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Novel Perspectives for Progesterone in Hormone Replacement Therapy, with Special Reference to the Nervous System

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The utility and safety of postmenopausal hormone replacement therapy has recently been put into question by large clinical trials. Their outcome has been extensively commented upon, but discussions have mainly been limited to the effects of estrogens. In fact, progestagens are generally only considered with respect to their usefulness in preventing estrogen stimulation of uterine hyperplasia and malignancy. In addition, various risks have been attributed to progestagens and their omission from hormone replacement therapy has been considered, but this may underestimate their potential benefits and therapeutic promises. A major reason for the controversial reputation of progestagens is that they are generally considered as a single class. Moreover, the term progesterone is often used as a generic one for the different types of both natural and synthetic progestagens. This is not appropriate because natural progesterone has properties very distinct from the synthetic progestins. Within the nervous system, the neuroprotective and promyelinating effects of progesterone are promising, not only for preventing but also for reversing age-dependent changes and dysfunctions. There is indeed strong evidence that the aging nervous system remains at least to some extent sensitive to these beneficial effects of progesterone. The actions of progesterone in peripheral target tissues including breast, blood vessels, and bones are less well understood, but there is evidence for the beneficial effects of progesterone. The variety of signaling mechanisms of progesterone offers exciting possibilities for the development of more selective, efficient, and safe progestagens. The recognition that progesterone is synthesized by neurons and glial cells requires a reevaluation of hormonal aging. (*Endocrine Reviews* 28: 387–439, 2007)

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Abbreviations: ADX, Adrenalectomized; ARK, aldo-keto reductase; BDNF, brain-derived neurotrophic factor; CBP, CREB-binding protein; CEE, conjugated equine estrogens; CNS, central nervous system; CREB, cAMP response element-binding protein; CSF, cerebrospinal fluid; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; ER, estrogen receptor (isoforms ERα and ERβ); GABA, γ-aminobutyric acid; GFAP, glial fibrillary acidic protein; HERS, Heart and Estrogen/Progestin Replacement Study; HRT, hormone replacement therapy; HSD, hydroxysteroid dehydrogenase; hu-mPRα, human membrane progesterone receptor α; MCAO, middle cerebral artery occlusion; MDN, mediodorsal thalamic nucleus; MPA, medroxyprogesterone acetate; mPR, membrane PR; MRI, magnetic resonance imaging; nAChR, nicotinic acetylcholine receptor; NBM, nucleus basalis magnocellularis; NMDA, *N*-methyl-D-aspartate; PAIRBP1, plasminogen activator inhibitor RNA binding protein-1; PBR, peripheral benzodiazepine receptor; PEPI, Postmenopausal Estrogen/Progestin Interventions trial; PGRMC1, progesterone membrane receptor component 1 (formerly 25-Dx); PNS, peripheral nervous system; PR, progesterone receptor (PR-A and PR-B); PREG, pregnenolone; RODH, retinol/sterol dehydrogenase; SDR, short-chain dehydrogenases/reductase; SRC, steroid receptor coactivator; SSRI, selective serotonin reuptake inhibitor; StAR, steroidogenic acute regulatory protein; TBI, traumatic brain injury; TSPO, translocator protein (18 kDa) (formerly PBR); WEST, Women's Estrogen for Stroke Trial; WHI, Women's Health Initiative.

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I. Introduction

A WIDELY USED therapeutic approach for relieving the symptoms, preventing the risks, and reversing some of the pathological changes related to the menopause is to compensate ovarian hormone deficiency by the administration of estrogens, alone or in combination with progestagens. Experimental research and observational clinical data have indeed provided evidence for the beneficial effects of postmenopausal hormone replacement therapy (HRT) on the aging nervous, vascular, and skeletal systems. However, more recently, the utility and safety of chronic hormone use in postmenopausal women has been seriously put into question by three large trials that showed no benefit and even potential hazards of postmenopausal HRT: the Heart and Estrogen/Progestin Replacement Study (HERS), the Women's Estrogen for Stroke Trial (WEST), and the Women's Health Initiative (WHI) (see *Section II*).

Their outcome has been extensively commented upon in the recent literature and has brought many fundamental issues of hormone therapies to light, but discussions were mainly limited to the effects of estrogens (1–5). In fact, progestagens are generally only considered with respect to their usefulness in preventing uterine hyperplasia and malignancy in response to estrogens. Thus, in a recent review on the clinical effects of progestagens, it was stated that "the only indication for the addition of progestins to estrogen-replacement therapy is endometrial protection" (6). There is even a debate about the real usefulness of progestagens in protecting the endometrium, and the possibility of omitting them from HRT has been considered (7). However, this may underestimate the potential therapeutic promises of progestagens, and in particular those of natural progesterone. These have been particularly well documented for the nervous system, where progesterone itself and its metabolites regulate vital neuronal and glial functions and, like estrogens, exert neuroprotective and neurotrophic effects (8–12). On the contrary, the effects of the natural hormone and its metabolites on peripheral tissues, including blood vessels, bone, and even classical targets such as the mammary glands, are still a matter of controversy and need to be studied further.

There are many excellent recent reviews on the effects of estrogens on the brain and on cognitive functions and their potential usefulness in HRT, but the effects of progestagens are surprisingly underrepresented in the literature. Two recent papers had the merit of at least calling attention to the potential usefulness of progestagens for HRT and of reminding readers that menopause is characterized by the concomitant loss of estradiol and progesterone (13, 14). The major aim of the present review is to discuss the pleiotropic effects of progesterone and its metabolites in the nervous system and their implications for preventing or treating age-dependent changes and dysfunctions of the brain and peripheral nerves.

Before discussing the neurotrophic, neuroprotective, and promyelinating actions of progesterone, a succinct description will be provided of the major recent HRT trials that have stimulated so much debate and also created some confusion. The commonly used nomenclature for progestagens will then be clarified, stressing the differences between natural progesterone and its synthetic analogs. Promising neural targets of progesterone within the aging nervous system will be examined in detail, including neurons, glial cells, and the myelin sheaths, and the question of whether the aging nervous system remains sensitive to its actions will be discussed, as will the question of whether it is meaningful to attempt the treatment of age-related changes with progestagens. As a matter of fact, when addressing such an important and fundamental problem, it is necessary to also refer to the work on estrogens (15–23). Indeed, progesterone and estradiol often act in a concerted manner within target cells, and both steroids frequently exhibit similar properties. However, there are also opposing effects; whereas estrogens increase the excitability of neurons, progesterone and its reduced metabolites in general reduce their activity, an effect that may significantly contribute to their neuroprotective actions (24). The effects of progesterone in peripheral tissues will be examined before drawing attention to novel perspectives for the use of progestagens in HRT, resulting from the recent discoveries of their multiple signaling mechanisms and of their local synthesis by neurons and glial cells.

II. The Recent HRT Trials

Three major prospective clinical trials that have led to the questioning of the usefulness of HRT are the HERS, the WEST, and the WHI. The HERS trial compared the effects of conjugated equine estrogens (CEE) plus medroxyprogesterone acetate (MPA) treatment with placebo on cardiovascular functions in 2763 women with prior coronary disease. Results showed an increase in coronary heart disease during the first year of hormone treatment and no overall cardiovascular benefit with longer follow-up (25). The WEST trial was a randomized, double-blind, placebo-controlled trial of estradiol therapy (1 mg/d) in 664 postmenopausal women (mean age, 71 yr), who already had an ischemic stroke or transient ischemic attack. This large trial found no benefit of estrogen treatment on cerebral stroke incidence, but it found an increased risk of fatal stroke (26).

The WHI trial comprised two very large placebo-controlled arms: combined estrogen plus progestin and estrogen only. The combined estrogen plus progestin WHI trial involved more than 16,000 women with an intact uterus who received either placebo or CEE (0.625 mg/d) plus MPA (2.5 mg/d) (mean age, 63 yr). This arm of the WHI, designed to continue until 2005, was already terminated in 2002 because the overall risks from use of combined HRT outweighed the benefits: there was a slight increase in the risks of breast cancer and of cardiovascular complications, a significant increase in the levels of inflammatory biomarkers, and an increased risk of ischemic stroke (27–30). Within this arm of the WHI trial, the effects of the combined HRT on cognitive functions were examined in a subgroup of 4532 women aged 65 yr or older. This so-called "Women's Health Initiative Memory Study" (WHIM) found no improvement of cognitive functions and no protection against mild cognitive im-

pairment. Instead, the study revealed a very small increase in the risk of cognitive decline and dementia, including Alzheimer's disease (women with dementia: placebo = 22; CEE+MPA = 45 per 10,000 person-years) (31, 32). The explanation for the small increase in dementia is unknown, but it may result from vascular events (33).

The estrogen-alone arm of the WHI trial compared the effects of CEE alone (0.625 mg/d) *vs.* placebo in 10,739 postmenopausal women with prior hysterectomy. The use of CEE in the absence of MPA had no incidence on breast cancer or on coronary heart disease, but again an increased risk of cerebral stroke was observed (34). Thus, in all the trials, HRT was found to be associated with an increased risk of cerebral accidents. A recent retrospective analysis of 28 trials, involving a total number of 39,769 women, was consistent with this conclusion and revealed that among women who had a stroke, those taking HRT had a worse outcome (35). As with CEE+MPA, estrogen alone was also found to have adverse effects on cognition in a smaller recent study involving 2808 women aged 65 yr or older (36).

Subsequent to these trials, many medical organizations have recommended that HRT should not be used for the prevention of age-related diseases and, when used for treating acute climacteric symptoms, only at the lowest dose and for the shortest time. These recommendations have recently been renewed by the French Agency for Health Product Safety. Accordingly, estrogen alone or combined estrogen-progestagen HRT has been relegated to strictly short-term treatment of symptoms such as hot flushes at the beginning of the menopause (37–41).

III. Progesterone, Progestagens, and Progestins

Before discussing the activities of progestagens in the nervous system, it is important to clarify the terminology and to call attention to the fact that not all progestagens behave the same. They do indeed exhibit profound differences according to their structure, and it is certainly not correct to consider them as equivalent compounds, as unfortunately continues to be done. Thus, after the WHI trials, concern has been directed toward progestagens as a single class. Worse, the term progesterone has even been used as a generic one for the different types of natural and synthetic progestagens in recent papers. "Progesterone" should in fact only be used to designate the natural hormone, produced in the corpus luteum of the ovary after ovulation, in the placenta during pregnancy, in the adrenal glands and, as shall be discussed later, also in the central and peripheral nervous systems (CNS and PNS). The term "progestagen" (also sometimes wrongly spelled "progestogen") corresponds to a functional definition and refers to natural or synthetic steroids which, like progesterone, possess progestational activity: preparing and maintaining the uterus for pregnancy. This generally accepted definition may be too restrictive in the light of the pleiotropic actions of progesterone, and in particular of its close metabolites, which do not bind to the intracellular progesterone receptors (PRs), but exert important biological activities. This is the case of allopregnanolone (3α,5α-tetrahydroprogesterone), which is a potent positive modulator of γ-aminobutyric acid (GABA) type A ($GABA_A$) receptors and has been qualified as "neuroactive" (42). The multiple functions of allopregnanolone and its interactions with $GABA_A$ receptors will be discussed in detail later.

Progesterone is indeed unidirectionally converted by steroid 5α-reductases to 5α-dihydroprogesterone, which also activates gene transcription via the intracellular PR (Fig. 1). These nicotinamide adenine dinucleotide phosphate (reduced form)-dependent enzymes convert a number of Δ4–3-ketosteroids, including progestagens, glucocorticoids, mineralocorticoids, and androgens, into their 5α-reduced metabolites. Two 5α-reductase isozymes are encoded by distinct genes. The type 1 isoform is expressed throughout the rat brain at all stages of development, whereas the type 2 isoform shows a more restricted distribution: it is expressed in the brain almost exclusively around birth, and it is present in the adult spinal cord mainly within gray matter (43, 44).

The bidirectional metabolism of 5α-dihydroprogesterone is catalyzed by two types of enzymes: the cytosolic nicotinamide adenine dinucleotide phosphate-dependent aldo-keto reductases (ARKs) and a subgroup of the membrane-bound nicotinamide adenine dinucleotide-dependent short-chain dehydrogenases/reductases (SDRs), the so-called retinol/sterol dehydrogenase (RODH)-like group of SDRs (45, 46) (Fig. 1). The four ARK1C1-ARK1C4 isoforms are frequently designated as hydroxysteroid dehydrogenases (HSDs), but also as hydroxysteroid oxidoreductases to insist on the supposedly bidirectional character of the enzyme reactions. However, although bidirectional *in vitro*, the ARKs may only function in the reductive direction in living cells, and they may thus be mainly responsible for the reduction of 3-ketosteroids to 3α-hydroxysteroids, and more specifically of 5α-dihydroprogesterone to allopregnanolone (47). On the other hand, the oxidation of 3α-hydroxysteroids to 3-ketosteroids, and more specifically of allopregnanolone to 5α-dihydroprogesterone, is thought to be catalyzed by the RODH-like SDRs (46). In humans, the RODH-like SDRs comprise four enzymes with 3α-HSD activity. Interestingly, two of them also exhibit 3$\alpha\rightarrow$3β-hydrosteroid epimerase activity, as shown both *in vitro* and in living cells, and they may thus play a crucial role in the control of the local concentrations of biologically active allopregnanolone (48, 49). Indeed, as described in detail in *Section X.B*, some of the neuromodulatory and protective effects of progesterone are mediated by allopregnanolone, a very potent modulator of $GABA_A$ receptor activity. On the contrary, the 3β-epimer of allopregnanolone, iso-allopregnanolone (3β,5α-tetrahydroprogesterone), is not only inactive at $GABA_A$ receptors but is also known to antagonize the effects of allopregnanolone (50–52).

The term "progestin" is not used in a consistent manner. It designates both natural and synthetic progestational molecules, including natural progesterone, or exclusively synthetic ones. In the present review, the term progestin will only be used to designate synthetically produced progestagens, including both C19 testosterone derivatives (19-nortestosterone derivatives) and progesterone derivatives (17α-hydroxyprogesterone derivatives and 19-norprogesterone derivatives) (Fig. 2 and Table 1). The pleonasms "natural progesterone" and "synthetic progestins" will be sometimes used to insist on the difference. The 19-norprogesterone de-

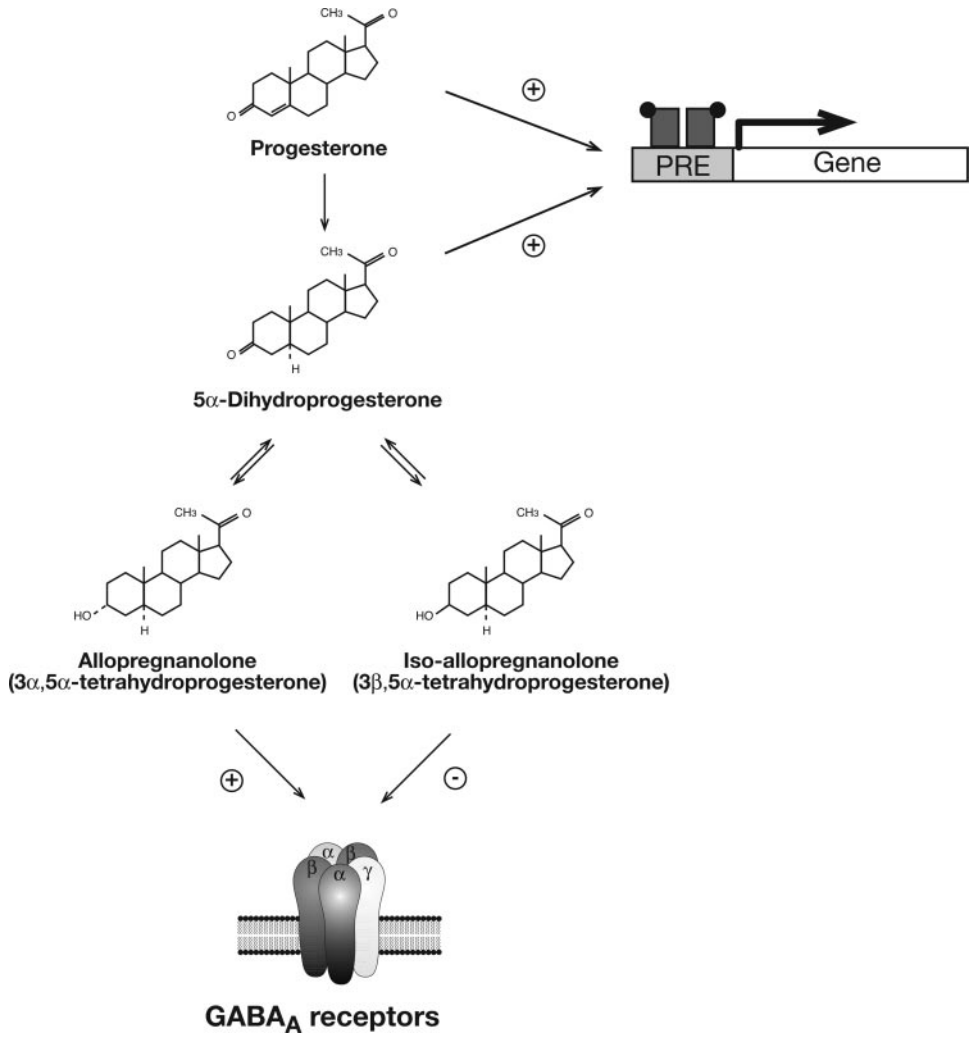

FIG. 1. Metabolism of progesterone. Progesterone is unidirectionally converted by the steroid 5α-reductases to 5α-dihydroprogesterone. Both progesterone and 5α-dihydroprogesterone bind to the intracellular progesterone receptors, which activate gene transcription by interacting with progesterone response elements (PRE, also referred to as glucocorticoid/progesterone response elements, GRE/PRE) often located in the promoter regions of target genes. The bidirectional metabolism of 5α-dihydroprogesterone is catalyzed by two types of enzymes: the cytosolic ARKs, which may only function in the reductive direction *in vivo*, and the membrane-bound RODH-like group of the SDRs, which oxidize allopregnanolone to 5α-dihydroprogesterone or epimerize it to iso-allopregnanolone. Whereas allopregnanolone is a positive modulator of GABA$_A$ receptors, iso-allopregnanolone is an inhibitor.

rivatives, such as 19-norprogesterone, promegestone (R5020), and nomegestrol acetate, are among the most selective agonists of the PR, and they are sometimes referred to as "pure" progestagens because as they do not in principle possess androgenic, estrogenic, or glucocorticoid activities (53–55). However, the other progestins bind to several steroid receptors and sometimes exhibit a wide range of nonprogestagenic biological effects. Thus, the 17α-hydroxyprogesterone derivative MPA, the most commonly prescribed replacement progestin in the United States and the one used in the recent large HRT trials, also has androgenic and glucocorticoid properties (56). The synthetic 19-nortestosterone-derived progestins, such as norethisterone acetate, a progestin commonly used in Europe, retain varying degrees of androgenic activity despite the removal of carbon 19 (57, 58).

In contrast to progesterone, progestins are not converted to the GABA$_A$ receptor-active metabolite allopregnanolone, whose importance in mediating some of the biological effects of progesterone will be discussed later. Nevertheless, progestins are also extensively metabolized in various tissues, but their metabolites are not well characterized. Some of the 19-norprogestins may have the potential to be converted to neuroactive metabolites. Thus, 19-nortestosterone-derived progestins including norethisterone, levonorgestrel, and gestodene are extensively converted to 5α-, 3α,5α- and 3β,5α-reduced metabolites (59, 60). Interestingly, whereas the 5α-reduction significantly increases the androgenic potency of testosterone, the 5α-reduction of norethisterone results in a significant diminution of androgenicity (61). The 3β,5α-reduced metabolites of norethisterone, levonorgestrel, and gestodene bind to ERα, although with a lower affinity than estradiol, and activate gene transcription via this receptor (60, 62, 63).

Whether A-ring-reduced metabolites of progestins act on GABA$_A$ receptors needs to be clarified. Norethisterone acetate and MPA were shown to produce some anxiolytic-like effects when rats were tested in the "elevated plus maze" and the "shock-probe burying test". In contrast, the norprogesterone derivative trimegestone only had little effect (64). However, these behavioral effects of the progestin metabolites do not necessarily result from the direct modulation of GABA$_A$ receptors. Indeed, some observations suggest that the administration of progestins affects the concentrations of endogenous allopregnanolone in the brain and influences the activity of enzymes involved in the metabolism of progestagens (65, 66). For example, MPA (Provera), which does not

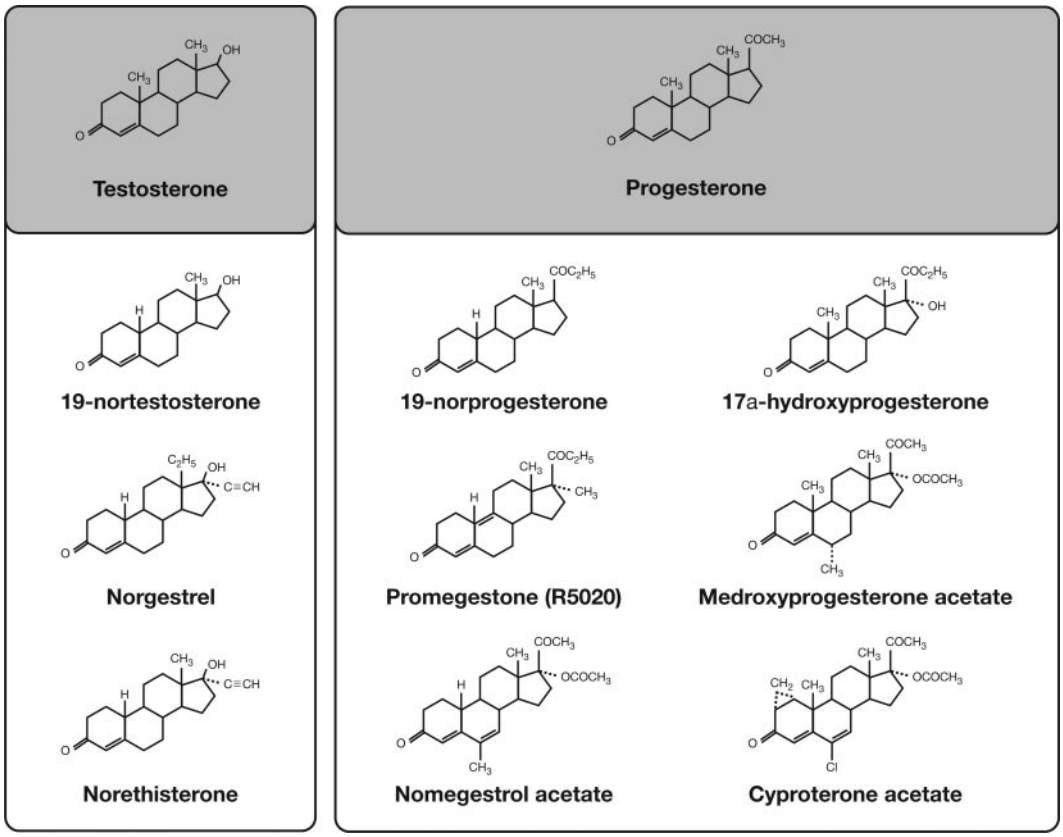

FIG. 2. Chemical structures of progestins. Comparison of the chemical structures of testosterone, progesterone, and progestins, comprising testosterone derivatives and progesterone derivatives (19-norprogesterone and 17α-hydroxyprogesterone derivatives). Small structural changes account for important differences in the effects of progestins.

directly act on $GABA_A$ receptors, enhances $GABA_A$ receptor-mediated inhibitory neurotransmission in the rat hippocampus by inhibiting the metabolism of allopregnanolone (67). Another recent study has shown that 2-wk oral treatment with MPA increases allopregnanolone levels within the hippocampus, cerebral cortex, and hypothalamus of ovariectomized female rats (68). The effect of MPA on the endogenous levels of brain allopregnanolone may explain why the progestin is therapeutically beneficial for catamenial epilepsy (69) and sometimes improves anxiety and mood in postmenopausal women (70, 71).

Only a few progestins have been tested for their effects on the nervous system, but concerns are particularly serious about the negative effects of MPA. Thus, MPA has been shown to antagonize the neuroprotective and promnesic effects of estrogen. Whereas progesterone and 19-norprogesterone, alone or in combination with estradiol, protected cultured hippocampal neurons against glutamate toxicity,

TABLE 1. Classification of synthetic progestins

19-Nortestosterone derivatives	19-Norprogesterone derivatives (C-20)	17α-Progesterone derivatives (C-21)
Estranes (C-18)	Promegestone (R5020)	Medroxyprogesterone acetate (MPA)
Norethisterone (NET) (= norethindrone)	Nomegestrol acetate (NOMAC)	Cyproterone acetate
Norethisterone acetate (NETA)	Trimegestone (TMG)	Chlormadione acetate
Gonanes (C-17)	Nestorone (NES)	Megestrol acetate
Levonorgestrel (LNG)	Demegestone	**Others**
Desogestrel (DSG)	Medrogestone	Drospirenone (DRSP)
Gestodene (GES)		Dydrogesterone
Norgestrel (NG)		
Norgestimate		
Etenogestrel		
Norgestimate		
Dienogest (DNG)		

Progestins are derived from either testosterone (19-nortestosterone derivatives) or progesterone (19-norprogesterone derivatives and 17α-hydroxyprogesterone derivatives). The 19-nortestosterone derivatives are further subdivided into estranes (with 18 carbons) and gonanes (with 17 carbons). The gonanes norethynodrel, lynestrenol, and ethynodiol are converted to norethisterone in the body. Other progestins are drospirenone, derived from spirolactone, and dydrogesterone, a highly selective stereoisomer of natural progesterone (a retroprogesterone with an additional double bond between C-6 and C-7) (adapted from Refs. 53, 55, 826, and 827). For the pharmacological characteristics of progestins, see Refs. 54 and 828–830.

MPA not only failed to be effective but also attenuated the estrogen-induced neuroprotection. At the molecular levels, MPA blocked estrogen-induced expression of the antiapoptotic protein Bcl-2 and antagonized estradiol-induced attenuation of the glutamate-induced rise in intracellular calcium (72, 73). Thus, one of the most prescribed progestins for HRT and contraception opposes some of the beneficial effects of estradiol in the brain and may even exacerbate the excitotoxic death of neurons (74). *In vivo*, MPA has recently been reported to diminish the ability of CEE to reduce stroke damage in subcortical regions of the rat brain (75). In female monkeys, treatment with MPA reduced the increase in sexual initiation induced by estradiol treatment and increased aggressive behavior, which may represent a serious behavioral side effect (76). MPA has also been shown to directly inhibit the activity of steroidogenic enzymes, in particular of the human type II 3β-HSD, an enzyme that converts pregnenolone (PREG) to progesterone, and the progestin thus interferes with steroid biosynthetic pathways (77).

It is important to draw attention to differences in HRT regimens between countries. In the United States, the most commonly used progestin is MPA, generally combined with CEE, an association of more than 10 different estrogens. Most of them are sulfated and distinct from the predominant endogenous estrogens in women, that is, estradiol before and estrone after menopause (78). Nevertheless, estrogen components of CEE have recently been shown to have potent antioxidant and neuroprotective effects and also to reduce the cortical infarction volume in a rodent model of stroke (75, 79–81). In the United Kingdom, the progestins mainly used are 19-nortestosterone derivatives (norethisterone acetate, norgestrel, and levonorgestrel). In central and southern Europe, both 19-nortestosterone derivatives and a range of progesterone derivatives are used. In France, micronized progesterone and 19-norprogesterone derivatives are commonly prescribed in combination with oral or transdermal estradiol (82–84). It would certainly be worthwhile to attempt retrospective comparisons of the different HRT formulations.

Oral micronized progesterone has been widely used in Europe, and in particular in France, since 1980. Micronized progesterone is natural progesterone, whose average particle size has been reduced, leading to decreased destruction in the gastrointestinal tract, a longer half-life, and enhanced bioavailability. Before, the discovery of the micronization process, progesterone could not be taken orally because it is poorly absorbed and rapidly metabolized. The use of micronized progesterone is well tolerated, with mild and transient sedation as a side effect that can be minimized by taking the hormone at bedtime (85). Moreover, the elevation of circulating levels of progesterone by oral administration of the micronized hormone has been shown to be as effective as the administration of progestins for the control of endometrial growth (86, 87). Earlier studies have also reported that micronized progesterone may improve mood in patients with premenstrual mood disturbances and in postmenopausal women (88, 89). When compared with the MPA-containing regimen, micronized progesterone was found to improve significantly vasomotor symptoms, somatic complaints, anxiety, and depressive symptoms in postmenopausal women (90). However, work by Bäckström and collaborators (91, 92) has shown that treatment with progesterone can also result in adverse mood changes (tension, irritability, depression), and that the metabolite allopregnanolone may be the mediator of these effects. Thus, in two studies of postmenopausal women with climacteric symptoms, negative mood effects during treatment with vaginal progesterone implants were related to the blood concentrations of allopregnanolone. During the progesterone treatment period, women had increased negative mood symptoms when compared with the estradiol-only period, but only when serum concentrations of allopregnanolone were increased to those seen during the midluteal phase of the menstrual cycle, not when they were either higher or lower (91, 92). These observations suggest a bimodal association between allopregnanolone and adverse mood, and they point to the importance of a well-dosed HRT.

IV. Trophic and Protective Effects of Progesterone in the Nervous System

A. Neuroprotective effects

Neuroprotective effects of progesterone have been demonstrated in different lesion models, notably in populations of neurons that are particularly sensitive to excitotoxic and ischemic damage. Such vulnerable neurons, which are generally characterized by high metabolic activity and abundant excitatory afferents, include the pyramidal neurons of hippocampus and cerebral cortex, dopaminergic neurons of the midbrain, Purkinje cells of the cerebellum, as well as neurons of the dorsal striatum and the caudate nucleus (93, 94). Thus, the administration of progesterone reduced the loss of neurons in the CA1 and CA2 subfields of the dorsal hippocampus and within the caudate nucleus after experimentally induced ischemia in cats (93, 95). In rats, progesterone given before middle cerebral artery occlusion (MCAO), decreased the infarct size and neurological deficits (96, 97). A recent study on functional outcomes after MCAO in male mice showed a beneficial effect of progesterone on survival rate, weight recovery, and motor ability evaluated by the grid and rotarod tests. Noteworthy, the spatial memory performance of the mice evaluated in the Morris water maze was also preserved by the progesterone treatment (98).

Beneficial effects of progesterone have also been demonstrated in experimental models of traumatic brain injury (TBI). A much-studied system corresponds to bilateral contusion lesion of the rat medial prefrontal cortex, which produces cognitive deficits typically observed after human frontal lobe injury (99, 100). The medial prefrontal cortex receives glutamatergic and cholinergic afferents, respectively, from the mediodorsal thalamic nucleus (MDN) and from the nucleus basalis magnocellularis (NBM). TBI leads to edema, to secondary excitotoxic neuronal death in the vicinity of the lesion, and subsequently to retrograde neuronal degeneration in both MDN and NBM (101). Edema is a very important negative factor for the outcome of TBI. Therefore, the observation that progesterone treatment reduced both edema and secondary neuronal losses and improved behavioral recovery after TBI in male rats was particularly encouraging. Females are protected by their high endogenous levels of pro-

gesterone, and their brains have much less water content after TBI when compared with males (102, 103). Following these important observations, a phase II, randomized, double-blind, placebo-controlled trial, named "ProTECT", has been conducted in Atlanta to test the usefulness of progesterone as a treatment for moderate to severe TBI. In this study, which included 100 trauma patients, stable progesterone levels were rapidly achieved after TBI by its iv infusion (104). The very promising outcome of the trial has now been published. Progesterone-treated patients had a lower 30-d mortality rate than controls, and survivors of moderate TBI who received progesterone had better outcomes. However, the administration of progesterone had no effect on the disability of severe TBI survivors. It is important to note that no adverse events could be attributed to progesterone in this trial (105).

What makes progesterone a particularly attractive neuroprotective agent for the treatment of brain lesions is its surprisingly large therapeutic window. Even when administered as late as 2 h after the onset of MCAO, progesterone still provided therapeutic benefit (106), and the steroid was effective in reducing edema and in protecting neurons after TBI when treatment was delayed as much as 24 h after injury (107). Pretreating ovariectomized female rats with low physiological concentrations of progesterone also allowed hippocampal neuron loss in response to TBI to be reduced (108). With respect to the duration of the progesterone treatment and its mode of administration, available experimental data show that both prolonged and continuous administration of the hormone leads to more complete behavioral recovery after TBI (109, 110).

An important finding was that administration of the enantiomer of progesterone (*ent*-progesterone) also decreased cerebral edema, neuron death, inflammatory cytokines, and reactive gliosis (111). Enantiomers of steroids indeed have a therapeutic potential for treating lesions and age-dependent dysfunctions of the nervous system (112, 113). An enantiomer is a mirror-symmetric, non-superimposable image of a molecule, with identical physical properties, except for the different rotation of polarized light, but with different biological actions (114). The protective effects of *ent*-progesterone were not mediated by the intracellular PR because the compound did not activate PR-mediated gene transcription, and its mechanisms of action, which may involve membrane receptors, need to be clarified. A previous study had shown that *ent*-progesterone is a potent competitive inhibitor of human enzymes involved in steroid metabolism, namely, the cytochromes P450c17 and P450c21 (115).

Neuroprotective effects of progesterone have also been demonstrated for midbrain dopaminergic neurons. Both progesterone and estradiol were found to protect dopaminergic neurons against degeneration induced by 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine, a finding consistent with a possible role of these hormones in the deterioration of dopaminergic functions with age and in the development of Parkinson's disease (116). There is indeed a higher incidence of Parkinson's disease in men when compared with women, and the risk of its occurrence is increased in women with an early onset of menopause (117).

In the spinal cord of male rats, chronic treatment with progesterone for 5 d reduced the size of the lesion and prevented secondary neuronal loss after contusion injury (118). Progesterone is indeed an important neuroprotective factor for spinal motoneurons, as has been shown after spinal cord lesion and in a genetic model of motoneuron disease, the Wobbler mouse (11, 119, 120). After spinal cord transection, progesterone treatment preserved the Nissl bodies of the ventral horn motoneurons, restored choline acetyltransferase levels, normalized the expression of the Na,K-ATPase, and increased growth-associated protein of 43 kDa (GAP-43) and brain-derived neurotrophic factor (BDNF) message and protein (121, 122).

The Wobbler mouse is a particularly useful model for the study of motoneuron diseases, including amyotropic lateral sclerosis. The Wobbler phenotype is due to a missense mutation in the gene encoding the vacuolar-vesicular protein sorting factor Vps54 (123). The first manifestations of the disease are already observed at 2–3 wk of age (124, 125). When 2-month-old, symptomatic Wobbler mice presenting tremor, ambulatory difficulty, and diminished muscle strength received sc implants of progesterone that produced constant high physiological levels of the hormone for only 2 wk, the neuropathological changes of spinal motoneurons were less severe, motoneuron vacuolation was reduced, and there was better preservation of the endoplasmic reticulum and of the mitochondria. Most importantly, progesterone treatment also had beneficial effects on muscle strength of the animals (126–128). The link between the mutant Vps54 and the severe impairment of Wobbler motoneurons needs to be explored, but it is likely that the mutation results in altered axonal transport. It was thus a significant finding that retrograde axonal transport is indeed impaired in Wobbler motoneurons, as shown by the injection of fluorogold into the limb muscles and its retrograde tracing, and that it can be restored by treating the animals for 8 wk with progesterone (119).

Only a few experimental studies have reported an absence of effect or negative actions of progesterone in the injured nervous system (attention has already been drawn in *Section III* to the disruptive effects of progestins such as MPA). Thus, one study did not find a beneficial effect of progesterone after ischemic insult in senescent female rats (129), and two dose-response studies have raised concerns about the possibility that high doses of progesterone (30–60 mg/d) may exacerbate the outcome of MCAO in ovariectomized female rats or of TBI in male rats (130, 131). The influence of sex hormones may also be dependent on the type and the severity of a brain lesion. Thus, sex differences in the outcome of TBI favoring female rats have been consistently observed after diffuse weight-drop-induced TBI, but these results could not be confirmed after more severe focal impact injury, qualified as "focal TBI" and characterized by a very fast evolution of neurodegeneration (132, 133). One study has reported that progesterone inhibited the neuroprotective effects of estradiol in the rat hippocampus observed after systemic kainate administration, which induces excitotoxic neuron death (134). Although very sparse, these negative results indicate that one should remain cautious and demonstrate the need for more research on the modes of action of progesterone and of its metabolites in the nervous system.

The mechanisms by which progesterone promotes morphological and functional recovery after brain injury are indeed not well understood, and they seem to involve multiple actions, some of which may be particularly relevant to the potential role of progesterone in the aging nervous system. In this regard, the above-mentioned observation that progesterone may improve impaired axonal transport is particularly interesting. There is indeed strong evidence that the slowing of axonal transport may be a key event in the aging process of the brain and of peripheral nerves (135–138). Reduced axonal transport has also been proposed to play an early and causative role in the development of Alzheimer's disease. That is, deficits in axonal transport are characteristic of the early stages of the disease in mouse models and in patients, and they may lead to aberrant amyloid-β peptide formation and subsequently to neurodegeneration (139).

Another important consequence of progesterone treatment is the reduction of lipid peroxidation, but the underlying mechanisms still need to be specified (140). The peroxidation of lipids is indeed a complex phenomenon, involving distinct enzymatic pathways as well as nonenzymatic mechanisms, such as free radical mediated peroxidation, and it is reduced by the actions of different antioxidant enzymes (141). During aging, there is an increase in the concentration of lipid peroxidation products, and the oxidative damage of lipids may play an important role in mediating or even initiating specific aspects of age-dependent changes (142). In addition, the oxidation of neuronal lipids resulting from an oxidative imbalance has been proposed to play a significant role in Alzheimer pathogenesis and, consequently, may represent an interesting therapeutic target at early stages of the disease (143). The increased exposure of aging tissues to oxidative stress partly results from decreased activity of antioxidant enzymes such as the superoxide dismutases. The prolonged treatment of middle-aged and old acyclic female rats (12, 18, and 24 months of age) with low doses of progesterone, estradiol, or a combination of both steroids increased superoxide dismutase activity and reduced lipid peroxidation (144, 145).

The age-dependent accumulation of oxidative damage is also a consequence of a decline in mitochondrial function, another major component of the normal aging process and of neurodegeneration (146–150). Many of the reactive oxygen species involved in oxidative stress are indeed toxic byproducts of the mitochondrial energy production pathway, and they damage not only lipids, but also proteins and nucleic acids. Over the past few years, attention has indeed focused on the role of the mitochondria in brain aging and neurodegeneration (151–153), and this cellular organelle is another important target for the actions of progesterone. One of the prevalent neuropathological changes found in spinal motoneurons of Wobbler mice is damaged mitochondria with severe vacuolation, and treatment with progesterone allowed the restoration of a normal appearance of the mitochondria (120). Progesterone can protect neurons against apoptotic cell death by increasing the expression of antiapoptotic proteins residing in the outer mitochondrial membrane, such as Bcl-2, and by down-regulating proapoptotic gene expression (bax and bad) and the caspase-3 enzyme (73, 154, 155). The expression of Bcl-2-family proteins is regulated by nuclear steroid actions, and the newly synthesized proteins are translocated to the outer mitochondrial membrane (156).

Some studies have suggested direct actions of steroids on mitochondria. Thus, estrogen and glucocorticoid receptors have been detected in mitochondria, and the mitochondrial genome contains nucleotide sequences with high similarity to known steroid-responsive elements (157–160). It has also been proposed that estradiol may protect against the formation of mitochondrial reactive oxygen species by directly acting on mitochondrial estrogen receptors (ERs) (161). Whether progesterone may also exert direct effects on mitochondria remains to be explored. In the low physiological range, progesterone has been shown to completely reverse postinjury alterations in mitochondrial respiration (108). The mitochondria is also a target for other steroids, such as dehydroepiandrosterone (DHEA), and it is the site where the first step in steroid hormone biosynthesis takes place, the conversion of cholesterol to PREG by the cytochrome P450scc, as shall be discussed later. There obviously exist complex relationships between steroids, mitochondrial activity, oxidative stress, the aging of brain cells, and neurodegenerative events. Unfortunately, the available data are still much too fragmentary for an integrated picture.

Progesterone exerts many other actions, which can be related to its neuroprotective effects and may also have implications for the aging brain. Thus, progesterone regulates the expression of aquaporin 4 in the injured brain, a membrane-channel protein involved in water homeostasis, which is largely distributed throughout the brain and may play a significant role during edema formation (162). It is puzzling that some of the actions of progesterone resemble those of the estrogens. For example, like estradiol, progesterone up-regulates the expression of antiapoptotic proteins such as Bcl-2 (163–165), reduces inflammation by repressing the activation of microglial cells and by inhibiting the production of proinflammatory cytokines (101, 166–168), up-regulates the expression of neurotrophins such as BDNF (122, 169, 170), and protects neurons against glucose deprivation and the toxicity of glutamate, $FeSO_4$, and β-amyloid peptides (72, 171, 172).

B. Promyelinating effects

Progesterone is also known to have a role in myelination and remyelination. By preserving or restoring the integrity of myelin sheaths, which insulate the large axons and are required for the efficient and rapid conduction of electrical impulses along nerve fibers, progesterone may not only play an important role in the efficient communication between neurons but also promote their viability. In fact, myelin has neuroprotective functions, and myelin-associated proteins influence the caliber of axons (173). Moreover, after injury, secondary demyelination of spared axons contributes to neuronal degeneration, the extension of the lesion, and functional deterioration (174).

A role of progesterone in myelination was first demonstrated in the regenerating mouse sciatic nerve after lesion as well as in explant cultures of rat dorsal root ganglia composed of sensory neurons and Schwann cells, which are the myelinating glial cells of the PNS (175). Progesterone also

enhanced the rate of myelin formation in dissociated cocultures of neurons and Schwann cells (176). Whether progesterone promotes myelination of peripheral nerves directly by acting on Schwann cells, or indirectly by acting on neurons, needs to be clarified because there are contradictory reports. Whole-cell radioligand binding assays suggested the presence of specific and saturable progesterone binding sites in Schwann cells (177). The PR has also been detected in Schwann cells either grown in culture or within the rat sciatic nerve by immunocytochemistry (178). On the contrary, in cocultures of dorsal root ganglia neurons and Schwann cells, the presence of PR mRNA and protein was only detected in the neurons, not in the Schwann cells (179). Also, in a recent study, purified rat Schwann cells and various Schwann cell lines were found to only express extremely low amounts of PR mRNA (180). In the mouse Schwann cell line MSC80, progesterone did not activate the transcription of a progesterone-sensitive reporter gene. Demonstration that the PR was the limiting factor was provided by the positive transcriptional responses obtained when exogenous receptors were transiently expressed (180). Whether present in neurons or glial cells, the PR in the peripheral nervous system is a potential pharmacological target for the therapy of inherited and acquired peripheral neuropathies. In a transgenic rat model of Charcot-Marie-Tooth disease overexpressing the peripheral myelin protein PMP22, the culprit of the disease, treatment with a progesterone antagonist reduced PMP22 expression and had a beneficial influence on the evolution of the disease (181). In a model of diabetic neuropathy, induced in rats by an injection of streptozotocin, prolonged treatment with progesterone or its reduced metabolites had beneficial effects on peripheral nerves at the neurophysiological, functional, and neuropathological levels (182).

In the CNS, brain, and spinal cord, axons are myelinated by oligodendrocytes (183, 184). That progesterone also promotes myelination by oligodendrocytes has been demonstrated in explant cultures of cerebellar slices taken from 7-d-old rats and mice (185). These organotypic cultures closely reproduce developmental events and provide a unique model for examining neuronal survival and maturation, as well as the myelination of axons (186, 187). In these explants, myelination is very intense during the second postnatal week, exactly at a time when endogenous levels of progesterone are elevated in the cerebellum (188, 189). A stimulatory effect of progesterone on myelination was observed in cerebellar slices of both sexes and involved the classical PR: 1) it could be mimicked by the selective progestin promegestone; 2) it was completely abolished by the PR antagonist mifepristone (RU486); and 3) it was not observed in cerebellar slice cultures from 7-d-old PR knockout mice (185). In these slices, progesterone was shown to stimulate the proliferation and maturation of oligodendrocyte progenitor cells (190). An earlier study had already shown that adding progesterone to cultures of glial cells isolated from neonatal rat brains increased the number of oligodendrocytes (191). More recently, the addition of progesterone to the medium of cultured oligodendrocytes has been shown to increase their branching, whereas estradiol stimulated myelin membrane formation (192). Progesterone also promotes remyelination by oligodendrocytes *in vivo*. After toxin-induced demyelination, the systematic administration of progesterone promoted the slow endogenous remyelination of axons within the cerebellar peduncle of aging male rats (193). In the lesioned rat spinal cord, treatment with progesterone was found to increase the density of $NG2^+$ oligodendrocyte progenitor cells and the expression of myelin basic protein (194).

The promyelinating effects of progesterone are particularly relevant when discussing the significance of the hormone in the aging nervous system. Indeed, it is less well appreciated that overall loss of myelin and altered integrity of myelin sheaths are among the most reliable markers of the aging nervous system, correlating with chronological age and cognitive decline (195, 196). Age-dependent changes in brain myelin, which have been extensively studied in rhesus monkeys, include alterations in oligodendrocytes, abnormalities and breakdown of the myelin sheaths, and loss of white matter (195, 197–199). Based on these observations, it has been proposed that myelin changes may significantly contribute to age-related cognitive decline by altering conduction velocities along axons (196). A postmortem study has provided evidence that aging in humans is also accompanied by the loss of myelinated fibers (200). By using the method of diffusion tensor magnetic resonance imaging (MRI), it has been shown that myelin disruption occurs in men even during normal aging. Most importantly, alterations of cortical myelin correlated with declined cognitive ability (201). In parallel with these morphological and functional studies, only a few reports have dealt with biochemical changes in myelin during aging. Increase in water and decrease in cholesterol within white matter have been described (202), and decreased expression of the major peripheral myelin proteins has also been reported in peripheral nerves of aged rodents and humans (203–206).

Importantly, remyelination continues to take place in the brains of aged monkeys, but the newly formed myelin sheaths are thin and have short internodes (207). Age is indeed a negative factor for the capacity to regenerate myelin sheaths, as has been demonstrated in the rodent CNS after demyelination induced by a gliotoxin; in old rats, the process of remyelination takes much longer than in young animals (208–210). The reasons for the age-associated slowing down of myelin repair are not well understood, but impaired recruitment of progenitor cells and their delayed differentiation into myelinating oligodendrocytes, as well as delayed expression of growth factors, may be responsible (211, 212). In humans, differences in the speed of remyelination could explain the much slower functional recovery in older patients after demyelinating diseases such as optic neuritis (213). In addition, a reduced capacity for myelin repair with age is consistent with the observation that the prognosis of multiple sclerosis is mainly age-dependent (214).

In conclusion, a substantial number of animal studies have documented neuroprotective effects of progesterone or its reduced metabolites in the lesioned or diseased nervous system of young adult rodents. Particularly promising for the treatment of traumatic lesions is the large therapeutic window of progesterone. Progesterone may exert neuroprotective effects and promote neuroregeneration by a dual action: by directly acting on neurons and increasing their survival, and by accelerating the formation of new myelin sheaths.

Progesterone and its metabolites may exert similar beneficial effects in the aging brain and peripheral nerves. The significance of progesterone in aging will be further explored when discussing the question of whether the aging nervous system remains sensitive to the beneficial effects of steroids.

V. Neurons and Glial Cells in the Aging Nervous System

A. Aging neurons

Early studies describing massive loss of neurons during nonpathological aging of the brain have been largely refuted by the use of more accurate stereological techniques for the precise counting of cells in histological sections (215). There is indeed no extensive loss of neurons during aging, as previously thought, even within vulnerable brain regions such as the cerebral cortex and the hippocampus (216). Stereological studies have also shown that there is no significant loss of neurons within the hypothalamic nuclei involved in the control of reproductive functions in older women, but rather a substantial remodeling of neuronal circuits and changes in neuropeptide expression (217, 218). Consequently, normal age-associated neuronal impairment is more likely to be mediated by synaptic alterations, which may be reversible, making the treatment of age-related dysfunctions of the brain a therapeutic possibility (219). Even in old rats with impaired spatial learning, no significant neuron loss was observed within the hippocampus (220). Also in patients with Alzheimer's disease, the loss of forebrain cholinergic neurons may not be as important as previously thought. Indeed, within the NBM, only a small subset of the neurons was found to die, but the large cholinergic neurons underwent atrophy and lost their markers (221). Neuronal death is indeed a relatively late stage event in Alzheimer's disease associated with dementia, and alteration of synapses is one of the early pathogenic processes (222–224).

However, brain structures may differ in the involvement of neuron loss, and some populations of neurons may be more affected by the aging process than others. For example, within subregions of the rat hippocampal formation, the number of neurons may significantly decrease at advanced ages, in particular within the subiculum and the hilus of the dentate gyrus (225). Among the most vulnerable cells of the nervous system are the cerebellar Purkinje cells, and there is consistent evidence for their significant loss during normal aging in rodents and humans. The age-dependent loss of Purkinje cells correlates with decreased eye-blink conditioning, a reflex pathway mediated by these neurons, and elderly people with very slow eye-blink conditioning may have an increased risk of becoming demented (226, 227).

B. Aging glial cells

Neurons have long been the main focus of studies on brain aging, and glial changes have been largely neglected. As already point out, there are significant deteriorations of the myelin sheaths with age, which may reflect age-dependent changes in the myelinating glial cells, oligodendrocytes in the CNS and Schwann cells in the PNS. However, the formation and maintenance of myelin sheaths are also dependent on neuronal signals, and there are complex reciprocal interactions between axons and myelinating glial cells in both compartments of the nervous system (228–231). Consequently, any age-dependent alterations of myelin sheaths may result from impaired neuronal functions, from changes in the myelinating glial cells themselves, or from both events.

Another type of glial cell also plays an essential role in brain aging. The general assumption was that the increased number of astrocytes (astrogliosis) during aging may be a consequence of neuron degeneration. However, there is now strong experimental evidence provided by Finch and collaborators (232) that changes in astrocytes are in fact a very early event in the aging process, and that increased glial fibrillary acidic protein (GFAP) expression by astrocytes may contribute to decreased synaptic functioning and plasticity in the aged brain. Indeed, in cocultures of neurons and old astrocytes, diminishing GFAP levels by RNA interference restored neurite outgrowth, whereas overexpression of GFAP in young astrocytes modeled the effects of aging by reducing neurite outgrowth (233). Consistent with these *in vitro* findings is the observation that inactivation of the GFAP gene in mice improves both neuronal survival and neurite growth (234, 235).

Important for the present discussion is the observation that astrocytes can mediate some of the effects of progesterone and estradiol on neuronal plasticity, and that steroids are a critical component of the cross-talk between neurons and glial cells (236, 237). Thus, enhanced neuronal sprouting after lesion in response to estradiol is mediated in part by the repression of GFAP expression in astrocytes (238). Astrocytes are also a target for the actions of progesterone; after a penetrating brain injury, treatment with progesterone decreased astrocyte accumulation in both female and male rats (239, 240). Progesterone was also shown to reduce astrocytic hypertrophy after TBI close to the lesion site (154). However, in two other models, progesterone was not found to modify astrocyte accumulation in rats, either after spinal cord transection or after medial frontal cortex contusion (241, 242).

VI. Gender Differences and Sensitivity to Progesterone

When studying the effects of progesterone on the nervous system, it is important to be vigilant about the possible contribution of structural and biochemical sex differences (243, 244). There is indeed increasing recognition that gender differences may influence the incidence and development of diseases and the responses to therapies (245). Accordingly, the effects of steroids may also differ between females and males, and data obtained for one gender may not necessarily apply to the other. Since the pioneering studies of Raisman and Field (246), important differences in brain structure between males and females have been widely recognized. In rodents, they arise in part through the permanent "organizational effects" of androgens secreted by the testis during sensitive periods in early life (247–249). Sex differences affecting brain structures and functions, including its asymmetry and functional lateralization, are also observed in hu-

mans (250–253). In addition, MRI analysis has revealed that age-specific changes within the human brain are also sexually differentiated (254). The sexual phenotype of brain cells is not determined exclusively by the exposure to steroid hormones during early development, but also by their genetic sex because some sex differences are already established before the maturation of the embryonic gonads (255, 256). More recently, the development of transgenic mouse models has allowed it to be shown that XX and XY brain cells are not equivalent, even when they have developed in a similar hormonal environment (257, 258).

Only a few studies have investigated the possible influence of gender on the response of the adult nervous system to the trophic and protective effects of ovarian steroids, and there are surprisingly few observations of sex differences. A recent study has revealed that the effects of progesterone and its 5α-reduced metabolites on the expression of peripheral myelin protein genes differs between males and females. This was shown by using sex-specific cultures of Schwann cells prepared from neonatal rats (259). On the other hand, the differential sensitivity of the male and female rodent brain to injury appears to be largely determined by the presence of different levels of progesterone. Thus, the more favorable outcome after cerebral stroke or TBI in female rats when compared with males mainly results from the presence of high endogenous levels of progesterone in the females. Similarly, treatment with progesterone provides similar neuroprotection in males and in females after TBI (102, 260).

It is worth mentioning here that a few studies have reported that some brain responses to estrogen are sexually dimorphic. In one study, estradiol was found to improve neurological outcome after TBI in male rats, but to exacerbate brain injury in females (261). In another study, although estrogen therapy protected both male and female brains against ischemic insult, the responses differed between sexes: acute exposure to estrogen was sufficient to ameliorate ischemic brain injury in males, whereas females required longer-term replacement (262). As will be discussed later, the response of aged hippocampal synapses to estrogen is also sexually dimorphic (263).

Very few studies have addressed the question of the effects of gender on the outcome of injury in the human nervous system, and in TBI patients the role of gender is still controversial. One clinical study has reported that female TBI patients have a better outcome than male patients (264). More recently, another group has shown that female patients have lower levels of cerebrospinal fluid (CSF) lipid peroxidation and oxidative damage products, consistent with the already discussed antioxidant properties of ovarian hormones (265, 266). However, other studies have not shown a beneficial effect of female gender on TBI outcome (267, 268).

Gender is also an influential factor in the incidence and progression of multiple sclerosis, a demyelinating disease that selectively affects the brain and spinal cord (214, 269–271). The questions of why more women have multiple sclerosis than men and why it affects women differently from men have been mainly addressed experimentally by examining hormonal influences on autoimmune responses (269, 272, 273). However, the recent observation showing that myelin is sexually dimorphic casts a new light on the role of gender and hormones in the maintenance, alterations, and diseases of myelin (274). In this study, gender differences in myelin components of white matter tracts of young and aged rodents were found to be so dramatic that it was possible to determine the sex of an animal from blind sections immunostained for oligodendrocyte-specific markers. Gonad-derived steroids appeared to be a major contributor to these sex differences because castration of adult males produced a female phenotype (274). Consistent with clinical observations showing that the course of multiple sclerosis is mainly age-dependent, and that women reach disability milestones at older ages than males (214, 275), the extent of oligodendrocyte remyelination after a demyelinating lesion was found to be significantly reduced in aging rats, and middle-aged males and females (12 months of age) differed in their capacity to remyelinate axons. This sex difference was not influenced by castration, suggesting a more stable sex difference (210).

VII. The Sensitivity of the Aging Nervous System to Progesterone and Estradiol

Two fundamental questions need to be addressed when discussing the usefulness of HRT: 1) does the aging nervous system remain sensitive to the actions of ovarian hormones; and 2) do these hormones continue to exert beneficial effects on the aging nervous system? Indeed, the majority of studies documenting beneficial effects of ovarian steroids have been carried out in young adult animals or in cultured cells isolated from embryonic or neonatal tissues. Data on aged animals or cells are rare, and only a few laboratories have examined the question as to whether responses of target tissues to steroids are preserved during the aging process. There is as yet no conclusive answer to this question, and the extent to which mechanisms of neuroprotection are similar in young adults and reproductively senescent animals remains to be clarified. There is however some experimental evidence that the aging nervous system remains, at least to some extent, sensitive to ovarian steroids, and that their administration may even allow reversal of some of the age-dependent structural abnormalities and dysfunctions. On the other hand, there are also indications that some responses of neural cells to hormones may change, even during the normal aging process. Such changes in hormone effects appear to be dependent on the steroid, brain region, and nervous function examined. The reader will notice that in this section explicit reference will also be made to the effects of estradiol. Indeed, a large number of the studies concerning steroid sensitivity of the aged nervous system has tested the effects of estradiol, and some of them are quoted here to exemplify the problem.

A. Maintained sensitivity to ovarian steroids

1. Progesterone. Beneficial effects of progesterone on the aging nervous system have been particularly well demonstrated for myelinated nerve fibers. In *Section IV.B*, progesterone plays an important role in peripheral nerve myelination, and recent studies have shown that treatment with progesterone or its 5α-reduced metabolites allows reversal of age-related

myelin abnormalities. Thus, in the sciatic nerves of aged male rats, a significant decrease in myelin-associated activity of the 5α-reductase, the enzyme that converts progesterone to 5α-dihydroprogesterone (or testosterone to 5α-dihydrotestosterone), is associated with a reduction in myelin gene expression. Treatment of the aged rats for 1 month with progesterone, 5α-dihydroprogesterone, or allopregnanolone allowed reversal of the age-dependent decline in peripheral myelin protein expression, whereas repeated injections of androgens were without effect (204, 205). The administration of progesterone not only counteracted the drop in myelin protein expression, it also allowed reversal of age-related structural abnormalities of the peripheral myelin sheaths. Indeed, the prolonged treatment of old male rats (22–24 months) with progesterone or its 5α-reduced metabolites significantly decreased the percentage of fibers with myelin abnormalities as well as the number of fibers with irregular shapes, and it increased the number of small myelinated fibers. Again, as previously observed for the normalization of myelin gene expression, the effects were specific for progesterone and its metabolites because the administration of androgens was inefficient (276).

As already mentioned, the capacity to repair myelin in the brain decreases with age; spontaneous remyelination after gliotoxin-induced demyelination is very rapid in the brain of young rats, but it is very much delayed in middle-aged rats. Whereas no beneficial effect of progesterone on central myelin repair could be observed in young males (10 wk old), because spontaneous remyelination was too rapid, the implantation of sc progesterone pellets stimulated a slow remyelination of axons in middle-aged animals (9 months old) (193, 277).

It has been proposed that the disappearance of the protection against ischemic brain injury in females after reproductive senescence may be a consequence of ovarian hormone deficiency. However, aging female rats remain responsive to the protective actions of ovarian hormones, at least until a certain age. Thus, in middle-aged female rats (16 months), the administration of either progesterone or estradiol alleviated cerebral stroke (278).

Animal models also support the axiolytic effects of progesterone, which are mediated by its conversion allopregnanolone, a potent positive modulator of $GABA_A$ receptors (see *Section X.B*) (279–281). In fact, the anxiolytic actions of progesterone do not require the intracellular PR because as they are still observed in PR knockout mice, which even exhibit a greater anxiolytic response than their wild-type littermates (282). However, progesterone does not enhance anxiolytic behavior in mice deficient of the type 5α-reductase (283). Most importantly, middle-aged (between 9 and 12 months of age) and old (between 18 and 24 months of age) wild-type and PR knockout mice continue to respond to the anxiety-reducing effects of progesterone (284). That the brain of senescent mice continues to be responsive to progesterone and its metabolites was also demonstrated by the results of another study showing that middle-aged and old female mice primed with estradiol show lordosis behavior after the intraventricular injection of progesterone or allopregnanolone (285). Lordosis is a stereotypic posture adopted by a sexually receptive female rodent in response to a mount by a male.

Another consequence of ovarian hormone deficiency is a decline in cognitive performance. Data concerning the effects of steroids on cognition, and in particular on memory, need to be interpreted cautiously because they are not always consistent. This may reflect the fact that complex behaviors are under the influence of multiple factors, and that behavioral testing procedures differ between laboratories. In particular, the behavioral effects of progestagens can be difficult to interpret, because progesterone and it metabolites can influence cognitive processes by multiple mechanisms. Thus, the rapid neuromodulatory effects of progesterone and allopregnanolone are expected to transiently impair memory performance: 1) progesterone behaves as an antagonist of σ1 receptors and inhibits the promesic effects of σ1 agonists (286); 2) allopregnanolone is a positive modulator of $GABA_A$ receptors, involved in the inhibition of memory processes within the hippocampus and at the level of cholinergic forebrain neurons (287–291) (see *Section X.B*). However, under stressful conditions, the effects of allopregnanolone may be beneficial for memory tasks because of its anxiolytic properties (292, 293). Moreover, although the immediate effect of progestagens may be a diminution of memory functions, because of the rapid modulation of neurotransmitter receptors, in the long run, they may have beneficial effects on cognitive performances because of their trophic and protective actions (294). Two studies have reported an influence of progesterone on cognition in aged female rats. In females ovariectomized at the age of 13 months, the prolonged weekly administration of estradiol and progesterone was found to be slightly more efficient in enhancing acquisition of a spatial memory task than treatment with estradiol alone (295). However, two other studies have reported negative effects of progesterone on cognition and working memory in aged female rats (296, 297).

2. Estradiol. As already mentioned, the administration of either progesterone or estradiol improved the outcome of cerebral stroke in middle-aged female rats (278). In both young (3–4 months) and middle-aged (9–12 months) female rats, replacement with physiological doses of estradiol decreased the extent of ischemic injury in the cerebral cortex (165, 298). It is important to note that these protective effects of the ovarian steroids were not mediated by changes in cerebral blood flow. In another study, the pretreatment of reproductively senescent, female rats (14–18 months) with estradiol alone or combined estradiol plus progesterone also reduced cortical infarct volume after MCAO (129).

Memory performance can also be restored in senescent female rats by estradiol if administered in an appropriate manner, even at an advanced age and after long-term hormone deficiency (299). Consistent with these behavioral findings, cholinergic pathways involved in memory processes can still be activated by estrogen in the brain of aged female rats (24 months) (300). In middle-aged nonhuman primates, multiple cognitive functions also remain sensitive to estrogen (301). Thus, in perimenopausal rhesus monkeys around 22 yr of age, which is at a time when cognitive functions start to decline, estrogen treatment improved memory (302). Even

after many years (up to 16) of estrogen deprivation, estradiol was still able to enhance some aspects of working memory in aged female rhesus monkeys (21–24 yr) (303, 304).

B. Modified sensitivity to ovarian steroids

1. Progesterone. In comparison with the estrogens, examples of age-related changes in the sensitivity to progestagens are sparse. This does not necessarily mean that the aging brain remains more sensitive to progesterone than to estradiol, although this a possibility, but it may simply reflect the fact that fewer studies have been devoted to the effects of progestagens. Among the few studies that have reported a loss in the sensitivity of the aging brain to progesterone, two have shown that acute administration of progesterone either before or after MCAO reduced cortical infarct in young, but not in reproductively senescent female rats (14–18 months old) (129, 305). It has been proposed that changes in the tissue metabolism of progesterone may be one means by which the effectiveness of progesterone decreases during aging (306).

2. The hypothalamic-pituitary-ovarian axis. An age-dependent reduction in the sensitivity to ovarian steroids has been well documented for hypothalamic nuclei involved in reproductive functions. The hypothalamic-pituitary-ovarian axis does indeed become less responsive to the positive feedback effects of ovarian hormones in middle-aged females, but the underlying mechanisms are only partly understood (307, 308). One possibility is that the hypothalamus does not respond to steroids in the same way in young and old females because of changes in the expression or functionality of steroid receptors, but discrepancies between studies make a general conclusion difficult. At least, there appears to be no global and marked decline in brain PR and ER with age (309–313). A recent stereological analysis has even reported an increase in ERα-immunoreactive neurons within specific hypothalamic nuclei of aged females (between 12 and 24 months of age) (314).

Within the hypothalamus, PRs involved in the regulation of reproductive functions are induced by estradiol (315, 316). Again, the ability of estradiol to induce hypothalamic PR seems not to be attenuated with age (309, 317). In a recent study, the induction of PR mRNA by estradiol within distinct nuclei of the hypothalamus was found to be at least as strong in 15-month-old as in young 3-month-old female rats (310). Obviously, decreased hypothalamic ER and PR expression does not provide a satisfactory explanation for reduced steroid sensitivity of the hypothalamus and reproductive senescence in female rats. However, binding and expression studies do not reveal very much about the functionality of steroid receptors. As will be discussed in *Section X.A*, work over the past 10 yr has revealed that the transcriptional efficiency of liganded steroid receptors is determined by nuclear coregulator proteins (318, 319). So far, there is little information concerning the effects of aging on the recruitment of coregulators by steroid receptors, and this is certainly a line of research worth exploring. One study has reported that expression of two coactivators, namely, steroid receptor coactivator-1 (SRC-1) and CREB-binding protein (CBP) are decreased in androgen-sensitive spinal motoneurons of aged rats (320). Another study has shown that low levels of SRC-1 within the olfactory bulb and forebrain of reproductively senescent female rats correlated with the reduced sensitivity of neurotrophins and their receptors to estrogens (321).

Other explanations than reduced steroid receptor expression have been proposed for the decreased ability of estradiol to generate a LH surge in aged females, such as alterations in circadian rhythmicity of the suprachiasmatic nucleus (308, 322). The age-dependent loss of astrocyte plasticity in the rostral preoptic area, where a subgroup of GnRH neurons resides, may also have an impact on the ability of estrogens to activate GnRH neurons because dynamic changes in the coverage of neurons by astrocytes play a key role in synaptic plasticity and efficacy (323, 324).

3. Estradiol. There is a series of studies which document that the response to estradiol of brain regions other than the hypothalamus is modified during the aging process. Within the medial septum and NBM, levels of choline acetyltransferase and of nerve growth factor receptor TrkA mRNA were substantially reduced in old long-term ovariectomized female rats (ovariectomy at the age of 13 months, histological analysis of the brains around 30 months of age). These changes could not be reversed by estradiol plus progesterone replacement, despite the fact that the cholinergic neurons were still present and that their number and size were apparently not affected (325). Other studies have provided evidence that the ability of estradiol to enhance cholinergic and cognitive functions declines with age in rats (299, 326). Thus, estrogen replacement can reduce memory deficits induced by the muscarinic receptor antagonist scopolamine in young and in middle-aged female rats with irregular cyclicity (12–13 months of age), but not at a more advanced age characterized by consistent estrus (20 months of age) (326). It has also been reported that estradiol replacement is not effective in improving working memory performance in female rats after long-term hormone deprivation by ovariectomy. However, when initiated immediately after ovariectomy, estradiol replacement significantly improved memory, even at 17 months of age (327). Based on these experimental findings, the existence of a "window of opportunity" after the loss of ovarian function has been proposed, during which hormone treatments may be most efficient for preventing a decline in cognitive functions (295). This hypothesis will be more closely addressed in *Section VIII*.

In the rodent hippocampus, estradiol regulates multiple aspects of synaptic plasticity involved in memory processes, such as an increase in the number of dendritic spines bearing excitatory *N*-methyl-D-aspartate (NMDA) synapses (328, 329). With age, there is a loss of CA1 synapses, which is not reversible by estrogen replacement: in contrast to young female rats (3–4 months old), estradiol failed to increase the number of dendritic spines in old females (23–24 months old) (330). However, although estradiol did not increase the number of dendritic spines in the hippocampus of aged female rats, it increased the number of NMDA receptors per synapse, which may be understood as a compensatory response (331, 332). It would be interesting to examine whether there is a relation between the increase in synaptic NMDA recep-

tors and the well-known increase in vulnerability of the aged hippocampus to excitotoxicity (333). In female rhesus monkeys, the number of dendritic spines on CA1 pyramidal neurons is also highly responsive to estrogen, but unlike aged female rats, hippocampal neurons of aged female rhesus monkeys (19–23 yr old) retain their capacity for spine induction in response to estrogen (334). Estradiol treatment also increased the density of dendritic spines of pyramidal neurons within the dorsolateral prefrontal cortex of both young and aged female rhesus monkeys, thus demonstrating a maintained responsiveness to estradiol and capacity for plasticity (5, 335).

Interestingly, the effects of steroids on the plasticity of aged synapses can differ between sexes, as has been reported for granule neurons of the dentate gyrus. Male and female rats were gonadectomized at 2 months of age. When examined at 16–20 months of age, the old females had a paucity of dendritic spines on granule neurons, but males showed no decrease in dendritic spines with age. A short-term treatment with estradiol allowed the density of dendritic spines in the old females to be increased, but on the contrary, decreased spine density in males (263).

Another example of reduced sensitivity of the aging nervous system to estrogens is the decrease in their ability to attenuate neuronal damage in response to unilateral entorhinal cortex lesion (336). After this type of lesion, which is used as a model of Alzheimer's disease-like deafferentation of the dentate gyrus, estradiol stimulated compensatory synaptic sprouting in young (3 months old), but not in middle-aged (18 months old) female rats (337). In the same study, it was shown that increased GFAP expression by hippocampal astrocytes in response to the lesion was reduced by estradiol in the young, but not in the middle-aged females. As already discussed, elevated GFAP expression in astrocytes contributes to the reduction in the neuronal sprouting response during aging (233). The increased expression of GFAP in astrocytes, which is progressive and begins before midlife, may thus play an important role in neuron atrophy and impaired synaptic plasticity (233).

Age-dependent impairment has also been documented for the regulation of neurotrophin signaling by estrogen. Estradiol increased the expression of BDNF and of the neurotrophin receptors TrkA and TrkB within distinct brain regions in young adult, but not in reproductively senescent female rats (321). However, another study has reported a comparable increase in TrkA mRNA expression by estrogen in the NBM of young (3 months old) and old (24 months old) rats, suggesting that the sensitivity of neurotrophin signaling to estradiol may be preserved in the aged brain (300).

C. Antagonistic pleiotropy

Some nervous functions may not only lose their sensitivity to steroids with progressing age, but may even become negatively affected by them. According to the "antagonistic pleiotropy" theory of senescence formulated by Williams in 1957, some genes with positive effects upon fitness early in life may become deleterious late in life. Antagonistic pleiotropic effects have been documented for various classes of genes, including those encoding growth factors, hormones, heat shock proteins, and apoptosis regulator proteins (338, 339).

With respect to steroid effects on the nervous system, this concept has only received support from a limited number of experimental and observational studies on estrogens (2), and there is no example of adverse effects of progestagens on the aging nervous system. Whether this situation is due to the smaller number of studies with progesterone or whether it reflects a better toleration of progesterone by the aged brain needs to be clarified. Indeed, in *Section VII.A*, the nervous system of even very old rodents remains surprisingly responsive to the beneficial effects of progesterone.

In contrast to young female rats, the treatment of reproductively senescent females with estradiol exacerbated neural injury and worsened the inflammatory response within forebrain circuits after an excitotoxic lesion (340). Estradiol, known to attenuate cytokine responses, was also shown to increase inflammatory cytokine expression by immune cells in acyclic rats (341). Similarly, effects of estradiol on neurotrophin expression can be opposite in young and aged females. Thus, estradiol increased BDNF expression in young female rats but decreased it in reproductively senescent females (321). The same group has also recently shown that the effects of estradiol on the blood-brain barrier are also dependent on age. Estrogen replacement to surgically castrated young female rodents reduced the permeability of the blood-brain barrier, but conversely made it more permissive in senescent females (342). Together, these findings point to the risk of estrogen replacement in the elderly. Within this line of thinking, an extreme view proposes that decreased estrogen levels may even become beneficial for some populations of neurons in the aged brain (221).

Concerning the cardiovascular system, ovarian steroids may become dangerous by inducing inflammatory responses and by having prothrombotic effects once atheromas and narrowing of blood vessels are established. This view is supported by mouse models of atherosclerosis, which have revealed that estradiol has an atheroprotective effect by acting on healthy endothelium, but induces inflammatory-immune responses once atheromatous plaques are formed (343). In monkeys, estradiol has been shown to inhibit atherosclerotic plaque formation when given directly after ovariectomy, but not 2 yr later (344).

In conclusion, the results of animal studies are rather encouraging because they show that some structures and functions of the aging nervous system remain sensitive to the beneficial actions of ovarian steroids, and in particular to those of progesterone, providing an experimental ground for the usefulness of HRT. Neuronal networks appear to remain in place during the aging process, and age-dependent structural changes and dysfunctions can be improved by the administration of ovarian steroids. However, there is also evidence that some responses to steroids are substantially modified with progressing age, and that aging and long-term deprivation of ovarian steroids may result in the insensitivity of specific brain functions to either progesterone or estradiol. Moreover, there is some concern about possible deleterious effects of estradiol on the brain and blood vessels in aged animals.

VIII. The Timing of HRT: A Therapeutic Window?

There may be therapeutic windows during which HRT is particularly efficient and during which the beneficial effects of hormones may prevail (345). Arguments in favor of this hypothesis have mainly been provided by the outcomes of estrogen-only or combined estrogen-progestin therapies. As in rodents, brain regions involved in the regulation of ovarian activity may also become less sensitive to estradiol during aging in women. Thus, data from the Women's Health Across the Nation (SWAN) study, a survey of women going through the menopause transition, suggest that secretion patterns of estradiol and LH in middle-aged women may reflect an increasing insensitivity to estradiol (346). In premenopausal women, estrogen levels are equivalent or higher than those observed in younger women, but conversely, LH pulse frequency is decreased, consistent with an altered positive estrogen feedback in the brain (347–349).

In particular, at very advanced ages or after very long hormone deprivation, the steroid responsiveness of specific neuronal circuits and cognitive functions may be altered in women. For them, the time when HRT starts relative to the onset of reproductive senescence may be a crucial factor. An accumulating body of evidence indeed suggests that the immediate postmenopausal period may constitute a window of opportunity for HRT to protect against cognitive decline and to reduce the risk of Alzheimer's disease (350, 351). In fact, a major difference between studies that found a protective effect of estrogens on cognitive functions and those that reported negative results was the time when the treatment started. This would be consistent with results of a series of animal studies suggesting that estrogens may be more efficient in preserving memory functions if treatment is started soon after the deprivation of ovarian steroids (304, 352, 353).

A large prospective trial conducted in Utah, named the Cache County Study, has examined the incidence of dementia among 1889 women (mean age, 74 yr) and 1357 men (mean age, 73 yr). Results for women showed that when HRT was initiated at the beginning of the menopause and lasted for at least 10 yr, the incidence of Alzheimer's disease was significantly decreased, and the age-dependent increase in the risk of Alzheimer's disease was abolished. On the contrary, in women who began HRT 10 yr or later after the onset of menopause, there was a tendency for an increased risk in developing Alzheimer's disease (354). The latter observation is thus consistent with the findings of the WHIM showing that HRT, initiated as late as 10 to 20 yr after the onset of menopause, increased the incidence of dementia and Alzheimer's disease (32, 355). Results of another recent study, involving a small number of postmenopausal women, confirm that women with few years since the onset of menopause benefit more from HRT with respect to cognitive functions than women starting later (356). The follow-up of another cohort of 343 women has shown that short-term HRT in the early phase of the menopause and lasting for only 2–3 yr may provide long-term protection against cognitive impairment, still observable 15 yr later (357). All these data are consistent with the view that initiation of HRT early in menopause may have cognitive benefits and reduce the risk of dementia, whereas hormone therapy initiated decades after the onset of hormone deficiency may be without benefit, and may even become unsafe.

It is noteworthy that a similar conclusion has been reached by examining steroid effects on the cardiovascular system. The time when HRT is started relative to the onset of menopause seems indeed to be crucial for its effectiveness on the vascular system (358, 359), and the effects of estrogens and progestagens on the vasculature may also depend on the stage of existing atherosclerosis (360). Moreover, longer exposure to endogenous ovarian hormones as a result of delayed onset of menopause may be protective: for each year's delay in the onset of menopause, the cardiovascular mortality risk was shown to decrease by 2% (358, 359). Thus, duration may also be a critical factor for HRT. In the Rancho Bernardo cohort, women who had used HRT for at least 10 yr had significantly less plaque burden than shorter term users (361). The analysis of data of the Nurses Health Study, which has followed 120,000 female nurses between 1976 and 2000, also suggested that starting HRT earlier may indeed make the difference; women who began HRT near menopause had a significantly reduced risk of coronary heart disease. On the contrary, no significant relation was found between HRT and coronary heart disease among women who initiated therapy at least 10 yr after menopause (362). A recent cohort study has shown that women who receive 2–3 yr of HRT after menopause do not have increased all-cause mortality, but have prolonged cardiovascular benefits (363). Windows of opportunity for the therapeutic benefits of HRT could explain the increased risk of dementia and of cerebro- and cardiovascular events observed in the HERS, WEST, and WHI trials, involving women with a mean age over 65 yr. That is, once atheromas and luminal narrowing of blood vessels are established, adverse effects of HRT may prevail, including vascular inflammation and prothrombotic effects (364, 365).

The importance of starting HRT early after the onset of menopause may also provide an explanation for some discrepancies between previous observational studies, most of which have reported beneficial effects of HRT on the nervous and cardiovascular systems, and the more recent prospective studies. In fact, in most observational studies, women started HRT early, during the perimenopause or at the beginning of the postmenopause period, for the relief of climacteric symptoms, whereas in the recent large trials, women had started HRT as late as 10 to 20 yr or even longer after the onset of menopause.

However, although appearing convincing, the hypothesis proposing a "window of opportunity for HRT" is still awaiting definitive proof. In fact, not all beneficial effects of hormones may be limited to a given time window. There are indeed experimental and clinical observations suggesting that even a delayed initiation of hormone treatment may still have beneficial effects on particular brain functions (366). This view is consistent with the animal studies described in *Section VII.A*, providing evidence that the nervous system of old animals remains at least to some extent sensitive to the beneficial effects of ovarian steroids, in particular of progesterone, and receives further support from recent imaging studies investigating the effects of HRT on the human brain.

Data obtained by high-resolution MRI indeed show that even in elderly postmenopausal women (age range, 57–79 yr), HRT attenuates the shrinkage of both gray and white matter within brain regions known for their sensitivity to age-related decline, such as the prefrontal, parietal, and temporal cortex, and the hippocampus (367). Most importantly, HRT had significantly greater effects on different brain structures with increasing age of the women. This finding is coherent with recent experimental studies in rodents showing that hormone treatments can reverse some age-related structural abnormalities of the nervous system. The MRI study also showed that the longer the duration of HRT, the better the sparing of brain tissue. Women had received unopposed estrogen or estrogen plus MPA, but because of the small number of women in the latter group, it was not possible to draw conclusions on the possible effects of the progestin. Unfortunately, these MRI results have not been related to cognitive measures (367).

Other recent MRI studies have reported significant effects of HRT on regional brain volumes. In elderly women (age range, 60–83 yr), HRT was shown to protect against hippocampal atrophy (368) and to have a positive effect on gray matter volume of cerebellum, parietal, and occipital cortex in women older than 50 yr (369). In another study of postmenopausal women (mean age, 60 yr), HRT was associated with greater gray matter volumes in cerebellum, the amygdaloid-hippocampal complex, and the frontal, temporal, parietal, and occipital cortex. Interestingly, women who underwent HRT sooner after menopause had greater volumes of gray matter compared with women under current treatment (369). This observation agrees with the already mentioned results of the Cache County Study, showing greater cognitive performance and reduced risk of Alzheimer's disease in women with earlier HRT (354). However, the fact that there are also MRI studies which did not find an effect of HRT on gray and white matter volumes, as was the case for a recent cross-sectional study involving 213 postmenopausal women aged 60–64, should not be passed over (370). On the other hand, most of the functional imaging studies, based on the measure of changes in regional cerebral blood flow by positron emission tomography, have consistently shown beneficial effects of HRT on brain activity, in particular within brain regions sensitive to neurodegenerative changes (371, 372).

In conclusion, clinical data indicate that there may be a therapeutic window for optimal efficiency and that it may be important to start HRT early after the onset of menopause. Once age-dependent alterations or diseases of the nervous or cardiovascular systems are established, steroids may lose their efficacy and may even develop adverse effects. However, some beneficial effects of HRT seem to persist even at advanced ages. Whether there is an optimal time interval after the loss of ovarian steroids for efficient hormone replacement intervention needs to be clarified by carefully designed animal and clinical studies. Two clinical trials, the Kronos Early Estrogen Prevention Study (KEEPS) and the Early *vs.* Late Intervention Trial with Estradiol (ELITE), have been launched to collect information on the usefulness of HRT in women at the beginning of their menopause (373, 374). KEEPS is testing whether starting HRT 6 months to 3 yr after the last menstrual period will prevent the progression of atherosclerosis. ELITE is comparing the effects of estrogen treatment started in early menopause with estrogen treatment begun 10 or more years after menopause. Another important factor to be taken into account is the duration of HRT, and it is important to clarify whether longer term HRT users gain more benefits than shorter term HRT users. However, the safety of long-term hormone use in younger women is a very important issue and is still difficult to appreciate.

IX. Effects of Progesterone in Peripheral Tissues

When considering the usefulness of progesterone for protecting aging neurons and for reversing age-related structural changes and dysfunctions of the nervous system, it is necessary to also examine its effects on peripheral tissues, especially on blood vessels, mammary glands, and bones. It is rather surprising for a steroid hormone isolated more than 70 yr ago (375–379) that our knowledge concerning the actions of progesterone in these target tissues is still very limited. Nevertheless, available data are rather encouraging because they point to the safety and also to beneficial effects of progesterone.

A. Blood vessels

In a study using video microscopic recording of blood flow, blood vessel morphology, and activities of various blood cells in live animals, the administration of progesterone did not result in vascular toxicity (380). Moreover, in other studies, progesterone and 19-norprogesterone derivatives were found not to interfere with estrogen protection against vasoconstriction (360, 381–383). A nongenomic endothelium-independent vasorelaxing action of the 19-nortetsosterone derivative norethisterone and of its reduced metabolite 3α,5α-norethisterone have recently been demonstrated (384).

However, there has been some concern with high doses of progesterone (385, 386). Also, a recent study has suggested that progesterone treatment may exacerbate the cerebrovascular inflammatory response to lipopolysaccharide (387). This finding contrasts with the reports of the beneficial effects of progesterone in experimentally induced cerebral ischemia, where inflammation of the cerebral vasculature is a key process (see *Section IV.A*). Obviously, more research on the effects of natural progesterone on the vascular system is needed (388, 389).

However, as for the nervous system, serious concerns have been raised about the use of synthetic progestins such as MPA, for which vascular toxicity has been reported (380). In both peripheral and cerebral vasculature, synthetic progestins were shown to cause endothelial disruption, accumulation of monocytes in the vessel wall, and platelet activation (380). There are many other reports demonstrating negative effects of MPA on the vascular system (390–394). Whereas progesterone and nomegestrol acetate increased nitric oxide synthesis by human endothelial cells, MPA lacked such an effect. Moreover, neither progesterone nor nomegestrol acetate interfered with the effects of physiological concentrations of estradiol, whereas MPA impaired estradiol signaling

(395). In comparison with MPA, norethisterone acetate caused less cerebrovascular tension in rabbits (396). Progestins indeed differ with respect to their influence on intracranial hemodynamics (397). Results of the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial, involving 596 nonhysterectomized postmenopausal women, showed that MPA attenuates the beneficial effects of estrogen on plasma lipoprotein levels (86).

B. Mammary glands

The prevailing opinion is that progesterone may contribute to the development of breast cancer, but the role of the natural hormone in breast epithelial proliferation is not completely understood (398). The assumption that progesterone may be an important breast mitogen initially came from the observation that the proliferation of breast epithelial cells is maximal during the luteal phase of the menstrual cycle. However, studies on normal human breast tissue implanted sc into adult female athymic nude mice found that progesterone had no mitogenic effect. Only estradiol at high levels, equivalent to those measured during the luteal phase, was found to stimulate the proliferation of breast epithelial cells (399–401).

However, in transgenic mouse models, PR-induced mammary epithelial proliferation has been shown to play a role in the initiation and progression of carcinogen-induced mammary tumors. In PR knockout mice, there was indeed a marked reduction in mammary tumor incidence when compared with wild-type mice after treatment with a carcinogen (402). Subsequently, the selective inactivation of either PR-A or PR-B has shown that the B isoform is required for a normal proliferative response of breast epithelial cells to progesterone (403). Alterations of progesterone signaling may play a significant role in breast cancer, as is strongly suggested by two observations: 1) the equilibrium of PR-A and PR-B isoform expression observed in normal breast tissue is disrupted in breast tumors; 2) the dissociation between steroid receptor expression and cell proliferation observed in normal mammary glands is lost after exposure to carcinogens and in breast tumors (401, 404–407). The conversion of progesterone to its 5α- and 20α-reduced metabolites, including allopregnanolone, may also play a role in regulating cell proliferation within breast tissue (408).

Results of a recent experimental study have revealed that exposure to progesterone pellets strongly increased mammary gland volume in mice deficient in the breast cancer susceptibility gene *BRCA1*, whereas the same treatment had little effect in wild-type mice (409). It was then shown that PRs are overexpressed in the mutant mammary gland because of a defect in their degradation by the proteasome pathway, and that treatment of the BRCA1-deficient mice with the PR antagonist mifepristone prevented mammary tumorigenesis (410).

What do clinical trials tell us about the role of progesterone in the development of breast cancer? Recent studies of large cohorts of premenopausal women strongly suggest a protective role for the endogenous hormone. Indeed, the risk of developing breast cancer was decreased in women with high luteal levels of progesterone (411, 412). Also, progesterone seems not to potentiate, but rather to protect against, the carcinogenic effect of estrogens during HRT (413). The recent analysis of a large French cohort of 54,548 postmenopausal women, named the E3N cohort, which is the French arm of the large European Prospective Investigation into Cancer and Nutrition (EPIC), has shown that both oral and transdermal estrogen use were associated with increased breast cancer risk after only 2 yr when combined with a synthetic progestin, but not with micronized progesterone (414). These results have been recently updated; even after 8 yr of treatment, there was no significant increase in breast cancer in women taking a combination of estrogen and micronized progesterone (415). A recent preclinical study performed in rhesus monkeys has also shown that estradiol plus micronized progesterone does not stimulate breast epithelial proliferation (416). However, in the same study, estradiol plus MPA was mitogenic for the cells (416).

The tumor-promoting role of synthetic progestins in general has indeed been demonstrated by recent studies (83). Earlier studies had failed to show such an effect, but this may be explained by the fact that the percentage of women who received combined HRT was relatively low (417). Results of the Million Women Study, which was set up to examine the effects of HRT on breast cancer incidence and mortality and involved 1,084,110 UK women aged 50–64, confirmed the suspicion that current and recent users of HRT may be at increased risk of breast cancer, and also showed that the relative risk rises with increasing duration of HRT (418). Moreover, the magnitude of the associated risk was significantly greater for estrogen-progestin than for estrogen-only treatment. Breast cancer risk was significantly increased for users of MPA, norethisterone, or nogestrel/levonorgestrel (418). These results are consistent with those of the WHI trial, showing a greater breast cancer incidence for the use of estrogen-MPA combination when compared with estrogen-only preparations (419). In addition, MPA has also been suspected of being the culprit for the recurrence of breast cancer associated with HRT (420, 421). These results have received further support from two recent reviews of the literature on breast cancer (422, 423). Because micronized progesterone has no harmful effects, it is likely that the increased risk of breast cancer found with progestins may be the consequence of their non-progesterone-like effects (84). Thus, interactions with the glucocorticoid receptor and disruption of protective androgen receptor signaling may both contribute to the increased breast cancer risk in response to MPA (424, 425).

C. Bones

It is now well established that the deficiency of ovarian steroids leads to the development of osteoporosis, one of the most serious age-related disturbances affecting more than 30% of postmenopausal women (426–428). Women have an increased risk of osteoporosis after a premature or surgical menopause and in the absence of HRT (429), and the most consistent beneficial effect of estrogen alone or estrogen plus progestin therapy concerns the bones.

As for most target tissues of ovarian hormones, the roles of progesterone in bone physiology and in preventing bone

loss are less well studied than those of estradiol, and most discussions have again focused on the role of estrogen administration. In addition to the estrogens, androgens have also been shown to have a profound influence on bone physiology (427, 429). In 1990, experimental, epidemiological, and clinical data supporting an important role of progestins in bone remodeling were reviewed (430). The author proposed that progesterone may play a role in the coupling of bone resorption with bone formation; whereas the main action of estrogens would be to decrease bone resorption, the predominant effect of progestins would be to promote bone formation. Progesterone may stimulate bone formation directly by regulating the expression of target genes and also indirectly by antagonizing the effects of glucocorticoids, well known to reduce bone formation (398, 430, 431).

Since the publication of Prior (430), data relating to the specific effects of progesterone and progestins on bone metabolism have remained relatively sparse, and their significance in bone physiology remains poorly understood. Again, this can be explained by the facts that: 1) many types of progestins with different pharmacological properties are used in HRT, and it is not always clear whether their effects can be ascribed to their actions on the PRs or their interference with other steroid receptors; 2) the positive effects of progesterone and progestins could be masked by the simultaneous use of high doses of estrogen, frequently together with other bone-promoting supplements such as vitamin D and calcium (432, 433); and 3) progestagens may act in synergy with estrogens and have no effect on their own. In one clinical study, the combination of a low-dose of ethinyl estradiol with the progestin norethisterone acetate (norethindrone acetate) had a greater bone-preserving effect than a higher dose of estrogen alone (434).

Concerning the actions of progesterone, intracellular PRs are present in cultures of human osteoblasts and osteoclasts (435–437). In addition, membrane actions of progesterone have been demonstrated in rat osteoblasts (438, 439). Progesterone stimulated the proliferation of human osteosarcoma cells and osteoblasts and up-regulated the transcription of early response genes, osteocalcin, and growth factors (437, 440). In rodents, progesterone has been shown to stimulate the proliferation of osteoprogenitors. Most importantly, progesterone stimulated the growth of osteoprogenitors and the expression of PR in these cells with a comparable efficiency in young and old female rats (441, 442).

There is also evidence that progesterone may act on bone cells in concert with estradiol and potentiate its actions. Thus, in cultured human osteoblasts, estradiol has been shown to increase the specific nuclear binding of progesterone (443), and PR expression was enhanced by the phytoestrogen genistein and by the selective estrogen receptor modulator raloxifene (444, 445). Both A and B isoforms of PR (PR-A and PR-B) were induced by estrogen in human osteoblasts (446). The progestin Org 2058 had no effect on the proliferation of human osteosarcoma cells and of primary rat osteoblasts when added alone to the culture medium, but strongly potentiated the mitogenic effect of estradiol. The authors of this study proposed the existence of a specific class of progesterone-sensitive osteoprogenitors, which may only proliferate in the presence of both estradiol and progesterone (435, 447). In human osteoblasts, a synergistic effect of estradiol and progesterone in up-regulating the expression of the insulin receptor substrate-2 has recently been reported (448). Insulin receptor substrate-2 is one of the substrates of receptor tyrosine kinases, and it plays an important role in bone formation (449).

There is so far no clear clinical evidence for a beneficial effect of progesterone or of synthetic progestins on bone in postmenopausal women, and estrogens continue to be considered as the primary bone-active agent in HRT. In the PEPI and in another recent double-blinded and placebo-controlled trial, the intake of micronized progesterone had no significant bone-protective effect, and in these studies, MPA was also found to be inefficient (450, 451). In the WHI trial, a reduction in hip fractures was observed, either after combined estrogen plus progestin or after estrogen-only treatment (27, 34). Lower doses of CEE with or without MPA than those administered during the WHI trial have also been shown to increase bone mineral density and bone mineral content (452). A prospective study of more than 138,000 postmenopausal women aged 50 to 69 yr and recruited within the above-mentioned Million Women Study revealed that all types of HRT studied, estrogen alone or in combination with MPA, norethisterone, or norgestrel/levonorgestrel, conferred substantial protection against fracture (453). Consistent favorable effects of HRT on bone density have also been confirmed by a large meta-analysis of 57 studies (454). One study has suggested that the progestin norethisterone, when administered alone, may prevent bone loss in postmenopausal osteoporosis by decreasing bone turnover (455).

However, not all studies have demonstrated an efficacy of HRT in the management of bone fractures, such as the HERS study, but women in this trial were not osteoporotic (25). Also, bone loss has been associated with MPA treatment, suspected to result from its glucocorticoid activity (456, 457), but in the WHI trial, combined CEE and MPA administration was found to reduce fractures (see previous paragraph). Based on the recent evidence, HRT has been recommended for the prevention and treatment of osteoporosis (458). However, because of the potential risks of HRT, its recommendation for osteoporosis prevention has been limited to the shortest possible time (usually 2 yr) and only in women with climacteric symptoms (428).

In conclusion, the significance of progesterone in peripheral tissues such as blood vessels, mammary gland, and bone still remains a matter of controversy. It is important to be aware that the choice of a progestin for HRT is very important with respect to efficiency and side effects. Micronized progesterone and the more selective 19-norprogesterone derivatives offer promising perspectives for more efficient and safe HRT. There is evidence that progesterone may not have cancer-promoting effects on normal breast tissue or deleterious effects on the blood vessel wall, and that it may eventually be protective.

X. Novel Perspectives for Progesterone in HRT: Multiple Signaling Mechanisms

Research over the past few years has revealed a multitude of signaling mechanisms of steroids, and in particular of

progesterone and estradiol (459–462). They offer exciting possibilities for the development of new ligands with more selective actions on specific targets, but they also point to the limitations and possible side effects of steroid compounds used at present in HRT. The still poor knowledge of the molecular mechanisms of steroid actions is hampering the development of more efficient therapies for the diseased, injured, and aging brain. The multiple mechanisms of action of progesterone and its metabolites comprise the regulation of gene transcription, the modulation of neurotransmitter receptors, and the activation of signaling cascades via new, recently identified membrane receptors. In particular the recognition of the significance of nuclear coregulator proteins in regulating the transcriptional activities of steroid receptors, as well as the cloning of new membrane steroid receptors, can be expected to completely change our vision of steroid actions on specific target tissues during the next few years.

A. Progesterone receptor isoforms and nuclear receptor coregulator proteins

The effects of progesterone on gene expression, the so-called "genomic effects," are mediated by at least two intracellular receptor isoforms (PR-A and PR-B), which are generated from a single gene (463, 464). They only differ by an additional 164 amino acid segment in the N-terminal region of PR-B, called the B-receptor upstream segment (465). It has been proposed that the PR isoforms undergo continuous nucleocytoplasmic shuttling, resulting from their active transport into the nucleus and their diffusion out into the cytoplasm (466). Binding of progesterone causes conformational changes of the PR, resulting in the dissociation of a chaperone protein complex, receptor dimerization, increased receptor phosphorylation, binding of the receptor dimer to specific hormone-responsive DNA elements located on target genes, and interactions of the receptor complex with nuclear coactivators (460, 467) (Fig. 3).

In vitro studies have provided evidence that PR-A and PR-B display distinct transactivational properties and that PR-B is a more active transactivator than PR-A (468–472). Moreover, in a promoter- and cell-specific manner, PR-A can even repress PR-B-mediated gene transcription, as it also does with the transcriptional activity of the estrogen and the other steroid receptors (469, 473, 474). The term "transrepression" has been coined to refer to the suppression of the transcriptional activities of other steroid receptors by PR-A, which involves the N-terminal domain of the receptors (475).

Engineered cells and transgenic mice have been used to demonstrate that PR-A and PR-B regulate the expression of different subsets of genes (476, 477). The study of transgenic

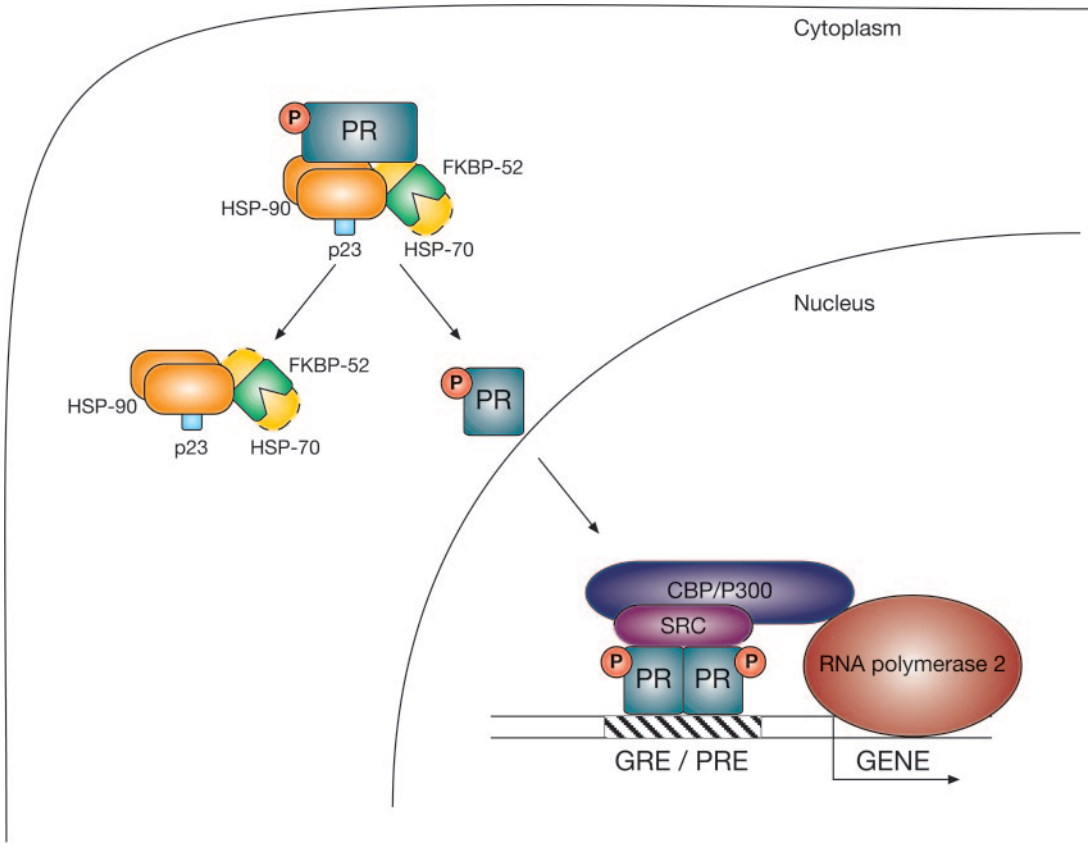

FIG. 3. Genomic actions of progesterone. Binding of progesterone (P) causes conformational changes of the PRs, resulting in the dissociation of a chaperone protein complex, composed of the heat-shock proteins (HSP), protein p23, and the immunophilin FKBP-52. The liganded PRs bind as homodimers to glucocorticoid/progesterone response elements (GRE/PRE), generally located in the promoter regions of target genes, and recruit nuclear coactivator proteins. The best characterized groups of coactivators are the p160 (SRCs) and the CBP/p300 families. Unfortunately, a heterogenous nomenclature is actually in use for the p160 coactivators (see text).

mice overexpressing either the A or the B form of the PR has shown that regulated expression of both isoforms *in vivo* is required for normal mammary gland development (478, 479). Subsequently, the creation of mice selectively lacking either PR-A or PR-B has revealed that the two PR isoforms function as distinct transcription factors, and that they mediate different but partially overlapping reproductive responses to progesterone (403). Inactivation of PR-B resulted in reduced pregnancy-associated mammary ductal morphogenesis, but did not affect ovarian and uterine development, consistent with the observation that PR-A is sufficient for the establishment and maintenance of pregnancy. On the contrary, female mice lacking PR-A have normal mammary glands but display severe ovarian abnormalities and uterine hyperplasia and are infertile (407, 480).

Because PR-B and PR-A show different responses to progestagens, have different transactivation functions, differently influence the actions of other steroid receptors, and also activate the transcription of different progesterone-responsive genes, their coordinated expression determines the effects of progestagens (406, 481, 482). However, their respective functions have so far only been explored in reproductive tissues. In the nervous system, their biological properties and their role in the cell-specific actions of progesterone are not well defined. Many studies performed over the past decade have documented that regulation of PR-A and PR-B expression varies between different brain regions according to sex, hormonal status, and age (483–488).

In addition to PR-B and PR-A, other transcripts are generated from the PR gene. First identified in malignant progesterone target tissues or cloned from cDNA libraries, their biological functions have not been studied very much (489–497). There is, however, evidence for a possible involvement of other PR isoforms, resulting from alternative splicing or the use of additional exons, in mediating the actions of progesterone (489, 498–500). The presence and biological significance of such additional PR variants has never been explored in the nervous system.

In the mid-1990s, the discovery of nuclear steroid receptor coregulators, comprising coactivators and corepressors, has added to the complexity of steroid signaling and has opened new avenues of research into the genomic effects of steroid hormones. The number of coregulators that can be recruited by steroid and other nuclear receptors is continuously increasing, and their cell-, promoter-, and ligand-specific actions have been extensively reviewed (318, 501–508). Agonist binding is believed to increase the affinity of steroid receptors for coactivators, providing the conditions for efficacious transcriptional activation. Coactivators include molecules that facilitate the access of the basal transcriptional machinery to gene promoters, such as histone acetyltransferase coactivators. The acetylation of histone lysines indeed disrupts molecular interactions, which maintain gene promoters in a closed state. The best characterized groups are the p160 (SRC-1a, SRC-1e, SRC-2, and SRC-3) and the CBP/p300 families, which act in concert with other factors and bring histone acetyltransferase activity to the vicinity of steroid receptor complexes (Fig. 3). Unfortunately, a heterogenous nomenclature is actually in use for the p160 coactivators (SRC-1 = NCoA-1; SRC-2 = NCoA-2 or GRIP1 or TIF-2; SRC-3 = p/CIP, ACTR, TRAM-1 or RAC-3) (509–511). For reasons of clarity, the SRC nomenclature will be exclusively used here. It should also be emphasized that numerous other coactivator complexes are involved in bridging the RNA polymerase II complex with basal transcription factors, in providing enzymatic activities modifying DNA structure, and in allowing interactions with other nuclear proteins, which have been reviewed elsewhere (506, 512).

Important functions of the SRCs in PR-mediated physiological processes have been revealed by the study of SRC knockout mice. Thus, the ability of the uterus to mount a decidual response is markedly impaired in SRC-1 knockout females, whereas in SRC-3 knockout mice, parity-associated development of the mammary gland was compromised (513, 514). These results suggested an important role for SRC-1 in the uterus and for SCR-3 in PR-mediated effects on the mammary gland. This model is also supported by the results of a study in which transgenic PR activity indicator mice, in which cell-specific PR activation is reflected by a fluorescent reporter gene, were crossed with SRC-1 knockout and SRC-3 knockout mice (515). A mouse model, in which SRC-2 function was selectively abrogated in PR-expressing cells by using a *cre-lox* engineering strategy, has allowed the demonstration that SRC-1 and SRC-2 cooperate in the progesterone-dependent decidualization of the mouse uterus (516).

Only a few studies have examined the functional relationship between steroid receptors and their coregulators in the nervous system. However, the still fragmentary findings show that coactivators of the p160 family are critically involved in the amplification of nuclear receptor actions within the brain in a temporally and spatially coordinated manner (512, 517, 518). Knockout mouse models for the three members of the p160 coactivator family show no major phenotypic defects, despite the fact that these coactivators are largely expressed throughout the nervous system. This lack of effect suggests adaptive compensations and functional redundancy between coactivators. Thus, SRC-$1^{-/-}$ mice are viable and only show partial hormone resistance and slight loss in reproductive functions, presumably thanks to the compensation by SRC-2 (519). Disruption of SRC-1 also slightly delays the development of cerebellar Purkinje cells, leading to moderate motor dysfunctions in adulthood (520).

However, the stereotaxic injection of antisense oligodeoxynucleotides, allowing the expression of specific coactivators to be decreased in a time- and brain region-specific manner, has allowed the demonstration of the critical role of nuclear coactivators in steroid receptor signaling within the brain. Thus, infusion of SRC-1 antisense oligodeoxynucleotides into the rat brain impaired the process of steroid-dependent sexual differentiation of reproductive behavior and brain morphology during development and inhibited progesterone-facilitated sexual receptivity in adult females (521, 522). The process of sexual differentiation of the brain and of behavior could also be disturbed by using CBP antisense oligodeoxynucleotides (523). Antisense oligodeoxynucleotides to SRC-1 and SRC-2, but not to SRC-3, inhibited lordosis behavior and the induction of the PR by estrogen within the ventromedial hypothalamus in rats and mice, consistent with the presence of SRC-1 and SRC-2 and the absence of SRC-3 within this brain region (524). That

coactivators are indeed limiting factors in steroid responses in the brain has been demonstrated in a recent elegant study. Decreasing SRC-1 expression in the preoptic area/hypothalamic region of the male quail by injecting antisense significantly blocked the activation by testosterone of both estrogen- and androgen-dependent male sexual behaviors, decreased the size of the preoptic medial nucleus, and reduced expression of the preoptic aromatase, responsible for the local conversion of androgens to estrogens. Most importantly, when injections of SRC-1 antisense were stopped, the coactivator became overexpressed in the hypothalamus, accompanied by an increase in sexual behavior and in the volume of the preoptic medial nucleus (518).

Recent studies on the interactions between steroid receptors and nuclear receptor coactivators of the p160 and CBP/p300 families in different cell types of the nervous system have revealed a novel gene- and cell-specific recruitment (525–527). These findings open new opportunities for the selective regulation of steroid actions within the nervous system. Indeed, an important concept that has emerged from recent steroid receptor and nuclear coregulator research is the influence of the cellular environment on the outcome of a particular ligand being bound. This has spurred research to develop novel synthetic ligands for steroid receptors, which would activate only a subset of the functions of natural hormones and ideally produce unique physiological effects in a particular cell type without undesired side effect activities (502, 503, 528). Such ligands have been referred to as "selective steroid receptor modulators," including the selective estrogen receptor modulators (SERMs) and the selective progesterone receptor modulators (SPRMs), which exhibit agonistic or antagonistic activities in a cell- or tissue-specific manner (529–532).

Steroid receptor actions have even become more complex with the discovery that they are targeted not only to the nucleus, where they function as ligand-dependent transcription factors, but also to the cell membrane, where they interact with cellular signaling pathways (533) (Fig. 4). Thus, the N-terminal domain common to PR-A and PR-B indeed contains a SH3 domain interaction motif, which was shown to selectively interact with Src tyrosine kinase family members. Interactions of the PR with the SH3 domain of these signaling molecules was found to be transient and ligand-dependent and corresponds to an unique feature of the PR (534, 535). A functional consequence of PR interaction with the SH3 domain of Src kinase is the activation of the Src/Ras/MAPK signaling pathway, as has been documented in MCF-7 breast cancer cells (536, 537).

B. The modulation of neurotransmitter receptors

The inhibition of neuronal excitability is an important component of the neuroprotective effects of progesterone and its metabolites. In this regard, they differ from the estrogens, which in general have excitatory effects by potentiating the actions of excitatory neurotransmitters (24). On the contrary, progesterone and its metabolites inhibit excitatory neurotransmitter receptors and stimulate inhibitory neurotransmitter receptors (538). Thus, the 3α,5α-reduced progesterone metabolite allopregnanolone is a potent positive modulator of $GABA_A$ receptors at physiological nanomolar concentrations (539, 540), which explains some of its psychopharmacological actions, in particular its anesthetic, analgesic, and anxiolytic effects, as well as its role in stress, depression, memory, seizure susceptibility, and alcohol dependence (541, 542) (Figs. 1 and 4).

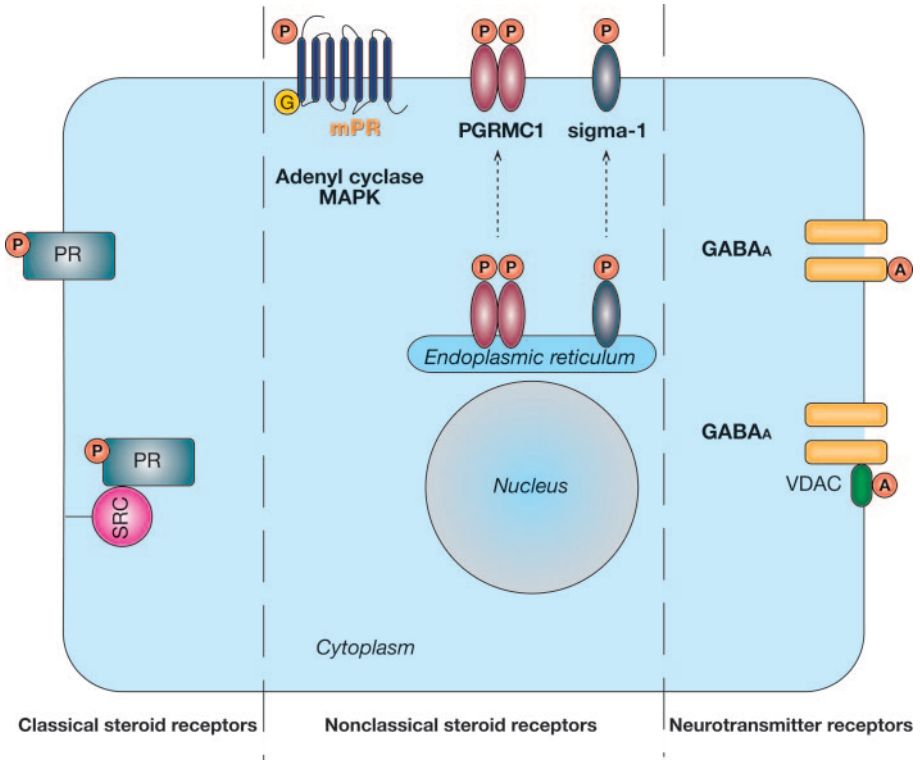

FIG. 4. Membrane actions of progestagens. Membrane actions of progestagens comprise: 1) The regulation of neurotransmitter receptors, in particular of $GABA_A$ receptors, by the progesterone metabolite allopregnanolone (A) and of the nAChR by progesterone (P) (*right*). Allopregnanolone directly binds to $GABA_A$ receptors, but some effects of allopregnanolone may be mediated by $GABA_A$ receptor-associated membrane proteins. 2) The binding to classical PRs after their translocation to the plasma membrane or their interaction with other membrane signaling components such as SRC (*left*). 3) The binding to novel G protein-coupled membrane receptors of progesterone (mPR) (*middle*). 4) The binding to σ1 receptors or to PGRMC1 (also called 25-Dx) (*middle*). Part of the σ1 receptors and of PGRMC1 may reside on membranes inside the cell and may translocate to the vicinity of the plasma membrane upon activation.

How neurosteroids interact with $GABA_A$ receptors has for long been an enigma. The existence of specific steroid binding sites on $GABA_A$ receptors has always been suspected because of the enantioselectivity of the effects of allopregnanolone (543). Moreover, a selective inhibitor of the GABA-modulatory effects of 5α-pregnane steroids has recently been developed (544). The expression of recombinant $GABA_A$ receptor subunits coupled with site directed mutagenesis had allowed the identification of structural motifs involved in steroid modulation (42, 538). However, it was only last year, precisely 20 yr after the discovery of the modulation of $GABA_A$ receptors by steroid hormones, that two discrete steroid binding sites were identified on $GABA_A$ receptors. Allopregnanolone potentiates GABA responses by binding to a cavity located between the transmembrane domains M1 and M4 of the α-subunits, whereas it directly activates $GABA_A$ receptors by binding to interfacial residues between the α- and β-subunits (545). Previously, it had been proposed that allopregnanolone may also modulate $GABA_A$ receptor activity by binding to associated membrane proteins, and this of course remains a possibility (546, 547).

It is important to note that allopregnanolone does not indiscriminately enhance neuronal inhibition via $GABA_A$ receptors throughout the nervous system. Indeed, accumulating evidence shows that the steroid-$GABA_A$ receptor interactions are specific to individual brain regions and different neurons. Thus, the modulation of $GABA_A$ receptors by allopregnanolone has been shown to be influenced by receptor subunit composition, phosphorylation, and the local steroid metabolism (42).

As was already pointed out, some of the biological effects of progesterone may be mediated via its conversion to allopregnanolone. Thus, allopregnanolone has antiapoptotic and antiastrogliotic effects, and its administration improves cognitive recovery after TBI (101, 154). The two brain nuclei where secondary neuron loss takes place after TBI, the MDN and NBM, are indeed under the control of GABAergic innervation, and its potentiation by allopregnanolone may preserve neurons from the effects of excessive excitatory neurotransmitter release in response to injury. Most importantly, the 3β,5α-reduced isomer epiallopregnanolone, which is inactive at $GABA_A$ receptors, neither reduced secondary neuronal loss nor improved behavioral recovery in this model. After transient MCAO in male rats, allopregnanolone was even more potent than progesterone in attenuating cortical damage (548). Allopregnanolone also protects neurons in the hilus of the hippocampus from kainic acid excitotoxicity (101, 549). In this lesion model, it was also observed that progesterone administration increased levels of 5α-dihydroprogesterone and allopregnanolone within the hippocampus, and that the neuroprotective effects of progesterone could be blocked by the administration of 5α-reductase inhibitors. In contrast, MPA did not increase hippocampal levels of allopregnanolone and did not prevent kainic acid-induced neuronal loss (550).

In vitro studies have provided further evidence for the neuroprotective actions of allopregnanolone. Thus, when grown in culture, mouse P19 neurons were protected by allopregnanolone against NMDA-induced apoptosis (551). Exposure of cultured rat Purkinje cells to increasing concentrations of progesterone during oxygen-glucose deprivation revealed a dose-dependent protection by progesterone. The neuroprotective effect of progesterone could be mimicked by allopregnanolone and blocked either by the 5α-reductase inhibitor finasteride or by the $GABA_A$ receptor antagonist picrotoxin (552).

Most experimental studies have reported beneficial effects of allopregnanolone on brain functions, and to our knowledge, there is only one report that allopregnanolone caused neurite regression in cultures of hippocampal neurons (553). However, as clarified by the results of a more recent study, allopregnanolone-induced neurite regression may reflect a mitogenic effect of the steroid on still immature neuronal cells. Indeed, allopregnanolone has been shown to stimulate the proliferation of cerebral and hippocampal neuroprogenitor cells via $GABA_A$ receptor-activated voltage-gated L-type calcium channels (554). An autocrine regulatory loop involved in the regulation of progenitor cell proliferation involving allopregnanolone and $GABA_A$ receptors has recently been described in expressing the polysialylated form of the neural cell adhesion molecule ($PSA\text{-}NCAM^+$) neural progenitors and isolated from neonatal rat brain. These multipotent progenitors, which spontaneously differentiate into oligodendrocytes, not only express $GABA_A$ receptors but also synthesize significant amounts of allopregnanolone and GABA. Both GABA and allopregnanolone were shown to increase progenitor proliferation through a $GABA_A$ receptor-mediated mechanism (555–557). The preclinical proof of concept for the therapeutic potential of allopregnanolone to promote neurogenesis has also been provided by a recent study (558). Neurons and neural progenitors are not the only cells in the nervous system that are directly influenced by the interactions between allopregnanolone and $GABA_A$ receptors. Thus, Schwann cells have been shown to contain the messengers of several $GABA_A$ receptor subunits, and it has been shown that allopregnanolone increases the expression of specific peripheral myelin proteins by acting on Schwann cell $GABA_A$ receptors (178).

Thus, progesterone could become part of the neuroprotective strategies that target $GABA_A$ receptor activity, some of which have proved to be quite efficacious (559, 560). Due to its hyperpolarizing properties, GABA protects hippocampal and cortical neurons against glutamate-induced neuronal loss both *in vitro* and in animal models of ischemia and epilepsy (559, 561). However, one should be aware that GABA does not always function as an inhibitory neurotransmitter. In the immature brain, when glutamatergic synapses are still quiescent, GABA functions as an excitatory neurotransmitter, and it only gradually converts to an inhibitory neurotransmitter. This switch from depolarizing to hyperpolarizing actions of GABA can take place very late during postnatal development for some types of neurons. Among them are the GnRH neurons in female rats, which only become hyperpolarized by GABA at the time of puberty (562). It is the transmembrane Cl^- ion gradient that determines whether the opening of $GABA_A$ receptors results in inhibition or excitation. $GABA_A$ receptors are indeed freely permeable to Cl^- ions, and GABA is excitatory during early development because of high intracellular Cl^- concentrations. During brain maturation, GABA progressively be-

comes inhibitory by the delayed expression of a transporter, which reduces intracellular Cl^- concentrations in neurons (563).

The excitatory effects of GABA during early life may explain its neurotrophic effects during brain development (564, 565). Since the pioneering studies by Wolff *et al.* (566), other laboratories have demonstrated that GABA acts as an important signaling molecule in neuronal proliferation, migration, and differentiation by causing membrane depolarization and by raising intracellular Ca^{2+} (567–569). In addition, GABA has been shown to inhibit proliferation of neuronal precursor cells, to influence the migration of neurons, and to promote morphological maturation of postmitotic neurons (570–573). However, excessive stimulation of $GABA_A$ receptors may become toxic for immature neurons (574). Thus, the $GABA_A$ receptor agonist muscimol can kill immature hippocampal neurons, and this neurotoxic effect is exacerbated by estradiol, consistent with the neuroexcitatory effects of this steroid (575, 576).

It is important to be aware that the depolarizing actions of GABA are not limited to the developing nervous system, because they can still take place in the adult brain, under either natural or pathological conditions (577). For instance, GABA has excitatory effects in adult dorsal root ganglia neurons and also in neurons of the cerebral cortex, depending on the resting membrane potential and on spatiotemporal interactions with excitatory amino acid inputs (578). In the suprachiasmatic nucleus, GABA acts as an excitatory transmitter during the day but is inhibitory at night (579). Most importantly, GABA can also cause the depolarization of hippocampal neurons under pathological conditions, as has been shown in temporal lobe epilepsy (580). Thus, in response to seizures, intense $GABA_A$ receptor activation can result in a transient accumulation of Cl^- ions within neurons, changing the GABAergic effect from inhibition to excitation (581). Increased concentrations of intracellular Cl^- ions can also result from axonal injury, because of alteration of the cation Cl^- cotransporter KCC2 in the lesioned neurons. As a consequence, the activation of $GABA_A$ receptors becomes excitatory; opening of the $GABA_A$ receptor channel results in Cl^- efflux and depolarization of the neuronal membrane (582). Under such particular circumstances, the stimulation of $GABA_A$ receptors could be expected to result in increased neuronal damage, and these recent observations raise the question of the safety of therapies aimed at increasing GABA concentration in the injured adult brain and during development.

However, all the trophic and protective effects of allopregnanolone are not necessarily mediated by $GABA_A$ receptors, as shown by the following findings. In the brain of the NP-C mouse, an animal model of Niemann-Pick type C disease characterized by neurological deficits and Purkinje cell loss, levels of 5α-dihydroprogesterone and allopregnanolone are decreased, as are the expressions of the enzymes involved in their synthesis, especially in the cerebellum. Most importantly, the systemic administration of allopregnanolone significantly delayed the onset of neurological symptoms and prolonged Purkinje cell survival (583). These neuroprotective effects of allopregnanolone could be blocked by bicuculline, an antagonist specific for $GABA_A$ receptors. Thus they seem to be mediated, at least in part, by $GABA_A$ receptors. However, it was then shown that the beneficial effects of allopregnanolone could be mimicked by its enantiomer (*ent*-allopregnanolone), which is inactive at $GABA_A$ receptors. Moreover, the efficacy of micromolar concentrations of allopregnanolone and *ent*-allopregnanolone correlated with their ability to activate pregnane X receptor-dependent gene expression (584). These findings point to a role for the pregnane X receptor in mediating protective effects of elevated levels of allopregnanolone in the nervous system.

In addition to $GABA_A$ receptors, other neurotransmitter systems are targets for the rapid modulatory effects of progestagens. Progesterone itself inhibits the activity of the neuronal nicotinic acetylcholine receptor (nAChR), but only at high micromolar concentrations (585, 586). However, it is not impossible that such concentrations are reached by locally synthesized progesterone (see *Section XI*), and a series of experimental observations are indeed compatible with the negative allosteric modulation of nAChR by progesterone (587). Unfortunately, even the most sensitive assay methods that are actually available do not allow a precise estimation of very localized steroid concentrations, for example at the level of synaptic clefts. The 19-norprogesterone derivative promegestone also behaves as an antagonist of the nAChR (588).

C. Novel membrane receptors of progesterone

More than 25 yr ago, a rapid action of progesterone at the level of the cell membrane, not requiring gene transcription, was described: the reinitiation of *Xenopus* oocyte meiosis (589, 590). This membrane effect involved a rapid increase in intracellular Ca^{2+}, inhibition of the adenylate cyclase/protein kinase A system, and activation of the MAPK cascade (591, 592). Recently, the amphibian homolog of the mammalian PR has been cloned and proposed to mediate nongenomic progesterone signaling in *Xenopus* oocytes after translocation into the cell membrane (593–595). However, this so-called *Xenopus* PR is not the only one mediating rapid membrane effects of progesterone on oocytes, as a recently cloned membrane progesterone receptor (mPR) is also involved in initiating the resumption of meiosis.

The characterization of membrane binding sites for progesterone has always been tentative and has usually been limited to immunostaining and molecular weight determinations. Another common approach for their study was the use of progesterone coupled to a protein or a polymer as a ligand, which supposedly does not enter cells and only acts at the cell surface. Thus, progesterone conjugated to radiolabeled BSA has been used to identify and to characterize binding sites on brain cell membranes (596). It was only in 1996 that a first putative membrane receptor of progesterone, distinct from the classical PR isoforms and comprising 194 amino acids with a single membrane-spanning domain, was isolated and cloned from porcine liver (597, 598). Binding of [^{3}H]progesterone to this new membrane protein was found to be reversible, saturable, and selective. Subsequently, homologous proteins were cloned in rat (named 25-Dx), cattle, and humans (599). The name 25-Dx has been used in recent

studies describing the expression and regulation of this membrane receptor of progesterone in the rat brain and spinal cord (600–603). However, in recent studies on the ovary, the protein has been referred to as "progesterone membrane receptor component 1" (PGRMC1) (604), and this nomenclature will be adopted here (Fig. 4).

In hepatocytes, the binding of progesterone to PGRMC1 was found to be associated with endomembranes rather than with plasma membranes. Moreover, expression of the cDNA of PGRMC1 in CHO (Chinese hamster ovary) cells resulted in increased microsomal progesterone binding (605). According to these earlier studies, PGRMC1 would rather qualify as an endomembrane progesterone binding protein. However, much progress has been made over past 2 yr in understanding the subcellular localization and the significance of this membrane receptor by studying the actions of progesterone in the rat ovary. There is now strong evidence that PGRMC1 mediates the antiapoptotic actions of progesterone in both rat granulosa and luteal cells. In the granulosa cells, PGRMC1 localizes to the nuclei, but after treatment of the cells with human chorionic gonadotropin, it is almost exclusively present at the plasma membrane (606). There, PGRMC1 interacts with another membrane protein, the plasminogen activator inhibitor RNA binding protein-1 (PAIRBP1; also known as RDA288 or SERBP1) and forms a complex required for transducing the antiapoptotic actions of progesterone in the ovary (607). The two proteins are also expressed in human granulosa and luteal cells, where they colocalize near the plasma membrane (608). The elucidation of the signal transduction pathway of the PGRMC1-PAIRBP1 progesterone membrane receptor complex has just begun. Available data strongly suggest that in the ovary, the membrane complex increases cGMP, which in turn activates protein kinase G (608). The cytoplasmic domain of PGRMC1 also has several potential Src homology 2 and Src homology 3 domains, through which progesterone activation could transduce an intracellular signal (606). Interestingly, PGRMC1 tends to form aggregates that can be as large as 200 kDa, although Western blots often detect PGRMC1 as a 56-kDa dimer or a 28-kDa monomer.

The presence of PGRMC1 has also been described on the cell surface of hypothalamic and spinal neurons (named 25-Dx in these studies) (600, 601). However, its signaling mechanisms have still not been studied in the nervous system. The distribution and regulation of PGRMC1 within different compartments of the nervous system may provide some clues concerning its functions. In the ventromedial hypothalamus of female rats, expression of PGRMC1 was shown to be increased by estrogen treatment, and the protein may thus play a role in the activation of female sexual behavior (600). A detailed immunohistochemical study of the distribution of PGRMC1 in the rat brain has confirmed the presence of the protein in the hypothalamus and has demonstrated its expression in circumventricular organs and ependymal cells of the ventricular walls as well as in vasopressin neurons of the paraventricular, supraoptic, and retrochiasmatic nuclei. Together with the observations that PGRMC1 was up-regulated in neurons and induced in astrocytes after TBI, these findings strongly suggested a role of the progesterone-binding protein in the maintenance of the water balance after injury (603). A role of PGRMC1 in mediating protective effects of progesterone in the nervous system is also supported by the observation that its mRNA and protein were up-regulated by progesterone treatment in dorsal horn neurons of spinal cord-injured male rats (601). In the cerebellum, PGRMC1 is present in Purkinje cells and in the external granule cell layer. The protein is particularly abundant during early postnatal life, suggesting a role in developmental processes. In the Purkinje cells, PGRMC1 immunoreactivity was found to be associated with membrane structures of the endoplasmic reticulum and the Golgi apparatus (602). Whether PGRMC1 undergoes translocation from intracellular membranes to the plasma membrane and associates with other membrane proteins such as PAIRBP1 in the nervous system needs to be clarified.

Another potential progesterone binding protein, which may also translocate from intracellular compartments to the cell membrane and which is distinct from PGRMC1, is the sigma-1 (σ1) receptor (Fig. 4). This receptor was first defined by its ability to bind with high affinity a variety of pharmacologically active drugs, named "sigma ligands" (609, 610). The molecular nature of the σ1 receptor remained enigmatic until the purification and cloning of the 223-amino acid receptor from guinea pig liver microsomes (611). Subsequently, the cDNAs for the orthologs of this orphan receptor were cloned from a human placental cell line, from mouse kidney, and from rat brain cDNA libraries (612–614). It has been proposed that this endoplasmic reticulum-anchored protein may, upon activation, translocate to the vicinity of the cell membrane, where it may regulate plasma membrane-bound signal transduction (615). Physiological roles of the σ1 receptor involve the modulation of intracellular Ca^{2+} levels and of various neurotransmitter systems (286, 616). Recent observations show that σ1 receptors continue to be expressed in the aging brain and that they may offer interesting ways to attenuate the progressive decrease of cognitive performance during normal and pathological aging and to protect neurons against β-amyloid-induced neurotoxicity (617–619).

The endogenous ligands of the σ1 receptor are unknown, but progesterone may be one of them because it acts as a competitive inhibitor of agonist binding (611, 620, 621). A role for the inhibition of σ1 receptor functions by progesterone has been documented in the dorsal hippocampus. There, the potentiation of the NMDA response of hippocampal neurons and the NMDA-evoked norepinephrine release from preloaded hippocampal slices by σ1 ligands were both strongly reduced in the presence of progesterone (622–624). Furthermore, progesterone has been shown to influence the behavioral efficacy of σ1 receptor ligands in mice (625, 626). A recent study has explored the influence of progesterone on σ1 receptor function during the aging process of the nervous system. Decreased levels of progesterone were measured in the hippocampus and cerebral cortex of aged, senescence-prone male SAMP/8 mice, correlating with an enhanced behavioral efficacy of σ1 ligands (617). Again, this observation is consistent with an inhibition of σ1 receptors by progesterone.

An important event was the cloning in 2003 of mPR from fish oocytes by the Thomas laboratory (627, 628) (Fig. 4). More than 20 genes closely related to the fish mPR have been

cloned from several vertebrate species including human, mouse, and pig. In humans, three mPR subtypes have been named mPRα, mPRβ, and mPRγ (628, 629). They are all unrelated to known nuclear steroid receptors and encode proteins with seven transmembrane domains, with the characteristics of G protein-coupled receptors, and belong to a large and ubiquitous family of proteins found in both prokaryotes and eukaryotes, termed "progestin and adiponectin receptors" (630, 631).

The mPRs are for the first time meeting the criteria of true membrane receptors: structure of a membrane-spanning protein, plasma membrane localization, expression in steroid target tissues, selective steroid binding, regulation of intracellular signaling pathways, regulation by hormones and biological functions. In the spotted sea trout, the first mPR gene cloned was found to be selectively expressed in reproductive endocrine tissues and in brain. Computer modeling predicted a protein with seven transmembrane domains, characteristic of G protein-coupled receptors. In fact, the fish mPR was shown to activate a pertussis toxin-sensitive inhibitory G protein, to inhibit adenylate cyclase activity and to activate the MAPK pathway, thus resembling the membrane actions of progesterone in *Xenopus* oocytes first described more than three decades ago (591). In fact, *Xenopus* oocytes express a mPRβ ortholog at the level of the plasma membrane, and the *Xenopus* mPRβ was shown to fulfill all the criteria for a progesterone receptor involved in oocyte maturation (632). The human mPRα (hu-mPRα) has also recently been shown to inhibit cAMP production in a pertussis toxin-sensitive manner and to inhibit membrane-bound adenylyl cyclase activity when transfected into the human breast cancer MDA-MB-231 cells (633).

The expression and regulation of these new membrane receptors have begun to be explored in the mammalian reproductive tract but have still not been explored in the nervous system. A recent study has provided evidence for the expression and regulation of mPRs in the rat corpus luteum, a tissue that interestingly does not contain detectable levels of intracellular PRs, but where actions of progesterone may be mediated by membrane receptors (634). Very recently, the presence of both mPRα and mPRβ has been demonstrated in human myometrium, where the activation of the mPRs leads to transactivation of PR-B and to a decrease in SRC-2 expression, thus suggesting a cross-talk between membrane and nuclear receptors of progesterone (635). Both mPRα mRNA and protein are also present in MCF-7 and SK-BR-3 human breast cancer cells. Interestingly, mPRα expression was found to be higher in breast tumor biopsies than in normal breast tissue, pointing to a possible role of membrane receptors in the development or progression of breast cancer (636).

The study of membrane receptors of progesterone, including PGRMC1 and the mPRs, is a very recent field, and it is thus not surprising that some controversies are being raised (637). However, we certainly are at the beginning of a great adventure. The novel membrane receptors may indeed mediate particular functions of progesterone, and they are likely to provide exciting opportunities for the development of novel receptor ligands specifically targeting the plasma membrane of cells. A very recent study has indeed shown that the binding characteristics of the hu-mPRα are very distinct from those of the classical PRs. In stably transfected human MDA-MB-231 cells, hu-mPRα localized to the plasma membrane and bound progesterone with high affinity and selectivity (Kd $\approx$ 7 nM). However, in contrast to the intracellular PRs, the recombinant hu-mPRα did not bind progestins commonly used as contraceptives or in HRT: 19-norprogesterone derivatives (promegestone, demegestone) and 19-nortestosterone derivatives (norethisterone, norgestrel). The hu-mPRα also had no affinity for the PR antagonist mifepristone (RU486) and for the very selective PR agonist Organon-2058. Thus, hu-mPRα showed a very specific pharmacological profile, very distinct from that of the classical PRs. Estradiol and cortisol did not bind to hu-mPRα, but interestingly, testosterone was able to displace the binding of [^{3}H]progesterone (IC_{50} = 390 nM) (633). At the functional level, G protein activation by hu-mPR was also specific for progesterone because promegestone and cortisol were ineffective (633). The binding characteristics of PGRMC1 are still less well characterized. The binding of [^{3}H]progesterone to microsomal and solubilized membrane fractions of porcine liver could be displaced by testosterone (IC_{50} = 3 μM), corticosterone (IC_{50} = 2 μM), cortisol (IC_{50} = 12 μM), and a higher dose of promegestone (IC_{50} = 20 μM), but not by estradiol, dexamethasone, or aldosterone. It is important to note that these membrane binding sites had much lower affinity for progesterone (Kd in the 100 nM) range than mPRs (10-nM range) and PRs (1-nM range) (638).

Like the progestagens, estrogens also exert direct effects on the cellular membrane (599, 639). It has been proposed that the orphan G protein-coupled receptor GPR30 may be an estrogen membrane receptor, unrelated to the nuclear ERs and regulated by progesterone (640). However, a recent study has cast some doubt by showing that estradiol fails to activate intracellular signaling pathways in cells that lack classical ER even when GPR30 is present (641). ER-X is another recently identified putative estrogen membrane receptor, which is enriched in caveolar-like microdomains of cellular membranes and interacts with kinases of the MAPK cascade and other signaling pathways (642). It is interesting that ER-X shows a particular pharmacological profile because it binds the 17α-isomer of estradiol and also progesterone at low micromolar concentrations (642, 643).

Thus, the binding specificities of steroid membrane receptors are very distinct from those of the classical intracellular receptors, perhaps requiring the creation of new categories of receptors. This is not further surprising because there is no homology in any region of the membrane receptors with the ligand binding domain of intracellular receptors. More work is needed to better characterize the binding and also the functional characteristics of the new steroid membrane receptors.

D. Dependence of steroid signaling on the physiopathological context

Recent observations strongly suggest that steroid actions may involve different signaling mechanisms depending on the physiopathological context. It has already been mentioned that $GABA_A$ receptors may become excitatory in re-

sponse to seizures or injury, and as a consequence, that the actions of the GABA-active steroid allopregnanolone on neuronal activity may shift from inhibitory to excitatory. Expression of the potential progesterone membrane binding protein PGRMC1 is increased after spinal cord transection in progesterone-treated rats. On the contrary, the classical intracellular PR was found to be down-regulated under these conditions (601). In response to TBI, expression of PGRMC1 was up-regulated in neurons and induced in astrocytes (603). Thus, membrane receptors of progesterone may play an important role in the neuroprotective effects of progesterone.

Another example illustrating alternative receptor signaling pathways in the injured nervous system concerns the putative estrogen membrane receptor ER-X, which is present in cortical and uterine plasma membranes of postnatal but not of adult animals, suggesting important functions in developmental processes. However, ER-X is again expressed after ischemic brain injury and may thus play a role in mediating the neuroprotective and neurotrophic effects of estrogen in the adult nervous system (642).

In conclusion, the variety of mechanisms by which progesterone and its metabolites exert their effects in the nervous system, including their genomic and rapid membrane actions, offer exciting new possibilities for the development of more efficient and safe steroid treatments, and in the future, it will be necessary to take into account this increasing complexity. An important emerging concept is that steroids may exert different actions and use different signaling mechanisms in the normal, injured, and perhaps also in aged nervous tissues. For example, membrane receptors of progesterone and estradiol are induced in response to lesion, which may mediate their protective and trophic effects. Also, the transcriptional effects of steroids mediated by classical intracellular receptors may differ between normal, lesioned, and aged nervous tissues, either because of changes in receptor expression or because of interactions with particular coregulator proteins or cooperative signaling pathways (644). In this regard, it is significant that some genes of the CNS involved in neuronal functions only become sensitive to progesterone after injury (10, 120).

XI. Novel Perspectives for Progesterone in HRT: Different Sources and Local Synthesis

Menopause is characterized by the rapid arrest of both progesterone and estradiol secretion by the ovaries, resulting in a marked decrease of their circulating levels (645). The idea of compensating for this loss is thus straightforward. However, this decline in circulating hormones does not necessarily reflect changes in steroid levels within specific target tissues, and even after menopause, women are not completely deprived of endogenous progesterone and estradiol. Indeed, these so-called "sex steroids" are not only derived from the ovaries; progesterone and its metabolites are also produced by the adrenal glands, and both progesterone and estrogens can be locally synthesized within hormone-sensitive tissues, including the nervous system. This concept is very important for fully appreciating the consequences of the decline in ovarian activity and steroidal aging in general.

A. Peripheral sources of progesterone

The adrenal glands are an important source of progesterone in rodents and in humans. Progesterone synthesized in the adrenal glands is indeed not only a precursor for the synthesis of gluco- and mineralocorticosteroids, but is also secreted into the bloodstream, stimulated by ACTH (646). Thus, progesterone continued be present in arterial plasma of female rats long after ovariectomy, but it rapidly became undetectable after combined ovariectomy and adrenalectomy (647). The secretion pattern of progesterone by the adrenal glands even shows a diurnal pattern in female rats, with peak values in the early morning hours (648). In ovariectomized and estrogen-primed rats and guinea pigs, the adrenal secretion of progesterone in response to ACTH stimulation can even become sufficient to facilitate sexual receptivity (649). Because progesterone production by the adrenal glands is regulated by ACTH, it is sensitive to stress; whereas in undisturbed female rats, adrenal progesterone secretion is about one fifth of the ovarian progesterone secretion measured during metestrous, it can become nearly as high as ovarian progesterone secretion in response to stress (650). Estradiol treatment has also been shown to stimulate progesterone secretion by the rat adrenal glands (651, 652).

Reproductive aging in female rats, one of the most commonly used animal models for studying the effects of hormone treatments, shows some particularities. When middle-aged female rats (9–10 months) enter an acyclic, persistent estrous state, levels of ovarian hormones show a marked decrease, but they still continue to have significant concentrations of circulating progesterone and estradiol provided by the adrenal glands, which again increase between 18 and 24 months of age (145, 647, 653, 654). When exposed to a male, early acyclic female rats display a mating-induced increase in progesterone from the adrenal glands along with small gonadotropin surges (655). Irregular estrous cycles can also be reestablished in old acyclic female rats by stimulation of the adrenal glands by ACTH or stress or by the administration of progesterone (656). Another means of restoring cyclic ovarian activity in aged female rats (15–20 months old) is to treat them with a nasal spray of male urine. This stimulus causes the release of progesterone from the adrenal glands, necessary for the increase in gonadotropins (657). It is noteworthy that even after several months of anovulation, old female rats can spontaneously resume ovulatory activity at irregular intervals, with the formation of functional corpus lutea, which are maintained for prolonged periods and secrete progesterone (654).

In fertile women, at least part of serum late follicular progesterone is derived from the adrenal glands (658), whereas in men plasma progesterone is exclusively of adrenal origin (659). The adrenal glands continue to be a source of progesterone in postmenopausal women. Progesterone released by the adrenal glands in response to mild inflammatory stress or to ACTH infusion was even sufficient to stimulate LH increase in postmenopausal women with estrogen replacement (660). Both the ovaries and adrenal glands contribute to serum allopregnanolone, whose levels do not change in women with age, in contrast to what is observed in men (661).

Concerning the postmenopausal ovary, it has been suggested that some steroid production may continue, in particular the synthesis of androgens, which can then be converted within target tissues to estrogens. Thus, the postmenopausal ovary has been reported to be a significant source of plasma testosterone and androstenedione, and there may be no abrupt decrease of ovarian androgen production at the time of menopause (662–664). The results of the Rancho Bernardo Study are consistent with these earlier findings; circulating levels of testosterone and androstenedione were lower in oophorectomized women when compared with unoperated postmenopausal women (665). However, the view that the climacteric ovary may be a major source of androgens has been challenged by a study showing that women averaging 12 yr after menopause with complete adrenal insufficiency had no detectable circulating androgens, strongly suggesting that the adrenal glands may be the major source. This finding was corroborated by the observation that levels of testosterone and androstenedione are very low in postmenopausal ovarian tissue, as is the expression of steroidogenic enzymes. Consistent with an adrenal origin of androgens was the observation that plasma androgen levels were strongly decreased after dexamethasone administration in postmenopausal women with normal adrenal glands (666). A recent analysis of steroidogenic enzymes in the postmenopausal ovary suggested a particular pattern of expression, which would favor the formation of Δ5 steroids (PREG, DHEA) over Δ4 steroids (progesterone, androgens). Expression of the type II 3β-HSD mRNA was found to be greatly reduced, and the enzyme could not be detected by Western blot analysis (667).

In addition to the steroidogenic endocrine glands, enzymes required for the synthesis and metabolism of progesterone are also expressed and functional in many peripheral tissues. Local synthesis and intracrine actions of progesterone have been proposed to take place in various tissues, including mammary gland, uterus, adipose tissue, bone, skin, kidney, liver, heart, blood vessels, brain, and peripheral nerves (9, 10, 457, 668–676). However, although expression of the 3β-HSD enzymes has been studied in a variety of tissues at the mRNA and protein levels, their activities and substrates have only been measured in a limited number of studies. This represents a serious gap because the 3β-HSDs can catalyze the conversion of a variety of steroid substrates; they not only convert PREG to progesterone, but they are involved in the biosynthesis of the major classes of steroid hormones by catalyzing the conversion of Δ5–3β hydroxysteroids to their corresponding Δ4–3-ketosteroids. As a consequence, the type of steroid produced by the 3β-HSDs in a given tissue will depend on the available substrates.

The 3β-HSD gene family has recently been reviewed in detail (677). The genes of two 3β-HSD isoenzymes have been cloned in humans: the type I 3β-HSD gene (*HSD3B1*), predominantly expressed in placenta and peripheral tissues including the mammary gland and skin; and the type II 3β-HSD gene (*HSD3B2*), expressed in the gonads, adrenal glands, and brain (677–679). Multiple 3β-HSD isoenzymes have been cloned from several other species: four members of the rat and six members of the mouse 3β-HSD family (680, 681). Unfortunately, for each of these species, the different 3β-HSD isoforms have been numbered according to the chronology of their discovery, and the isoforms of different species do not correspond by number.

Estradiol can also be synthesized in significant amounts from circulating precursor androgens in extragonadal sites such as the breast, adipose tissue, bone, and brain. In these tissues, the aromatase enzyme, which converts testosterone to estradiol or androstenedione to estrone, is present and becomes the major source of estrogens after the menopause (682). The significance of local estrogen formation in peripheral tissues is well documented by the benefits of aromatase inhibitors in breast cancer (683–685). Thus, the very low levels of estrogen that persist in the plasma of postmenopausal women result from their release by a variety of tissues, but they do not necessarily reflect the local concentrations of the steroid within these tissues. Moreover, the capacity of peripheral tissues to produce estrogens, such as adipose tissue, seems to increase with age (686). Consequently, after menopause, estrogens mainly become paracrine/autocrine signaling molecules within extragonadal tissues (687, 688).

The circulating substrates for the local formation of estrogens in postmenopausal women are androgens mainly derived from the adrenal glands, and DHEA, also of adrenal origin (688). As a consequence of the progressive decline in the circulating levels of DHEA and DHEA sulfate (DHEAS) with progressing age, substrate availability for the local synthesis of androgens and estrogens decreases with age (689–691). The measurement of DHEAS in a cohort of 3029 women from the SWAN study has revealed that its levels do not steadily decline during the transition to late perimenopause, and that they may even transiently increase in some women at this stage. Interestingly, changes in circulating testosterone and estradiol correlated with changes in DHEAS (692). These observations are consistent with results of a study in female macaques, showing a transient increase in DHEAS levels during the time of perimenopause (693). These observations point to an important role of the precursor steroid DHEA during the menopause transition period.

B. Synthesis of progesterone in the nervous system

The nervous system is an important site of steroid formation; neurons and glial cells not only have the capacity to convert circulating steroid precursors to neuroactive steroids, but they can also synthesize them *de novo* from cholesterol (669, 694). Steroids that are synthesized within the CNS or PNS have been named "neurosteroids" (695, 696). To qualify as a neurosteroid, there are two requirements: 1) persistence of the steroid in the nervous system in the absence of the steroidogenic endocrine glands (gonads and adrenal glands); and 2) expression and activity of the enzymes involved in their synthesis within the nervous system.

From an evolutionary point of view, progesterone can be synthesized within the nervous system of all species studied so far, and 3β-HSD enzymes are widely distributed throughout the brain of fishes, amphibians, birds, and mammals (189, 671, 697–704). Estradiol can also be formed within the brain in all these phyla, but particularly high amounts of estradiol are synthesized in the brains of fishes and birds. Thus, in goldfish, the activity of the aromatase enzyme is about 10-

fold higher in the hypothalamus than in the ovaries (705, 706). In songbird species, such as the zebrafinch, the brain synthesizes very large quantities of estradiol from androgens and is the exclusive source of circulating estradiol (707, 708). The presence, regulation, and functional significance of the aromatase has been studied in detail in the bird brain (709, 710).

In the rodent nervous system, the synthesis of neurosteroids has been extensively reviewed (696, 697, 703, 711) (for an overview of steroidogenic enzymes in the pathways from cholesterol to the different steroid hormones, see Ref. 712) (Fig. 5). The mitochondrial cytochrome P450scc, the cholesterol side chain cleavage enzyme that catalyzes the *de novo* synthesis of PREG, is expressed at low levels throughout the rodent brain and has been detected in most cell types (697, 713–717). Its presence and activity have recently been demonstrated within rat pain pathways: dorsal root ganglia, dorsal horns of the spinal cord, and somatosensory cortex. Results are consistent with an important role of neurosteroids in the control of pain mechanisms (718–720). The second enzyme necessary for the synthesis of progesterone, the 3β-HSD, is also present in neurons and glial cells. The enzyme is present in the cytoplasm and can also form a catalytically active molecular complex with the cytochrome P450scc at the inner mitochondrial membrane (721). Detailed *in situ* hybridization studies have shown its widespread expression in the rat CNS during development and adulthood (704, 722, 723). By measuring the cerebral accumulation of PREG in

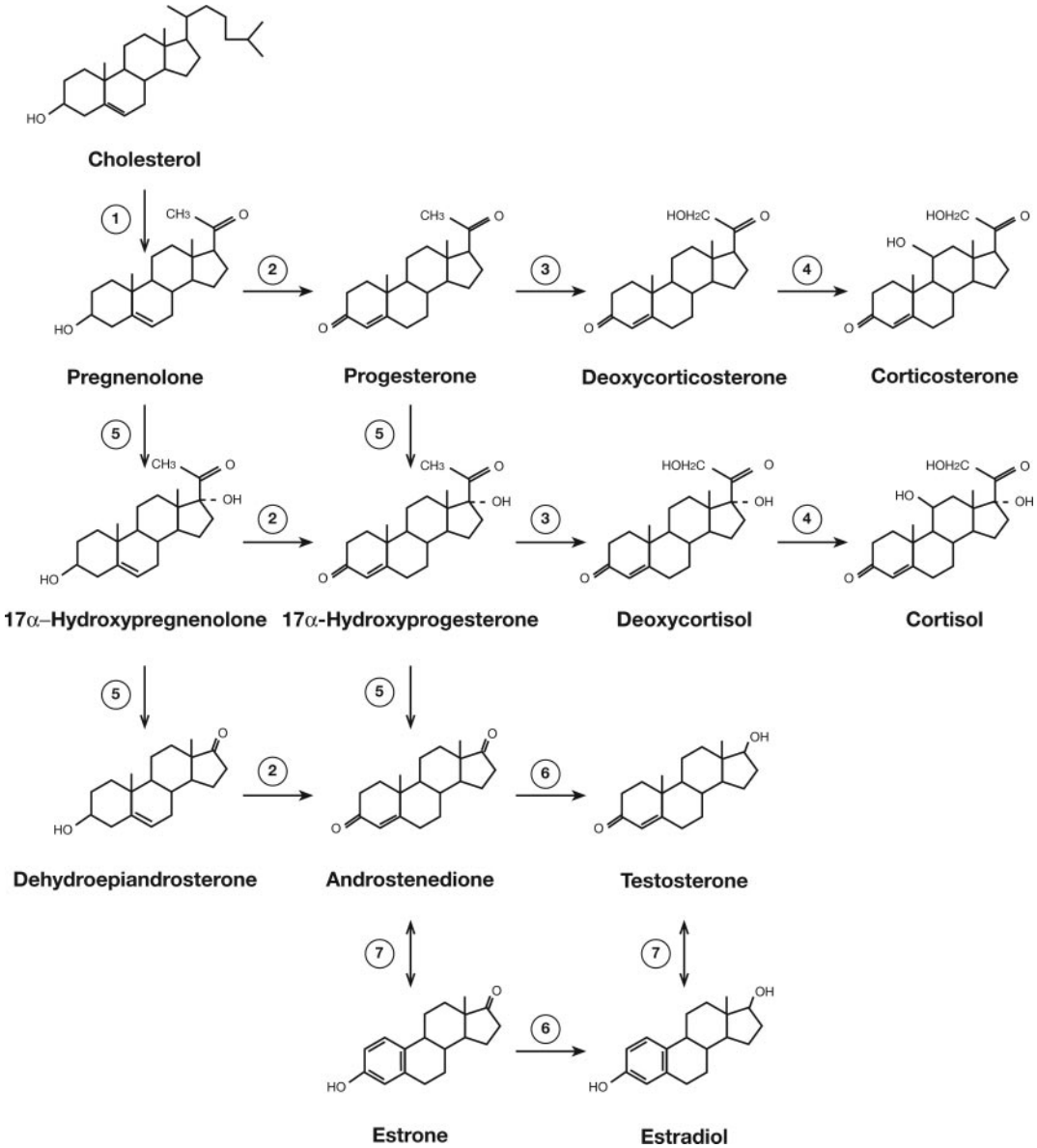

FIG. 5. Biosynthetic pathways of steroids. Conversion of cholesterol to pregnenolone is catalyzed within the mitochondria by the cytochrome P450scc (scc = side chain cleavage) (1). The other enzymes involved in steroid metabolism are: 2) 3β-HSD; 3) cytochrome P450c21 (21-hydroxylase); 4) cytochrome $P450_{11\beta}$ (11β-hydroxylase); 5) cytochrome P450c17 (17α-hydroxylase/17,20-lyase); 6) 17β-hydroxysteroid oxidoreductases (17β-hydroxysteroid oxidoreductase); and 7) aromatase. Note that most enzymes accept several substrates and can catalyze multiple reactions. It is thus necessary to know which steroid substrate is available in a cell before concluding which metabolite is formed.

castrated and adrenalectomized rats after administration of the 3β-HSD inhibitor trilostane, it has been demonstrated that the conversion of PREG to progesterone is a very active metabolic pathway in the rat brain (724). The synthesis and functions of progesterone have been extensively studied in Purkinje cells. These neurons also express the aromatase enzyme, suggesting the local formation of estradiol (725).

Which steroids can be locally synthesized within the mammalian nervous system? This question needs to be answered cautiously, and results of related studies should always be interpreted within their precise context. In fact, the expression and activity of steroidogenic enzymes are subject to complex regulations, and they may be expressed and functional in a defined compartment of the nervous system only under particular conditions, depending on environmental influences, cellular interactions, the presence of neurotransmitters or neuropeptides, the developmental stage, or the integrity of the nervous tissue. Its is also important to be aware of some technical constraints. Thus, the relevance of studies on the formation of neurosteroids by cultured neural cells isolated from embryonic or newborn animals always needs to be examined *in vivo* because of the phenotypic plasticity these cells exhibit *in vitro*. Also, studies of the expression of steroidogenic enzymes by *in situ* hybridization, RT-PCR, or immunocytochemistry, although they provide very important information, do not demonstrate their functionality.

The synthesis of progesterone and its 5α-reduced metabolites by neurons and glial cells is now well established (726–730). Expression and activity of the cytochrome P450c17 in the brain and the possibility of a local synthesis of DHEA and androstenedione, the obligatory precursors of androgens and estrogens, has long remained controversial (in contrast to humans, DHEA is not secreted by the rat and mouse adrenal glands). Thus, P450c17 expression within specific sites of the rat PNS and CNS was found to be restricted to embryonic and early neonatal life (731). However, the mRNA of the enzyme has also been detected by RT-PCR in the adult rodent brain (715), and neurons and astrocytes in culture have been shown to convert PREG to DHEA if grown under specific conditions (732). By combining molecular, anatomical, and neurochemical approaches, expression and activity of the cytochrome P450c17 have recently been demonstrated in the rat spinal cord (733). Two laboratories have even provided evidence for a local synthesis of glucocorticoids (734) and estradiol (735) in the rat brain, at least within specific regions, and the term "synaptocrinology" has been suggested to refer to the effects of locally synthesized neurosteroids on synaptic plasticity (736).

A recent study has shown that an increase in PREG and progesterone synthesis is part of the responses of nervous tissues to injury, consistent with an important role of these neurosteroids in the protection and regeneration of nerve cells (730). Expression of the 3β-HSD and levels of progesterone are also strongly up-regulated in the brains of dysmyelinating jimpy and shiverer mouse mutants (737) and in the spinal cord of streptozotocin-treated diabetic rats (738). Neuropathic pain has also been shown to increase the neosynthesis of PREG and allopregnanolone in the rat spinal cord, suggesting a role for these neurosteroids in nociception and neuroprotection (739, 740). All these observations provide strong evidence for the concept that an increase in the synthesis of progesterone and its metabolites may be part of the mechanisms by which nerve cells cope with neurodegeneration. A recent study has provided evidence that the synthesis of progesterone and other neuroprotective neurosteroids is affected by Alzheimer's disease key proteins. Thus, the overexpression of amyloid precursor protein in human neuroblastoma cells inhibited progesterone synthesis. On the contrary, overexpression of human native tau protein enhanced progesterone formation, but tau protein with a pathogenic mutation was devoid of actions on neurosteroidogenesis (741).

Under normal circumstances, expression of the brain aromatase is restricted to specific neuronal populations. These aromatase-containing neurons are located in brain areas involved in neuroendocrine control. However, in response to different types of injury, aromatase expression and activity is induced in reactive astrocytes, strongly suggesting a role for local astroglial estrogen formation in brain repair (23, 742). Taken together, these results point to an important role for the local formation of progesterone and estradiol in the lesioned or diseased nervous system, which may complement an insufficient supply or override an inappropriate supply of steroid hormones by the endocrine glands. An important area for future investigation is changes in the endogenous capacity of the aged brain to synthesize and metabolize steroids (743–745).

C. Neurosteroids in the human nervous system

Evidence has accumulated over the past few years that neurosteroids are also synthesized and metabolized in the human nervous system (9, 746–748). The presence of the cytochrome P450scc was first detected in the human brain by immunocytochemistry (749), and subsequently, several studies have described the presence of P450scc mRNA in different brain regions (678, 750–752). A recent study has shown that P450scc and StAR are coexpressed in cells of the human brain, consistent with the active sites of neurosteroidogenesis (753). The type II isoform of the human 3β-HSD is largely expressed in different parts of the brain and spinal cord (678, 752), and the enzymes necessary for the metabolism of progesterone are also present in the human brain (754, 755). The activity of the aromatase has been characterized in microsomal preparations of temporal lobe biopsies as well as in cerebral cortex and subcortical white matter samples of adults and children with epilepsy (756, 757).

Because both the endocrine glands and a local production contribute to the pool of steroids present within nervous tissues, the age-dependent decrease in circulating levels of steroids may not necessarily reflect changes in their availability for neural cells. It is not known whether the capacity of neurosteroid synthesis changes with age in humans or whether neurosteroids can compensate for the age-dependent decrease in the activity of the steroidogenic endocrine glands. So far, only a few studies have investigated the distribution of steroid concentrations in aged human brain by RIA. The data of three studies show that elevated levels of PREG and DHEA remain present in the brains of the elderly

(758–760). In the study of Lacroix *et al.* (760), based on nine women and one man (ages, 76–93 yr), levels of PREG, progesterone, androstenedione, and DHEA varied little among different brain regions and were about seven to nine times higher in brain tissue when compared with plasma, consistent with their accumulation or local synthesis. Very high levels of PREG have also been measured in human peripheral nerves, where its mean concentration was about two orders of magnitude higher than in blood (761).

In a more recent study, brain levels of progesterone, 5α-dihydroprogesterone, and allopregnanolone were found to be higher in fertile women in their luteal phase (ages 18–42 yr) compared with the postmenopausal women (ages 59–85 yr), and were thus obviously dependent on ovarian production (762). However, despite the correlation between blood and brain levels of progesterone, it is noteworthy that brain concentrations of progesterone and its metabolites remained elevated and that they were only decreased by about half in the postmenopausal women with very low serum concentrations. In another study, postmortem concentrations of estradiol were also found to be higher in the blood and in specific brain regions of fertile women when compared with postmenopausal women, but again, significant levels of estradiol were measured in the brains of the latter (763).

Levels of steroids have also been measured by RIA in brain and CSF of Alzheimer's patients and aged controls. Some of these studies have reported differences in brain steroid levels between controls and Alzheimer's patients, whereas others found no differences (764, 765). Brain progesterone and estradiol may have a direct influence on Alzheimer's disease. Thus, by crossing aromatase knockout mice with a transgenic mouse model of Alzheimer's disease, it has been demonstrated that β-amyloid peptide is more rapidly deposited in estrogen-deficient brains (765). Progesterone may also play a significant role in senile plaque formation because transcription of the gene encoding neprilysin, one of the major enzymes involved in β-amyloid degradation, is up-regulated by progesterone (766).

The development of very sensitive and precise analysis of steroids by gas chromatography/mass spectrometry corresponded to a major technological breakthrough (767). In fact, because of its great sensitivity, this method allows the analysis of small amounts of neurosteroids in nervous tissues with great precision and reproducibility. The different steps of the assay have been optimized to allow the simultaneous measure of a large range of free and conjugated neurosteroids within distinct brain regions (768, 769). A comparative analysis of the concentrations of several neurosteroids in various brain regions between aged Alzheimer's patients and aged nondemented controls has been recently reported (770). This study was also the first to use gas chromatography/mass spectrometry technology to quantify neurosteroids in human brain and thus provided reference values. In agreement with previous studies using RIA, PREG was the most abundant neurosteroid in the different brain regions analyzed. Steroids found at the highest concentrations were, in decreasing order, PREG > DHEA > progesterone > PREG sulfate > DHEAS > allopregnanolone. It is important to note that levels of all these steroids, except for allopregnanolone, were found to be elevated in the brains of the old patients, and that they were much higher than previously reported blood levels (690, 771, 772). There was also a general trend toward lower levels of the steroids in different brain regions of Alzheimer's patients when compared with controls (770).

D. Regulation of the local synthesis of progesterone in the nervous system

Stimulating the local synthesis of neurosteroids with neuroprotective or neuroregenerative potentials may offer novel perspectives not only for treating lesions and diseases of the nervous system, but also for hormone therapies in the elderly. This line of thinking indeed receives support from observations that the formation of neuroactive steroids, and in particular of progesterone and its reduced metabolites as well as of estradiol, is strongly up-regulated in the lesioned and diseased nervous system (see *Section XI.B*). However, there are still serious gaps in our knowledge of the regulatory mechanisms involved in the biosynthetic pathways of neurosteroids, some of which may be distinct from those described for the steroidogenic endocrine glands. Thus, particular regulatory mechanisms of P450scc transcription have been identified in the nervous system (773, 774). Interactions between neural cells obviously play an important role in the regulation of neurosteroid biosynthesis. Thus, progesterone synthesis is regulated in astrocytes by a still unidentified autocrine factor (775) and in Schwann cells by diffusible neuronal molecules (727).

Furthermore, neurotransmitters and neuropeptides play a particularly important role in modulating the synthesis of steroids within the nervous system. Thus, the $GABA_A$ receptor agonist muscimol and the central-type benzodiazepine receptor agonist clonazepam both stimulated PREG synthesis in retinal ganglion cells (776). Several neurotransmitters and neuropeptides involved in neurosteroid regulation have been identified in the frog brain, where all the major steroidogenic enzymes are expressed and functional (702). In the frog hypothalamus, 3β-HSD-immunoreactive neurons express $GABA_A$ receptors involved in the inhibition of the conversion of PREG to progesterone or to DHEA (777), whereas activation of central-type benzodiazepine receptors by octadecaneuropeptide was found to increase the synthesis of these neurosteroids (778). Steroid-producing cells of the frog diencephalon are innervated by neuropeptide Y-immunoreactive fibers, and neuropeptide Y was shown to inhibit the sulfation of both PREG and DHEA (779). On the contrary, vasotocin and mesotocin, the respective amphibian orthologs of mammalian vasopressin and oxytocin, were found to stimulate the synthesis of progesterone and DHEA in diencephalic nuclei (780). These studies in an amphibian have demonstrated the important role of neuropeptides in the regulation of neurosteroid biosynthesis, but their relevance for mammalian species needs to be examined. A recent study has shown that in spinal sensory circuits of the rat, substance P, a major mediator of pain signals, inhibits the conversion of progesterone to allopregnanolone (719). In the rodent hippocampus, stimulation of neurons with NMDA induced a significant production of estradiol (735).

Circulating steroid hormones are also involved in the regulation of neurosteroid biosynthesis within the brain. A nice

example, which also illustrates well the functional significance of locally synthesized neurosteroids, is the induction of progesterone synthesis by estradiol within the hypothalamus. The Micevych group has indeed shown that systemic estrogen treatment of ovariectomized and adrenalectomized (ADX) rats increases levels of progesterone in the hypothalamus. Estradiol failed to induce an LH surge in the ovariectomized-ADX females if the increase in hypothalamic progesterone was blocked by an inhibitor of the 3β-HSD (676, 781). These observations strongly suggest that progesterone synthesis in the hypothalamus is critically involved in the positive feedback mechanisms of estradiol that trigger the LH surge. Other experiments have shown that estradiol induces the synthesis of progesterone in hypothalamic astrocytes by acting through a membrane-associated receptor and by releasing intracellular stores of Ca^{2+} (782). Noteworthy, estradiol increased the production of progesterone in astrocytes from postpubertal, but not from neonatal, female rats (782). Also, castrated/ADX male rats, which in contrast to females do not show an estrogen-induced LH surge, had no increase in hypothalamic progesterone after estrogen treatment (781).

Intramitochondrial cholesterol transporters offer very promising possibilities for stimulating the synthesis of neurosteroids and for promoting neuroprotection and neuroregeneration. One of them, the peripheral benzodiazepine receptor (PBR), is a mitochondrial protein particularly abundant in steroid-producing tissues and also in glial cells. Recently, the PBR has been renamed "translocator protein (18 kDa)" (TSPO) (783), and this nomenclature will be adopted here. Both *in vitro* and *in vivo* studies have demonstrated that the TSPO is necessary for the transport of cholesterol from the outer to the inner mitochondrial membrane, where the cytochrome P450scc is located. This intramitochondrial transport of cholesterol is a rate-limiting step in the biosynthesis of steroids (784, 785).

Ligands of the TSPO not only increase the synthesis of steroids by the steroidogenic endocrine glands, but they also allow the stimulation of the synthesis of neurosteroids, as has been shown in cultured glial cells and in the brain of castrated and ADX rats (729, 786–789). The possibility of increasing the synthesis of neuroactive steroids has stimulated recent efforts to develop more selective and efficient TSPO ligands (790–792). Interestingly, in response to injury, TSPO expression is increased in the brain and in peripheral nerves (793–795). A strong up-regulation of TSPO expression is also observed during neurodegenerative diseases (Alzheimer's disease) and demyelinating diseases (multiple sclerosis) (785, 796). In the hippocampus of Alzheimer's patients, increased TSPO expression correlated with increased levels of PREG (785). These observations suggest that TSPO ligands may offer novel means for neuroprotection and for improving age-dependent dysfunctions of the nervous system. TSPO ligands have indeed been shown to protect neurons from excitotoxic injury, to promote their regeneration, to reduce inflammatory responses, to decrease reactive gliosis, and to reduce aging-associated myelin degeneration (792, 797–800). However, a role for neurosteroids in mediating these beneficial effects of TSPO ligands awaits demonstration. That is, TSPO ligands can influence many aspects of mitochondrial activity and not only steroidogenesis because the TSPO is physically associated with the voltage-dependent anion channel and the adenosine nucleotide translocase, which form the backbone of the mitochondrial permeability pore (801, 802).

A second protein necessary for the intramitochondrial transport of cholesterol and steroidogenesis is the steroidogenic acute regulatory protein (StAR) (803, 804). Clinical studies of patients suffering from congenital lipoid adrenal hyperplasia, as well as studies of StAR null mice, have demonstrated the indispensable role of StAR in regulated steroidogenesis (805). Recent studies have shown that there is a functional interaction between StAR and TSPO required for cholesterol delivery into the mitochondria and steroid formation (806). In response to excitotoxic injury, StAR is strongly induced in hippocampal neurons (807, 808). Interestingly, whereas TSPO and StAR expression are decreased in the aging gonad (809), StAR expression has been reported to be up-regulated in the brains of aged animals (807). Whether the increased StAR expression reflects a compensatory increase in local neurosteroid synthesis within the aged brain would be an interesting working hypothesis. It is important to note that StAR expression may be regulated in a specific manner within distinct compartments of the nervous system, and as for the TSPO, in a different manner than in the endocrine glands (810). Thus, StAR expression is induced by cAMP within the gonads but down-regulated in Schwann cells (811).

A molecule that efficiently stimulates the synthesis of progesterone and its reduced metabolites in the rat brain is the anxiolytic drug etifoxin [2-ethylamino-6-chloro-4-methyl-4-phenyl-4*H*-3,1-benzoxazine hydrochloride (Stresam)], which is a ligand for both $GABA_A$ receptors and TSPO (729, 812, 813). An increase in allopregnanolone formation indeed contributes to the anxiolytic effects of etifoxin, as has been demonstrated in the Vogel conflict test (729). As already mentioned, the anxiolytic properties of allopregnanolone have been studied in different stress models and also in aged animals (284, 814, 815).

Other drugs used in medicine directly affect the biosynthesis or metabolism of neurosteroids. For example, fluoxetine (Prozac) and other selective serotonin reuptake inhibitors (SSRIs), which are widely used for the treatment of depression, enhance allopregnanolone levels in the rat brain (816, 817). At the molecular level, SSRIs have been shown to stimulate the accumulation of allopregnanolone in the brain by increasing substrate affinity of the human ARK1C2 enzyme (type III 3α-HSD), which converts 5α-dihydroprogesterone to allopregnanolone. Sertraline also blocked the reverse oxidative reaction, the conversion of allopregnanolone to 5α-dihydroprogesterone catalyzed by microsomal RODH-like SDRs (818). Like the SSRI, the antidepressant mirtazapine enhanced the formation of allopregnanolone and inhibited the oxidation of allopregnanolone into 5α-dihydroprogesterone.

Preclinical observations strongly suggested a key role for neurosteroids in depression and an antidepressant potential of allopregnanolone: levels of allopregnanolone were shown to be decreased in human CSF and plasma during major depression, and their levels were restored by antidepressant

treatment (819, 820). However, more recent studies have shown that increased levels of allopregnanolone and other neuroactive steroids observed in response to antidepressant treatment merely reflect a specific pharmacological effect of the drugs, which may contribute but is not essential for the clinical responses: 1) changes in neurosteroid levels were found to be comparable between responders and nonresponders during antidepressant treatments; and 2) nonpharmacological antidepressive treatments, such as sleep deprivation and transcranial magnetic stimulation, can have beneficial effects without affecting steroid levels (815, 821, 822).

In conclusion, progesterone and estradiol continue to be locally produced within a variety of peripheral tissues, long after the arrest of ovarian functions. Age-dependent changes in their circulating levels thus do not necessarily reflect changes in their concentrations within hormone-sensitive tissues. This is particularly true for the brain, spinal cord, and peripheral nerves, where neurosteroids can be synthesized *de novo* from cholesterol. Only very limited information is available concerning changes in neurosteroid levels with age. The use of pharmaceutical agents that increase the synthesis of biologically active steroids within the nervous system offers novel therapeutic perspectives for promoting healthy aging and for treating age-related dysfunctions of the nervous system. Anxiolytic and antidepressant drugs also increase levels of neurosteroids, especially of 3α-reduced pregnane steroids, which in turn appear to play an important role in the pathophysiology of psychiatric disorders (815). Neurosteroids thus offer interesting perspectives for pharmacological intervention on age-associated psychiatric disorders, including mood and anxiety disorders.

XII. Conclusions

Whereas progesterone elicits increasing interest for its usefulness in treating lesions and degenerative diseases of the nervous system (13, 105, 270), its therapeutic potential for HRT is far from being completely appreciated. There are two major reasons for this situation: 1) little knowledge concerning the physiopathological effects of natural progesterone and of its metabolites in many tissues, which is rather surprising more than 70 yr after the discovery of the hormone; and 2) unjustified generalizations of the effects of specific compounds, often with an inappropriate nomenclature. In fact, different synthetic progestins have very different pharmacological and biological properties, sometimes very distinct from progesterone, and referring to them as a single class is not acceptable. Most serious are the risks associated with the use of some of the currently available progestins, such as increased risk of breast cancer and of cardiovascular complications. Within the nervous system, MPA has been shown to inhibit the beneficial effects of estradiol and even to exert damaging effects. On the contrary, these risks are not found with the use of progesterone, and until the development of more selective and safe progestins, micronized progesterone may be an option for efficient and safe HRT, although such an option may not be very attractive for the pharmaceutical industry.

At least in the nervous system, the beneficial pleiotropic actions of progesterone and its close reduced metabolites are now well recognized, including their neuroprotective, neurotrophic, and promyelinating effects, thus offering interesting perspectives for the protection and recovery of aged nerve cells. Particularly encouraging are recent experimental observations obtained in rodents, showing that the aging nervous tissue remains to some extent sensitive to trophic effects of progesterone, and that treatment with progesterone even allows reversal of age-related structural abnormalities and dysfunctions. These findings are coherent with recent neuroanatomical studies, which have demonstrated that the aging process is not associated with a massive loss of neurons, but rather with more subtle changes in neuronal circuits and alterations of the myelin sheaths. Some of these age-dependent changes appear to be reversible, making attempts to treat them meaningful.

However, one should be aware that information in the literature concerning the effects of ovarian steroids on the aging nervous system are still fragmentary and that they do not allow final conclusions concerning the preservation or modifications of steroid responses during aging to be drawn. This is obviously a fundamental question when considering HRT, and hormone therapies in general, for the elderly. Some reports indeed advise caution with respect to hormone actions in the aging nervous system. In particular at advanced ages or after long-term hormone deficiency, some brain functions may become less sensitive to steroids. Under such circumstances, the administration of steroids may even become dangerous by increasing the risk of cerebral stroke and by precipitating the development of cognitive dysfunctions and of neurodegenerative diseases. For some steroid effects, there may be windows of opportunity, during which hormone treatments may be most efficient. But this concept awaits further demonstration, and safety of prolonged hormone treatment remains an important issue.

It is also important to improve our knowledge concerning the molecular signaling mechanisms of progesterone. Although much progress has been achieved over the past few years, they are still poorly explored in the nervous system. For example, whereas the functions of the two PR isoforms have been extensively studied in reproductive tissues, their respective significance in the brain and peripheral nerves remains largely unknown. The recognition of gene- and cell-specific recruitment of nuclear coregulator proteins by steroid receptors and the recent identification and cloning of membrane receptors of progesterone open completely new perspectives for the development of more efficient, selective, and safe progestagen treatments. An important emerging concept is that steroids may use different signaling mechanisms in the normal, injured, and perhaps also aged nervous tissues. In the future, this increasing complexity will need to be taken into account when examining the usefulness of hormone replacement strategies.

When appreciating the consequences of steroidal aging, it is important to be aware that steroids are not only produced by the endocrine glands, but that they are also locally formed within hormone-sensitive tissues, where they act as autocrine/paracrine signaling molecules. As a consequence, the decline in circulating steroids does not provide information

concerning changes in steroid levels within specific tissues, and even after the menopause, women are not completely deprived of endogenous progesterone and estradiol. In the nervous system, progesterone and estradiol are synthesized by neurons and glial cells. The stimulation of their synthesis by pharmaceutical agents such as TSPO ligands offers another perspective for promoting healthy aging and for treating age-related dysfunctions of the nervous system.

Taken together, the rapid development of knowledge concerning the biosynthesis, mechanisms of action and effects of progesterone in the nervous system offer very promising possibilities for HRT. However, experimental and clinical data are still insufficient for making definitive therapeutic recommendations, and more basic research and carefully designed clinical trials are needed for better understanding hormone actions in the aging nervous system.

The quest for more selective and safe steroid compounds for HRT is a very important issue. Laboratories are searching for chemical modifications that eliminate the side effects associated with chronic hormone use, while enhancing their beneficial actions in the nervous system. Future HRT strategies will also need to take into account the marked individual differences in aging. Indeed, whereas some old individuals exhibit performances similar to those of young subjects, others are severely impaired (288). In addition, one should be aware that steroids such as progesterone are only part of the complex signaling systems, and that they exert their effects in concert with other hormones and growth factors, which all undergo major changes during the aging process (37, 823, 824). This may contribute to the influence of environmental factors and lifestyle choices, which also influence the outcome of hormone therapies (825).

Acknowledgments

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