PERGAMON

Journal of Steroid Biochemistry and Molecular Biology 69 (1999) 97–107

The Journal of Steroid Biochemistry & Molecular Biology

Progesterone as a neurosteroid: synthesis and actions in rat glial cells☆

I. Jung-Testas[a,*], A. Do Thi[a], H. Koenig[b], F. Désarnaud[a], K. Shazand[a], M. Schumacher[a], E.E. Baulieu[a]

[a]*INSERM U 488, 80 rue du Général Leclerc, 94276 Le Kremlin-Bicêtre Cedex, France*
[b]*Université Bordeaux I, 33405 Talence, France*

Abstract

The central nervous system (CNS) and the peripheral nervous system (PNS) are targets for steroid hormones where they regulate important neuronal functions. Some steroid hormones are synthesized within the nervous system, either *de novo* from cholesterol, or by the metabolism of precursors originating from the circulation, and they were termed '*neurosteroids*'. The sex steroid progesterone can also be considered as a neurosteroid since its synthesis was demonstrated in rat glial cell cultures of the CNS (oligodendrocytes and astrocytes) and of the PNS (Schwann cells). Both types of glial cells express steroid hormone receptors, ER, GR and PR. As in target tissue, e.g. the uterus, PR is estrogen-inducible in brain glial cell cultures. In the PNS, similar PR-induction could not be seen in pure Schwann cells derived from sciatic nerves. However, a significant PR-induction by estradiol was demonstrated in Schwann cells cocultured with dorsal root ganglia (DRG), and we will present evidence that neuronal signal(s) are required for this estrogen-mediated PR-induction. Progesterone has multiple effects on glial cells, it influences growth, differentiation and increases the expression of myelin-specific proteins in oligodendrocytes, and potentiates the formation of new myelin sheaths by Schwann cells *in vivo*. Progesterone and progesterone analogues also promotes myelination of DRG-Neurites in tissue culture, strongly suggesting a role for this neurosteroid in myelinating processes in the CNS and in the PNS.

1. Introduction

The nervous system is a target for steroid hormones. In late foetal and early postnatal life, gonadal steroids influence the differentiation and connectivity of specific neuronal populations in both the brain and spinal cord [1]. In the adult, steroid hormones still influence neuronal functions, mainly by regulating synaptic transmission, and they exert neurotrophic effects by influencing changes in the morphology and connections of nerve cells. For example, estrogens increase the density of dendritic spines of hypothalamic and hippocampal neurons [2]. Steroid hormones also act in neural tissues to affect brain development and behavior, as for example the induction or inhibition of sexual behavior by progesterone in the female rat [3,4]. The actions of steroid hormones are mediated by specific intracellular receptors and it has become obvious that such receptors are more widely distributed throughout the nervous system than has been thought. Thus steroid hormone receptors were demonstrated in different regions of the brain [5], and as in target organs, e.g. the uterus, estradiol modulates progestin receptor concentrations in some brain regions [6,7]. Our group has demonstrated the presence of progesterone, glucocorticoid, estrogen and androgen receptors (PR, GR, ER and AR) in rat glial cells of the central nervous system (CNS) and the presence of PR and ER in rat glial cells of the peripheral nervous system (PNS) [8–11]. Among these receptors, only PR was increased by estrogen. The distribution of

☆ Proceedings of Xth International Congress on Hormonal Steroids, Quebec, Canada, 17–21 June 1998.

* Corresponding author. Tel.: 1 4959 1803; fax: 1 4521 1940.

0960-0760/99 $ - see front matter
PII: S0960-0760(98)00149-6

these intracellular receptors in glial cells was determined by ligand binding experiments and immunohistochemistry of receptor proteins. These techniques do not distinguish between receptors for peripheral steroids and steroids synthesized within the brain (neurosteroids), and we do not know whether receptors in the nervous system are or are not the same as in peripheral target tissues. The estrogen-inducibility of PR in glial cells as well as sex-differences in this PR-induction [9] would suggest that the glial PR is identical to that of the reproductive PR in terms of hormonal regulation. However, the cloning and sequencing of the ligand binding domain (LBD) from rat glial cells and rat uterus (R. Fiddès and K. Shazand, in preparation) indicated that the glial cell PR differs from that of the uterus. In glial cells, two forms of PRs were identified, one with a LBD corresponding to that of the uterus, and another with several amino acid differences. This raised the question whether glial cells express two different PRs. In attempt to answer this question, ligand binding studies and immunohistochemistry of receptor proteins were undertaken in mice lacking progesterone receptor (PR KO-mice), kindly provided by B.W. O'Malley [12]. The result will be described in this paper.

Research performed over the past few years has shown that some steroids, called neurosteroids, are synthesized within the brain and peripheral nerves by glial cells [13–15]. The term neurosteroid does not designate a particular class of steroids, but only refers to their site of synthesis — the nervous system. Steroidogenesis begins with the conversion of cholesterol to pregnenolone by the cytochrome P450scc. With the use of specific antibodies and immunocytochemistry, we have demonstrated the presence of P450scc in brain glial cells [16], and these results have recently been confirmed by the detection of P450scc mRNA in oligodendrocytes [17]. The production of neurosteroids is thus a feature of myelinating glial cells and an important question is the biological significance of such neurosteroids. That glial cells are a target for steroid hormones was shown in cell culture experiments, in which different effects of progesterone and estradiol on cell growth, cell adhesiveness and cell morphology were demonstrated. However, the most striking effect of progesterone was the stimulation of myelin-specific protein synthesis in oligodendrocytes during primary culture [18] and its trophic activity on myelination of DRG-neurites in DRG-rat Schwann cell cultures, as well as in regenerating sciatic nerves after cryolesions in *in vivo* studies [19]. Additional data of the trophic action of progesterone and progesterone analogues on the induction of myelin protein synthesis in glial cells of the CNS and the PNS will be described.

2. Progesterone as a neurosteroid

The term 'neurosteroid' was applied to steroids synthesized in the brain '*de novo*' from cholesterol [20]. The first step in steroidogenesis is the conversion of cholesterol to pregnenolone by the side chain cleavage enzyme cytochrome P450scc in the inner mitochondrial membrane, a conversion characteristic of all steroidogenic cells. The expression of this mitochondrial enzyme was demonstrated in brain glial cell cultures of newborn rats, composed of oligodendrocytes (the myelinating glial cells of the CNS) and of astrocytes. Immunohistochemical staining showed the presence of P450scc in both cell types, oligodendrocytes displayed more intense staining than astrocytes [16] (Fig. 1). Such glial cells have the capacity to synthesize pregnenolone and progesterone. This was first demonstrated in glial cell cultures, after incubating cells with labeled mevalonate as steroid precursor, or with labeled cholesterol-linoleate, incorporated into reconstituted low-density lipoproteins [13, 21].

The synthesis of pregnenolone and progesterone in the nervous system is not restricted to the CNS. Indeed, high levels of pregnenolone, the immediate precursor of progesterone, were found in the human sciatic nerve [22] and in the rodent sciatic nerve [23]. Progesterone was also abundant in rodent sciatic nerve and its biosynthesis was demonstrated after incubation of Schwann cells with labeled pregnenolone. However, only Schwann cells derived from fetal dorsal root ganglion (DRG-Schwann cells) were able to produce progesterone. In the absence of sensory neurons, as in Schwann cell cultures derived from sciatic nerves, no progesterone synthesis was observed [24].

The production of neurosteroids appears to be a feature of myelinating glial cells in the CNS and in the PNS, and the synthesis of progesterone suggests a direct action of this steroid hormone in the nervous system. The biological actions of steroid hormones are mediated by specific intracellular steroid hormone receptors and therefore their presence was investigated in rat glial cells.

3. Progesterone receptors in rat glial cells

Our first demonstration of the presence of intracellular steroid hormone receptors in glial cells was done in primary cultures derived from newborn rat forebrain. On the basis of ligand binding experiments, we have identified progestin-, estrogen-, glucocorticoid and androgen receptors. Among these receptors, only the progestin receptor was estrogen-inducible and its presence within glial cell nucleus was confirmed by immunocytochemical staining of the receptor protein [8, 9].

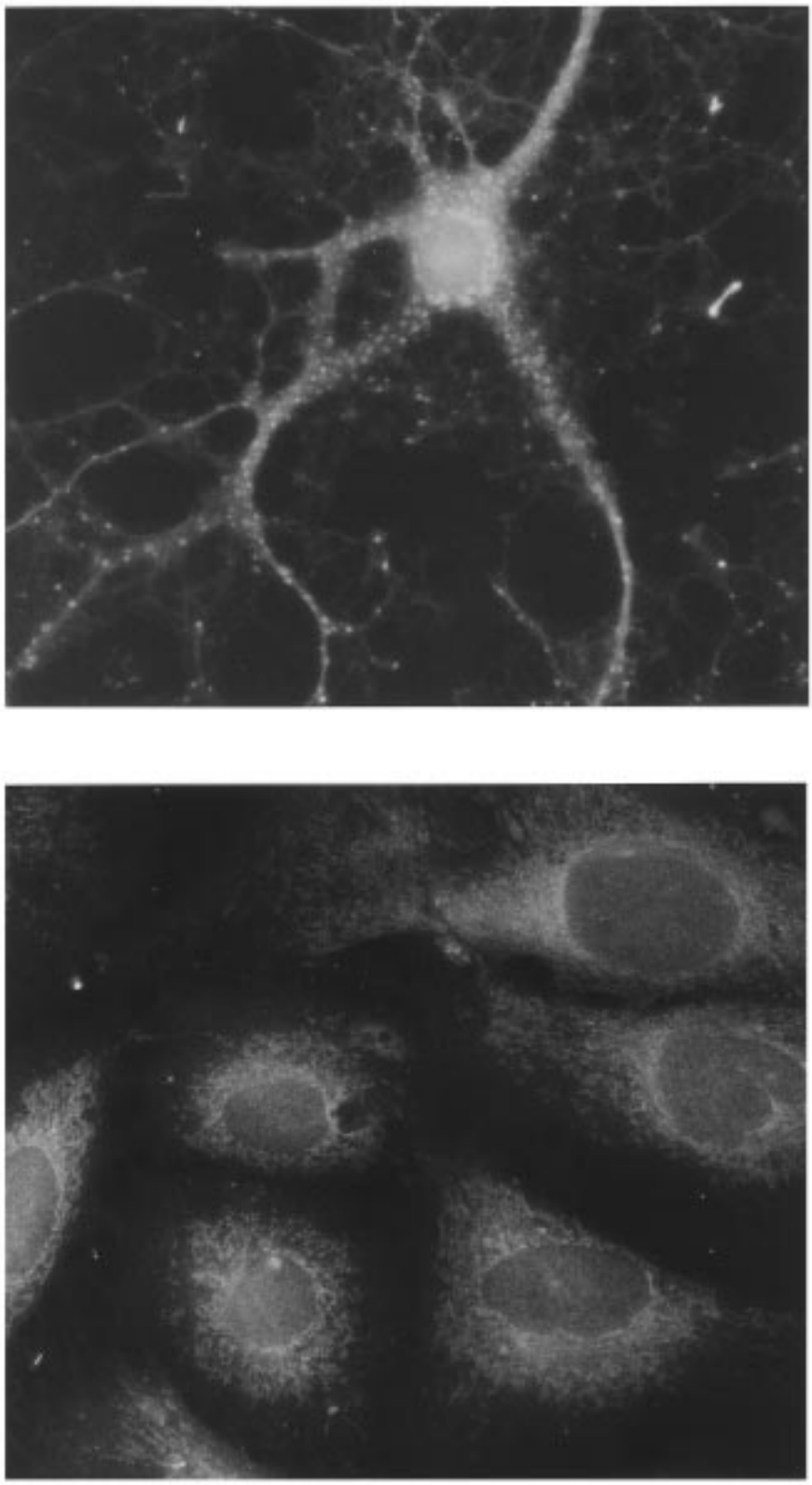

Fig. 1. Indirect immunofluorescence staining of cholesterol side chain cleavage enzyme cytochrome P450 scc in glial cells of the CNS. *Upper part*: rat oligodendrocyte and *lower part*: rat astrocytes.Primary cultures were established from newborn rat forebrain and immunostained with specific anti-P-450ssc antibodies, as described [8, 16]. Note the dotted images of immunostaining (especially in oligodendrocyte), revealing mitochondrial localization of the enzyme.(magnitude ×1750).

In further studies, the presence of estrogen- and progestin receptors was also demonstrated in glial cells of the PNS, in Schwann cell cultures, derived from newborn rat sciatic nerves. Under such culture conditions, Schwann cells remain undifferentiated and do not express myelin proteins. In contrast to glial cells of the CNS, the concentration of PRs, after treatment of Schwann cell cultures with estradiol, had not increased, despite the presence of estrogen receptors [11]. To determine whether this lack of PR-induction was due to culture conditions, Schwann cells were derived from fetal dorsal root ganglion (DRG-Schwann cells), as was first described by Wood [25]. As the ganglia taken from embryonic rats mature *in vitro*, they recapitulate many of the developmental stages: nerve fibers grow out initially and Schwann cells migrate out from the explant. To obtain pure Schwann cells, the general strategy was to excise the neuronal soma, confined to the explant, allowing the neurites to degenerate and leaving an outgrowth containing only Schwann cells. The day after excision of the ganglia, the concentration of PRs was investigated in DRG-Schwann cells, treated or not with estradiol, by ligand binding experiments. After estrogen treatment of the cultures, PR concentration had significantly increased and the increase was dose-dependent, maximal induction was obtained at 50 nM estradiol concentration. This estrogen-mediated induction of PR was specific, since in presence of the antiestrogen ICI 164,384, PR-induction was completely inhibited (Fig. 2). However, this estrogen-mediated PR-induction could not be observed any more after withdrawal of the ganglion from DRG-Schwann cells for several days, despite the presence of estradiol in the culture medium. In contrast, the concentration of non-inducible PR-binding in control cultures remained unchanged after withdrawal of the neuronal mass for 1–14 days [26]. These results suggest that neurons or neuronal signal(s) or factor(s) are necessary for Schwann cells to express estrogen-inducible progestin receptors. Until now, the nature of such neuronal factor(s) is not known and work is in progress for their identification.

The presence of steroid hormone receptors in both types of glial cells (CNS and PNS) had been demonstrated by ligand binding experiments and immunocytochemistry of receptor proteins. These techniques do not distinguish between receptors for peripheral steroids and neurosteroids. To determine whether the PR in glial cells was the same as in peripheral target tissues, we have undertaken the cloning of the ligand binding domain (LBD) from glial cell- and rat uterus progestin receptors (R. Fiddès and K. Shazand, in preparation). In glial cells, two forms of PR were found, one identical to that of the uterus, and another with several amino acid differences in the LBD, and

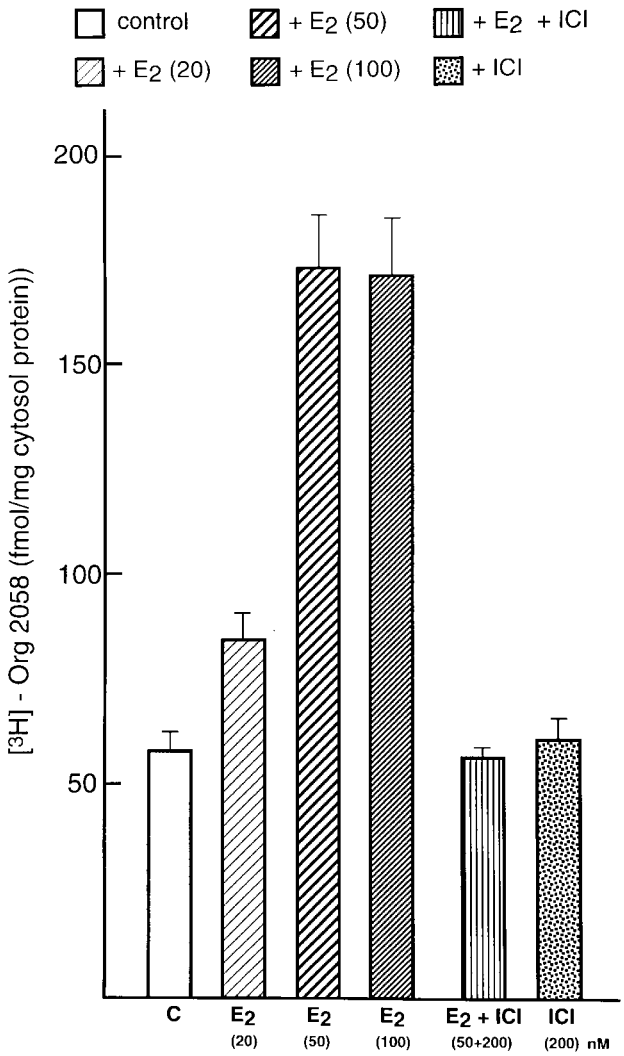

Fig. 2. Progesterone receptors in DRG-Schwann cells. DRG-Schwann cell cultures were prepared from 18-day old rat embryos as described in Table 1. Three weeks after explanting dorsal root ganglia, the DRG-Schwann cells were cultured for two other weeks in the presence (or in absence) of increasing concentration of estradiol (E2) from 20 to 100 nM; or in the presence of E2, 50 nM, plus ICI 164,384, 200 nM; or in the presence of ICI 164,384 (200 nM), alone. After excision of the ganglia, Schwann cells were recovered the following day and incubated at 37°C with saturing concentration of labeled Organon 2058 (5 nM), in presence of 20 nM unlabeled cortisol and with or without excess (1 μM) of unlabeled progesterone to determine specific ligand binding. Cell cytosol was prepared with a glass–glass potter homogenizer after extensive washing the cells with PBS at 0°C, following the incubation. The homogenate was centrifuged at 10,000× *g*. Results are expressed as fmol bound [^{3}H]-Organon 2058/mg cytosol protein. Each data point was done in duplicate. The mean values ± SD of three separate experiments, corresponding to three different primary cultures, are indicated.

this raised the question whether the glial PR was different from that of peripheral target tissue.

In trying to answer this question, we measured PRs in progesterone receptor null mutant mice (PRKO) and wild type mice (WT) from the same strain by

ligand binding and immunocytochemistry. The castrated male mice were treated with estradiol (10 μg sc-injection in sesam oil) two days prior the PR-measurements, and competitive [^{3}H]-Organon 2058 binding assays for PR in medial basal hypothalamus and brain cortex cytosolic extracts indicated respectively 66 and 9 fmol bound Organon per mg protein in cytosol from WT mice. In cytosolic extracts of PR KO mice, no specific binding could be measured (Fig. 3). Unfortunately, PR-binding studies were not possible in sciatic nerves due to lack of material. However, primary Schwann cell cultures could be obtained after dissociating the sciatic nerves, and immunocytochemical staining showed the presence of PR in Schwann cell nuclei of WT mice, but only background staining was seen in Schwann cells from PRKO mice [Fig. 4(a)]. Similar results were obtained after immunohistochemical PR staining of cross sections from brain cortex. Only brain cortex from wild type animals displayed PR-staining [Fig. 4(b)]. These results, and the fact that the glial PR is identical to that of the reproductive PR in terms of hormonal regulation, suggest that glial cells

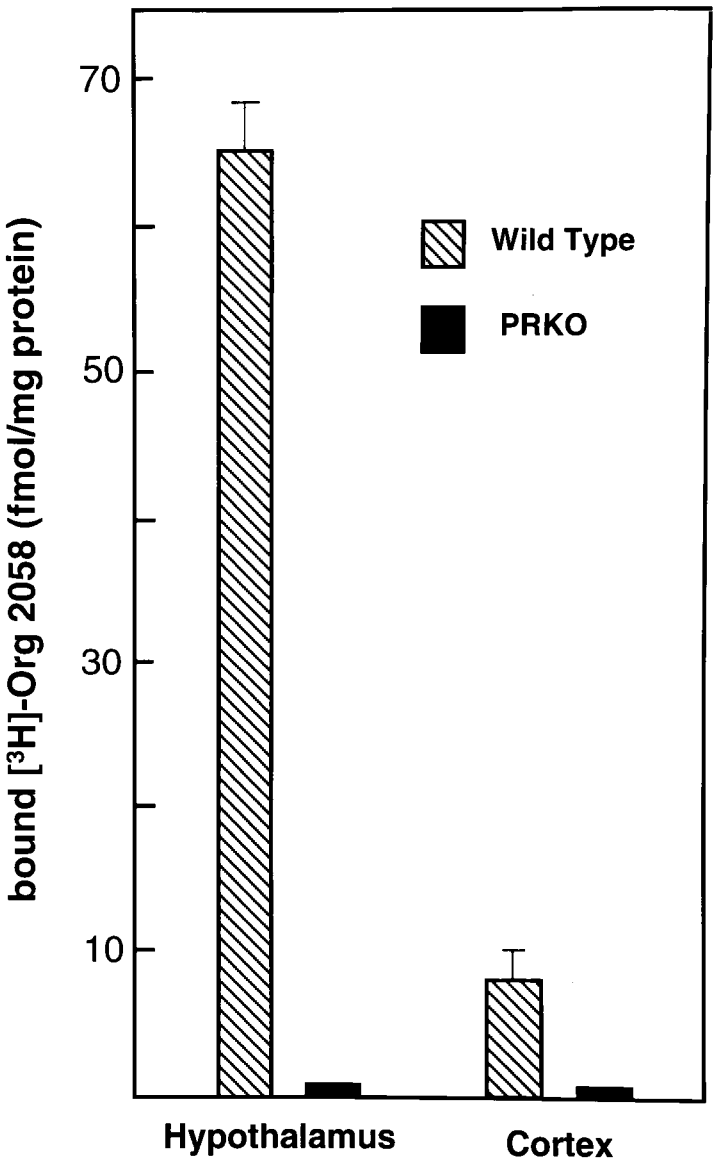

Fig. 3. Progesterone receptors in cytosolic extracts of medial basal hypothalamus and brain cortex of WT and PRKO mice. The castrated male mice were SC injected with 10 μg estradiol in sesame oil, 24 h prior the PR-measurements. After decapitation, hypothalamus and cortex were dissected and homogenized in 4 vol. of TET-buffer (Tris 10 mM, EDTA 1 mM, Dithioerythritol 1 mM, glycerol 10%, pH 7.4). The homogenates were centrifuged at 105,000× *g* for 30 min at 2°C and aliquots of the supernatant (cytosol) were incubated with [^{3}H]-Organon 2058 (1 nM and 4 nM), in the presence or in absence of excess unlabeled progesterone, for 6 h at 0°C. Specific binding was determined after charcoal treatment of the aliquots. Results were expressed as specific [3H]-Organon bound per mg cytosol protein. Each data point was done in triplicate. The mean values ± SD are indicated.

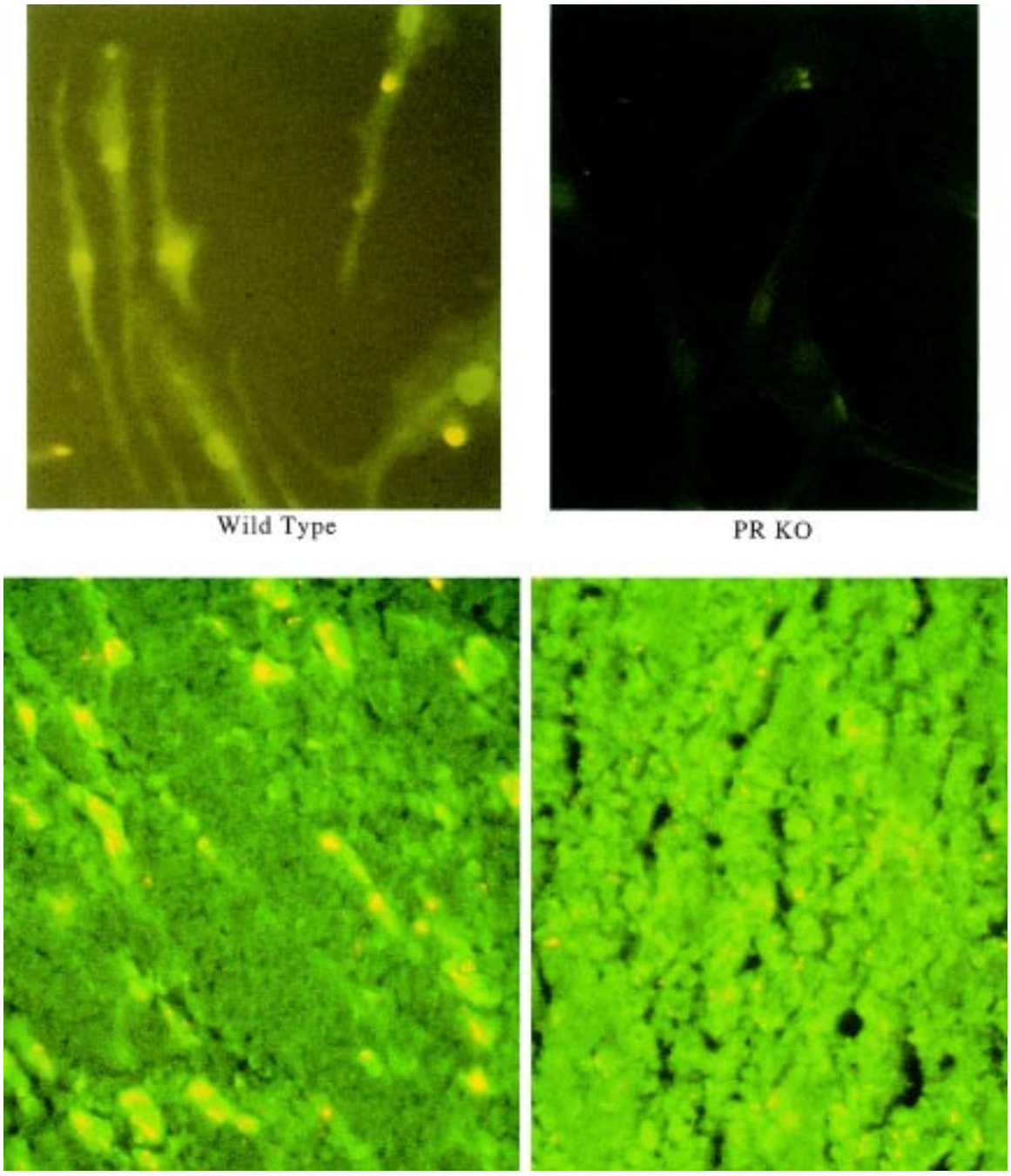

Fig. 4. Immunofluorescence staining of progesterone receptors in Schwann cells from adult mouse sciatic nerves, and in brain cortex sections. *Upper part*: Schwann cells were prepared after dissociating sciatic nerves from WT (left) or PRKO mice (right). Cells were plated on glass cover slips and grown in DME-medium containing 10% charcoal-treated calf serum and insulin (5 μg/ml). After 10 days of culture, immunostaining of PR was done with a monoclonal PR-antibody, (magnitude $\times$400). *Lower part*: PR-immunostaining of cross sections of fixed brain cortex from WT (left), or PRKO mice (right). Note that only background staining can be seen in PRKO mice.

express the classical PR found in peripheral target tissues.

Interestingly, ultrastructural analysis of semithin cross sections and ultrathin cross section of sciatic nerve fibers showed no apparent differences in the myelin sheaths in sciatic nerves of WT- and PRKO mice. In both situations, sciatic nerves were surrounded by compact myelin sheaths whose thickness was similar. The average number of myelin lamellae was 51 ± 17 in PRKO mice and 53 ± 17 in their WT-littermates. As for myelin, no abnormalities were observed in PRKO mice when compared to WT mice: the diameter of the myelinated fibers (3–6 μm) were similar, Schwann cells were surrounded by basal laminas in both cases, the

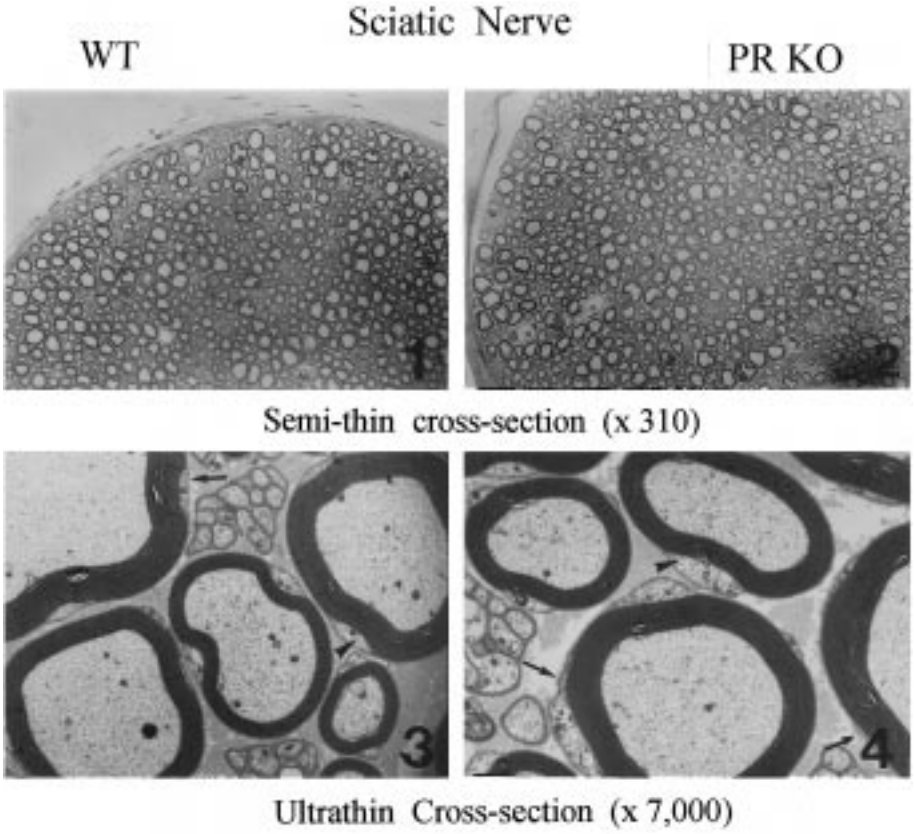

Fig. 5. Cross sections of sciatic nerves from WT- and PRKO mice. Sciatic nerves were fixed in 2.5% glutaraldehyde and cut in 1 mm pieces before osmication in 2% osnium tetroxide for 1 h at 4°C followed by embedding in epon. Ultrathin cross sections of the sciatic nerves were obtained with a Leitz ultramicrotome and were counterstained with uranyl acetate and lead citrate. Semithin sections, obtained from the same embedded nerves, were stained with toluidine blue. *Upper part*: semithin cross sections (×310) of WT (left) and PRKO mice (right). Lower part: ultrathin cross sections (×7000). Note the similar number of lamellae and their compaction in both situations. The myelin sheaths are surrounded by the Schwann cell cytoplasm and basal lamina of Schwann cells.

morphology of the non-myelinated axons was not modified and the concentration of collagen fibrils in endoneural regions was similar (Fig. 5).

4. Action of progesterone and progesterone analogues on myelin protein synthesis

Myelin formation involves complex interactions between different cell types, namely the neuron and the myelinating glial cell. In the CNS, the myelinating cell is the oligodendrocyte, while in the PNS, it is the Schwann cell. Central myelin is composed of about 70% lipids, among which galactocerebroside (Gal C) is a specific marker for oligodendrocytes, and of about 30% proteins, mainly proteolipid protein, myelin basic protein (MBP), 2′3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) and myelin-associated glycoproteins [27]. In newborn rats, the myelin-specific components as MBP, CNPase and Gal C are expressed early in oligodendrocytes and their expression can be followed in primary cultures of newborn rat forebrain by specific immunofluorescence staining. During the first 10 days of primary culture (day one corresponding to the day of birth), the number of oligodendrocytes expressing myelin antigens can be determined by counting the fluorescent cells amidst unlabelled oligodendrocytes. The treatment of glial cell cultures from day one onwards with progesterone (100 nM) had considerably increased the number of MBP- and CNPase expressing oligodendrocytes when compared to control cultures, and this was our first observation of the trophic action of progesterone on myelin protein synthesis [9]. In further studies, the stimulation of myelin protein synthesis by progesterone was confirmed by immunoblotting analysis of CNPase. Single bands of the protein could be detected from day 11 of primary culture onwards, and its synthesis gradually increased thereafter. Treatment of the cultures with progesterone had accelerated CNPase expression and the intensity of the bands had significantly increased when compared to control cultures [8, 29].

The effect of progesterone analogues on myelin protein synthesis in rat oligodendrocytes was also tested. Three orally active progestogens whose pharmacological profile resembles the natural hormone were chosen:

Megestrol acetate (17-hydroxy-6-methylpregna-4,6-diene-3,20-dione acetate), Chlormadinone acetate (17-acetyloxy-6-chloro-pregna-4,6-diene-3,20-dione acetate) and Drospirenone (6β,7β,15β,16β-dimethylene-3-oxo-17a-pregn-4-ene-21,17-carbo-lactone). They were kindly given by Dr. E. Schillinger, Schering, Berlin. The three progestogens had similar high affinity to the glial PR as was shown by competitive ligand binding assays [29]. Among the three compounds, Megestrol acetate was found as potent as progesterone on myelin protein synthesis, as can be seen on Fig. 6. The antiprogestin RU 486 had no antagonistic effect on progesterone-induced myelin protein synthesis and displayed even some agonistic action by itself (Fig. 6).

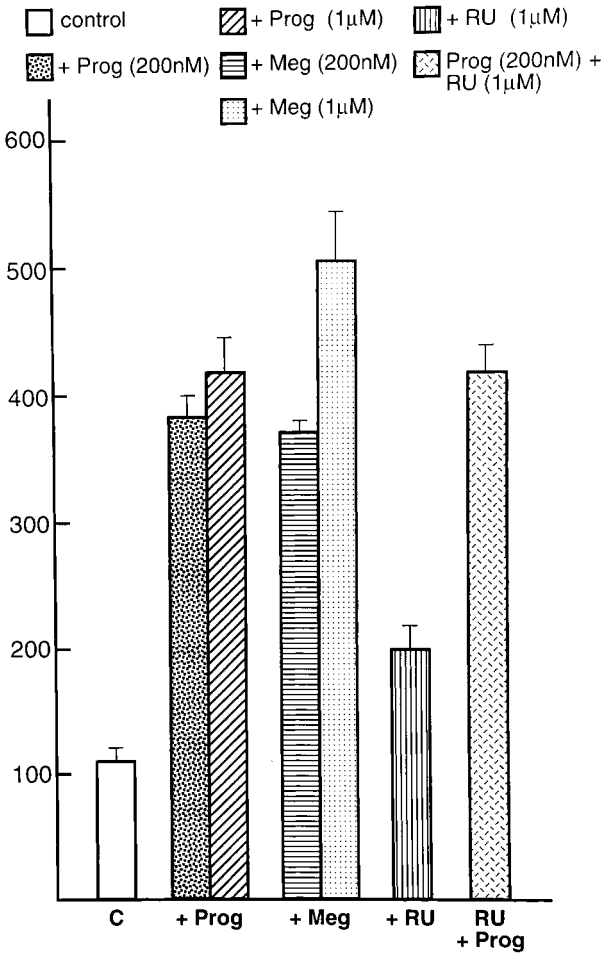

Fig. 6. Effect of progesterone, megestrol acetate and the antiprogestin RU486 on MBP-expression in rat oligodendrocytes. Primary rat glial cell cultures were prepared at postnatal day one, as described [8]. Cells were plated on glass cover slips and cultured in DME-medium containing 10% charcoal-treated calf serum and insulin (5 μg/ml), in the absence (control), or the presence of progesterone (200 nM or 1 μm), megestrol acetate (200 nM or 1 μM), RU 486 (1 μM) and progesterone plus RU 486 (200 nM + 1 μM). At day 11 of culture, immunofluorescence staining of MBP was performed. Labeled and unlabeled oligodendrocytes were counted and the number of antigen-expressing cells amidst 2000 oligodendrocytes are indicated. The mean values $\pm$ SD of three independent measures are indicated.

In the PNS, similar studies cannot be done in Schwann cell cultures, since Schwann cells do not express myelin proteins in usual culture conditions. However, in DRG-Schwann cell cultures, myelination of sensory axons occurs in presence of serum and ascorbic acid [30], and the addition of progesterone to the culture medium had significantly increased the number of myelin segments [19].

The effect of progesterone, the antiprogestin RU 486 and the three progestogens was then tested on myelin formation in such DRG-Schwann cell cultures. After 3 weeks treatment with progesterone (200 nM), a 65% increase of myelin density, expressed as the total length of myelinated segments per mm^2 of the culture dish surface, was observed. RU 486 (400 nM) did not influence myelin formation, but it completely inhibited the progesterone-induced myelin increase when it was added together with the progestin (Table 1). Megestrol acetate and Drospirenone (200 nM) slightly promoted myelin formation, but Chlormadinone was almost as effective as progesterone, as is shown on Table 2.

Taken together, the results from these data and from our earlier studies are a clear demonstration of the trophic action of progesterone on central- and peripheral myelin protein synthesis in *in vitro* experiments.

Table 1
Effect of progesterone and the antiprogestin RU 486 on myelin synthesis in DRG-Schwann cell cultures

Culture conditions	Density of myelin (μm/mm2)	% Control
Control	8846 $\pm$ 1600	100
Progesterone (200 nM)	14616 $\pm$ 1865	165
RU486 (400 nM)	7865 $\pm$ 1267	89
RU + Progesterone (400 + 200 nM)	8013 $\pm$ 1945	91

DRG-Schwann cell cultures were prepared as described [26, 30]. DRG explants were placed on 35 mm Petri dishes coated with rat tail collagen and cultured in Dulbecco's modified Eagle's medium-Ham's F-12 (50:50; v/v) containing 10% fetal calf serum (FCS) and nerve growth factor (30 ng/ml). During the first week, the cultures were treated two times with 10 μM cytosine arabinoside to eliminate fibroblasts. Subsequently, the explants were grown for an additional 3 weeks in DME/F-12 medium containing transferrin (100 μg/ml), insulin (5 μg/ml), putrescine (100 μg/ml), NGF (30 ng/ml) and selenium (30 μg/ml). Thereafter, the DRG-Schwann cells were cultured three more weeks in the same medium, to which FCS (15%) and ascorbic acid (50 μg/ml) were added, as well as the corresponding hormones or antihormones. Ascorbic acid and hormones were added daily. Myelinated fibers were stained with Sudan black (0.5%) after fixing the cultures with glutaraldehyde (2.5%). The analysis of myelin density was performed using the public domain NIH image program 1.62. Myelin formation was quantified as the total length of myelin segments per mm^2 of the culture dish surface. For each point, six sets of culture dishes were analyzed, twelve images/picture of 1 mm^2 were taken at random from each dish.

Table 2
Effect of progesterone and progesterone analogues on myelin synthesis in DRG-Schwann cell cultures

Culture conditions	Density of myelin (μm/mm2)	%/Control
Control	5466 ± 1230	100
Progesterone	10676 ± 2005	196
Megestrolacetate	6876 ± 1900	126
Chlormadinone	8924 ± 2200	163
Drospirenone	6892 ± 2350	126

DRG-Schwann cell cultures were prepared as described in Table 1 and treated for the last 3 weeks of culture either with progesterone, megestrolacetate, chlormadinone or drospinerone (200 nM). Control cultures received vehicle only (ethanol). The density of myelinated fibers was determined as described in Table 1.

Moreover, the role of progesterone on myelin formation was also shown *in vivo*, in the regenerating sciatic nerve of male mice after cryolesions. In this *in vivo* system, the application of progesterone to the lesioned nerve accelerated myelin repair [19].

We are currently studying the effect of progesterone on the expression of myelin specific proteins. A different approach was to study promoter activity of peripheral myelin protein-22 (PMP22) and protein zero (Po) genes in cultured Schwann cells, transiently transfected with reporter constructs in which luciferase expression was controlled by the promoter region of either peripheral protein gene. Progesterone stimulated the Po promoter and promoter 1 of PMP 22. The antiprogestin RU 486 did not abolish the effect of progesterone, but stimulated promoter activity by itself [31], (Fig. 7). The effect of progesterone was specific, as estradiol and testosterone only weakly activated the promoters. These effects of progesterone are likely to be relevant to the process of myelination, since both promoters are involved in myelin formation. These findings are in agreement with recent reports that progesterone can increase Po and MBP RNA levels in Schwann cell cultures [32].

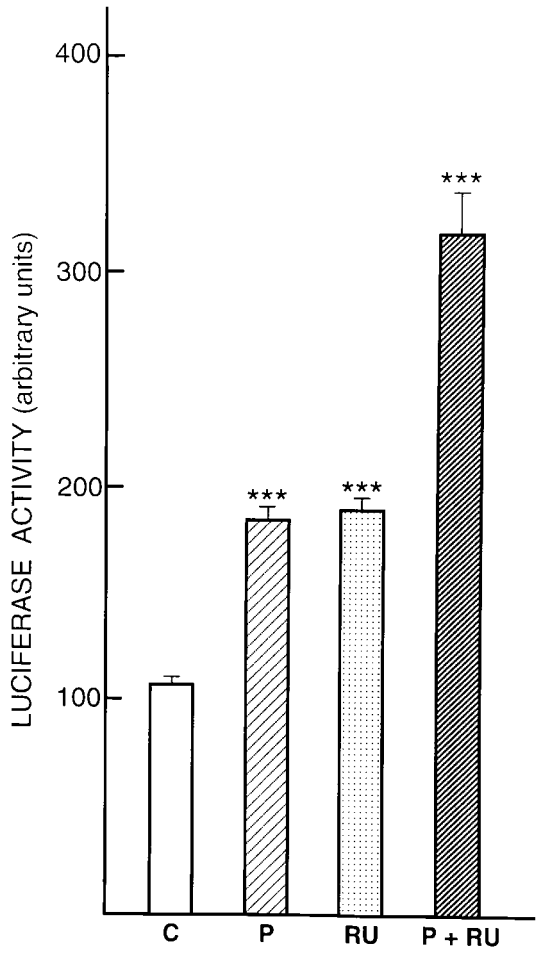

Fig. 7. Effect of progesterone and the antiprogestin RU486 on PMP-22 promoter 1-luciferase activity in cultured rat Schwann cells. Primary cultures of rat Schwann cells were prepared from newborn rat sciatic nerves as described [10]. Cells were grown to 90–100% confluence in DME-medium, containing 10% heat-inactivated FCS, forskolin (5 μM) and insulin (4.8 μg/ml). Schwann cells were transiently transfected using the polyethylenimine (PEI) method with a ratio PEI/DNA of seven equivalents. After transfection, Schwann cells were incubated in culture medium containing 10% charcoal-treated FCS and either progesterone, 1 nM (P), or RU486, 10 μM (RU), or progesterone, 1 μM, plus RU486, 10 μM (P + RU). Control cultures received vehicle only. Luciferase activity was assayed according to standard procedures. Data are given as mean ± standard error (SEM). They were analyzed by one-way ANOVA followed by Newmann–Keuls multiple comparisons tests. ($^{***}p < 0.001$ when compared to control).

5. Conclusions

We have demonstrated that neurosteroids are synthesized in the central and peripheral nervous system, particularly in myelinating glial cells. Synthetic pathways may start from cholesterol or from steroidal precursor(s) imported from peripheral sources. The sex steroid progesterone, the classical female reproductive hormone, can also be classified as a neurosteroid, since its synthesis within both the CNS and the PNS was demonstrated. In the nervous system, progesterone regulates neurotransmission and exerts trophic effects on both neurons and glia through autocrine and/or paracrine actions [33]. Whether neurosteroids formed within nervous tissues significantly contribute to the pool of circulating steroids is unknown. The concentration of neurosteroids in the nervous system are compatible with their playing a physiological neuromodulatory role and are consistent with the affinities of steroid hormone receptors with which they interact. Using a combination of hormone-binding assays and immunocytochemical techniques, we have demonstrated the presence of steroid hormone receptors in glial cells of the CNS and the PNS. They resemble to

those found in peripheral target organs, as for their similar ligand binding affinity and the modulation of PRs by estradiol. Moreover, in PRKO mice, no PRs were detected in brain cortex or sciatic nerve-Schwann cells, whereas their presence was demonstrated in corresponding tissue from WT mice.

Different effects of neurosteroids on glial cells were shown, especially interesting is the trophic action of progesterone and progesterone analogues on central and peripheral myelin protein synthesis, providing compelling support for an important role of progestins in the myelinating process. A very first observation of the role of steroids on myelination was made by Rawlins *et al.* [34], when the authors observed retardation of peripheral nerve myelination in mice treated with inhibitors of cholesterol biosynthesis.

As progesterone is produced by glial cells, it may increase myelination by acting on adjacent neurons and activate the expression of neuronal signaling molecules required for myelination, or it may function as an autocrine trophic factor and directly stimulate myelin formation. Further investigations should assess the physiological significance of our findings.

An important observation is the trophic action of some progesterone analogues on myelin synthesis, and glial cells provide an essential research tool for studying a wide range of such compounds, which may have clinical implications in the treatment of demyelinating diseases.

Acknowledgements

We are very grateful to Professor B. W. O'Malley for providing us the PRKO and WT mice. We also thank Corinne Legris for efficient and excellent assistance in preparing the manuscript and J. C. Lambert for preparing the figures. This work was supported by INSERM.

References

[1] De Vries, G. J., De Bruin, J. P. C., Uylings, H. B. M. and Corner, M. A., Sex differences in the brain. In *Progress in Brain Research, Vol. 61*. Elsevier, Amsterdam, 1984.

[2] McEwen, B. S., Oestrogens and structural and functional plasticity of neurons: implications for memory, ageing and neurodegenerative process. In *Nonreproductive Actions of Sex Steroids*, ed. G. R. Bock and J. A. Goode. John Wiley and Sons, 1995, pp. 52–73.

[3] H.H. Feder, B.L. Marrone, Progesterone: its role in the central nervous system as a facilitator and inhibitor of sexual behavior and gonadotropin release, Ann. NY Acad. Sci. 286 (1977) 286–331.

[4] L.P. Morin, Progesterone: inhibition of rodent sexual behavior, Physiol. Behav. 18 (1977) 701–708.

[5] B.C. McEwen, A. Biegon, P.G. David, L.C. Krey, V.N. Luine, Y. McGinnis, C.M. Paden, B. Parson, T.C. Rainbow, Steroid hormone: humoral signals after brain cell properties and functions, Recent Prog. Horm. Res. 38 (1982) 41–83.

[6] N.J. MacLusky, B.S. McEwen, Oestrogen modulates progestin receptor concentrations in some brain regions but not in others, Nature 274 (1978) 276–278.

[7] M. Moguilewsky, J.P. Raynaud, The relevance of hypothalamic and hypophyseal progestin receptor regulation in the induction and inhibition of sexual behavior in the female rat, Endocrinology 105 (1979) 516–522.

[8] I. Jung-Testas, J.M. Renoir, J.M. Gasc, E.E. Baulieu, Estrogen-inducible progesterone receptor in primary cultures of rat glial cells, Exp. Cell Res. 193 (1991) 12–19.

[9] I. Jung-Testas, M. Renoir, H. Bugnard, G.L. Greene, E.E. Baulieu, Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells, J. Steroid Biochem. Mol. Biol. 41 (1992) 621–631.

[10] I. Jung-Testas, M. Schumacher, H. Bugnard, E.E. Baulieu, Stimulation of rat Schwann cell proliferation by estradiol: synergism between the estrogen and cAMP, Dev. Brain Res. 72 (1993) 282–290.

[11] I. Jung-Testas, M. Schumacher, P. Robel, E.E. Baulieu, Demonstration of progesterone receptors in rat Schwann cells, J. Steroid Biochem. Mol. Biol. 58 (1996) 77–82.

[12] J.P. Lydon, F.J. DeMayo, C.R. Funk, S.K. Mani, A.R. Hughes, C.A. Montgomery Jr.., G. Shyamala, O.M. Conneely, B.W. O'Malley, Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities, Genes Dev. 9 (1995) 2266–2278.

[13] I. Jung-Testas, Z.Y. Hu, E.E. Baulieu, P. Robel, Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells, Endocrinology 125 (1989) 2083–2091.

[14] E.E. Baulieu, Neurosteroids: a new function in the brain, Biol. Cell 71 (1991) 3–10.

[15] P. Robel, E.E. Baulieu, Neurosteroids: biosynthesis and function, Trends Endocrinol. Metab. 5 (1994) 1–8.

[16] I. Jung-Testas, F. Alliot, B. Pessac, P. Robel, E.E. Baulieu, Localisation immunocytochimique du cytochrome P450scc dans les oligodendrocytes de rat en culture, C. R. Acad. Sci. Paris 308 (1989) 165–170.

[17] S.H. Mellon, C.F. Deschepper, Neurosteroid biosynthesis: genes for adrenal steroidogenic enzymes are expressed in the brain, Brain Res. 629 (1993) 283–292.

[18] I. Jung-Testas, M. Schumacher, P. Robel, E.E. Baulieu, Action of steroid hormones and growth factors on glial cells of the central and peripheral nervous system, J. Steroid Biochem. Mol. Biol. 48 (1994) 145–154.

[19] H.L. Koenig, M. Schumacher, B. Ferzaz, N.A. Do Thi, A. Ressouches, R. Guennoun, I. Jung-Testas, P. Robel, Y. Akwa, E.E. Baulieu, Progesterone synthesis and myelin formation by Schwann cells, Science 268 (1995) 1500–1503.

[20] E.E. Baulieu, Steroid hormones in the brain: several mechanisms? In *Steroid Hormone Regulation of the Brain*, ed. K. Fuxe, J. A. Gustafsson and L. Wetterberg. Pergamon Press, Oxford, 1981, pp. 3–14.

[21] I. Jung-Testas, H. Weintraub, D. Dupuis, B. Eychenne, E.E. Baulieu, P. Robel, Low density lipoprotein-receptors in primary cultures of rat glial cells, J. Steroid Biochem. Mol. Biol. 42 (1992) 597–605.

[22] R. Morfin, J. Young, C. Corpéchot, B. Egestad, J. Sjövall, E.E. Baulieu, Neurosteroids: pregnenolone in human sciatic nerves, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 6790–6793.

[23] Y. Akwa, M. Schumacher, I. Jung-Testas, E.E. Baulieu, Neurosteroids in rat sciatic nerves and Schwann cells, C. R. Acad. Sci. Paris 316 (1993) 410–414.

[24] E.E. Baulieu, M. Schumacher, Neurosteroids, with special reference to the effect of progesterone on myelination in peripheral nerves, Multiple Sclerosis 3 (1997) 105–112.
[25] P. Wood, Separation of functional Schwann cells and neurons from peripheral nerve tissue, Brain Res. 115 (1976) 361–375.
[26] A. Do Thi, I. Jung-Testas, E.E. Baulieu, Neuronal signals are required for estrogen-mediated induction of progesterone receptor in cultured rat Schwann cells. *J. Steroid Biochem. Mol. Biol.*, 67(3) (1998) 201–211.
[27] W.T. Norton, Biochemistry of myelin. In *Advances in Neurobiology*, ed. S. G. Waxman and J. M. Ritchie. Raven Press, New York, 1981, pp. 93–121.
[28] I. Jung-Testas, M. Schumacher, P. Robel, E.E. Baulieu, The neurosteroid progesterone increases the expression of myelin proteins (MBP and CNPase) in rat oligodendrocytes in primary culture, Cell. Mol. Neurobiol. 16 (1996) 439–443.
[29] I. Jung-Testas, E.E. Baulieu, Steroid hormone receptors and steroid action in rat glial cells of the central and peripheral nervous system, J. Steroid Biochem. Mol. Biol. 65 (1998) 243–251.
[30] C.F. Eldridge, M. Bartlett, R.P. Bunge, P.M. Wood, Differentiation of axon-related Schwann cells *in vitro*. I. Ascorbic acid regulates basal lamina assembly and myelin formation, J. Cell Biol. 105 (1987) 1023–1034.
[31] F. Désarnaud, A. Do Thi, A.M. Brown, G. Lemke, U. Suter, E.E. Baulieu, M.J. Schumacher, *Neurochem.*, 71(4) (1998) 1765–1768.
[32] R.C. Melcangi, V. Magnaghi, L. Cavaretta, L. Martini, F. Piva, Age-induced decrease of glycoprotein Po and myelin basic protein gene expression in the rat sciatic nerve: repair by steroid derivatives, Neuroscience 23 (1998) 569–578.
[33] E.E. Baulieu, Neurosteroids: of the nervous system, by the nervous system, for the nervous system, Recent Prog. Horm. Res. 52 (1997) 1–32.
[34] F.A. Rawlins, B.G. Uzman, Retardation of peripheral nerve myelination in mice treated with inhibitors of cholesterol biosynthesis: a quantitative electron microscopic study, J. Cell Biol. 16 (1970) 505–517.