PREG, such as PROG, remains to be determined. In any case, these observations strongly suggest that neurosteroids, and in particular PREG and PROG, may be useful to attenuate the consequences of traumatic brain and spinal cord injuries, though the mechanisms by which these steroids exert their beneficial effects remain to be elucidated. They may do so by regulating neurotransmission, by exerting trophic effects on the damaged nervous tissue or by potentiating the effects of peptide growth factors. For example, estradiol has been shown to increase the expression of brain-derived nerve factor (BDNF) within the hippocampus [Singh et al., 1995] and estrogen receptors are co-localized with low-affinity NGF receptors in cholinergic neurons of the basal forebrain [Toran-Allerand et al., 1992] and in DRG neurons [Sohrabji et al., 1994]. Studying the possible neurotrophomodulatory functions of neurosteroids is a promising field of research with possible clinical implications.

Effects on Glial Cells

In response to brain injury, astrocytes and microglia proliferate, participate in the repair of the disrupted blood-brain barrier and promote neuronal survival and neuritic growth by secreting growth factors and cytokines. On the other hand, excessive proliferation of reactive astrocytes forms a physiological barrier which impedes axonal regrowth. Only few reports describe the effects of neurosteroids on glial functions during regeneration. PROG has been shown to inhibit the proliferation of astrocytes in culture [Jung-Testas et al., 1992]. As astrocytes can synthesize PROG from PREG, this neurosteroid may be an autocrine regulator of astroglial proliferation after injury. A recent study has shown that PREG, PREG S and DHEA regulate the morphology of astrocytes in hippocampal slice cultures from adult castrated male rats. Both PREG and PREG S increased GFAP-immunoreactive processes, PREG S being the most potent steroid. Although DHEA did not affect the extension of GFAP-immunoreactive processes from