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Review

Peripheral nerves: a target for the action of neuroactive steroids

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Abstract

Peripheral nervous system possesses both classical and non-classical steroid receptors and consequently may represent a target for the action of neuroactive steroids. The present review summarizes the state of art of this intriguing field of research reporting data which indicate that neuroactive steroids, like for instance progesterone, dihydroprogesterone, tetrahydroprogesterone, dihydrotestosterone and 3α-diol, stimulate the expression of two important proteins of the myelin of peripheral nerves, the glycoprotein P0 (P0) and the peripheral myelin protein 22 (PMP22). Interestingly, the mechanisms by which neuroactive steroids exert their effects involve classical steroid receptors, like for instance progesterone and androgen receptors, in case of P0 and non-classical steroid receptors, like $GABA_A$ receptor, in case of PMP22. Moreover, neuroactive steroids not only control the expression of these specific myelin proteins, but also influence the morphology of myelin sheaths and axons suggesting that these molecules may represent an interesting new therapeutic approach to maintain peripheral nerve integrity during neurodegenerative events.

Theme: Cellular and molecular biology
Topic: Neuroglia and myelin

Keywords: Glycoprotein P0; Peripheral myelin protein 22; Neuroactive steroids; Myelin; Schwann cells

Contents

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1. Introduction

Recent data obtained in our and other laboratories have indicated that peripheral nerves represent an important target for the effects of neuroactive steroids. Namely, as will be reported here, peripheral nerves synthesize neuroactive steroids and express both classical and non-classical steroid receptors which are able to interact with them. Due to this capability, peripheral nerves and particularly, their glial component, the Schwann cells, respond to neuroactive steroids with changes in cell proliferation and the elaboration of cellular products (e.g., myelin membranes, myelin proteins, transcription factors involved in the myelination process, etc.). The present review summarizes these observations suggesting the possibility that neuroactive steroids themselves, or their synthetic receptor modulators, might represent a therapeutic approach aimed to counteract neurodegenerative events in peripheral nerves.

2. Peripheral nerves are able to synthesize neuroactive steroids

It is now well ascertained that the capability to synthesize steroids is not only a peculiarity of the classical steroidogenic tissues, like for instance the gonads and the adrenal glands, but is also present in the nervous system with the formation of the so-called *neuroactive steroids*. Namely, several observations have demonstrated that enzymes involved in the steroidogenic process are present in the non-neuronal compartment of the brain (e.g., astrocytes and oligodendrocytes) giving origin to pregnenolone, progesterone (PROG), dehydroepiandrosterone, androstenedione, testosterone (T) and estradiol [5,74–76,82,91]. Interestingly, it has been recently demonstrated that also the peripheral nervous system (PNS), and particularly Schwann cells, is able to form neuroactive steroids. For instance, Schwann cells express molecules able to participate in the transport of cholesterol from intracellular stores to the inner mitochondrial membrane where the cytochrome *P*450scc (i.e., the enzyme that converts cholesterol to pregnenolone) is located. Examples of such molecules are the peripheral benzodiazepine receptor (PBR), its endogenous ligand, octadecaneuropeptide (ODN) [45,46,90], and the steroidogenic acute regulatory protein (StAR) [6]. Moreover, it has been demonstrated that cytochrome *P*450scc and 3β-hydroxysteroid dehydrogenase (3β-HSD), which converts pregnenolone into PROG, are expressed in Schwann cells [11–13,32,43,85,90]. It is interesting to note that the formation of PROG is neuronal dependent. Namely, the expression and activity of the 3β-HSD present in Schwann cells cultured alone is very low; however, when these cells are cultured in contact with sensory neurons, both the expression and activity of this steroidogenic enzyme are induced [90]. Finally, peripheral nerves and Schwann cells are also able to metabolize native steroids into their 5α- and 3α-hydroxy-5α reduced derivatives via the enzymatic complex formed by the 5α-reductase (5α-R) and the 3α-hydroxysteroid dehydrogenase (3α-HSD) [66,70,73,106]. This enzymatic complex is very versatile, since every steroid possessing the delta 4–3keto configuration may be first 5α-reduced and subsequently 3α-hydroxylated. In particular, T can be converted into dihydrotestosterone (DHT) and then into 5α-androstane-3α, 17β-diol (3α-diol), PROG into dihydroprogesterone (5α-DH PROG) and subsequently into tetrahydroprogesterone (3α, 5α-TH PROG) [66,70,73]. In Schwann cells the formation of 5α-DH PROG from PROG (i.e., 5α-R activity) is at least four times higher than that present in oligodendrocytes. On the contrary, the formation of 3α, 5α-TH PROG from 5α-DH PROG (i.e., 3α-HSD activity) is lower than that present in oligodendrocytes [64].

3. Classical and non-classical steroid receptors are expressed in peripheral nerves

Peripheral nerves and Schwann cells not only possess the capability to form neuroactive steroids, but they are also a possible target for some of them. Several observations obtained in our and other laboratories have shown that peripheral nerves express classical and non-classical steroid receptors. For instance, rat sciatic nerve, and in particular, Schwann cells, express classical steroid receptors like for instance PROG (PR), estrogen, glucocorticoid and mineralocorticoid receptors [35,40,55,56,69,74]. Also, androgen receptor (AR) is expressed at the level of rat sciatic nerve [39,55]. However, at variance with what occurs in case of other classical steroid receptors, AR does not seem to be present in Schwann cells, but in the endoneurial compartment [39,55].

At the level of central nervous system, neuroactive steroids have been reported to modulate also neurotransmitter receptors, like for instance γ-amino butyric acid type A and B ($GABA_A$ receptor, $GABA_B$ receptor), serotonin type 3 (5-HT3), *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate receptor, and an atypical receptor like the sigma 1 [1,2,17,23,24,28,41,42,47,48,61,86–88]. Interestingly, some of these non-classical steroid receptors are also expressed in peripheral nerves and in Schwann cells. For instance, we have demonstrated that rat sciatic nerve and Schwann cells express

several subunits of the $GABA_A$ receptor (i.e., α2, α3, β1, β2 and β3 subunits) [65]. Moreover, we have recently demonstrated that also $GABA_B$ receptor isoforms (i.e., $GABA_{B1}$ and $GABA_{B2}$) are specifically localized in the rat Schwann cell population of the sciatic nerve [57]. Furthermore, rat sural nerve expresses NMDA receptor 1 subunit, glutamate receptor 1 (GluR1) AMPA subunit, and GluR5, 6, 7 kainate subunits [14,102], and the Schwann cells of mammalian peripheral vestibular system express GluR2, 3, 4 [15,102]. Finally, the presence of sigma 1 receptor has been recently confirmed at the level of Schwann cells of rat sciatic nerve [81]. Altogether, these observations indicate that peripheral nerves and Schwann cells may be considered a target for the action of neuroactive steroids.

4. Effects of neuroactive steroids on myelin proteins of peripheral nerves

In the last few years, several observations have indicated that neuroactive steroids are able to modulate cellular and molecular parameters of Schwann cells, like for instance their cell proliferation and cellular products [4,11,12,18,19,33,54,55,56,57,58,64–74,78,85,98,99]. In particular, the effects exerted by PROG, T and their neuroactive derivatives, respectively 5α-DH PROG and 3α, 5α-TH PROG in case of PROG and DHT and 3α-diol in case of T, on the expression of peripheral myelin proteins have been extensively investigated by our and other laboratories. The myelin proteins so far considered as a possible target for neuroactive steroids have been the glycoprotein P0 (P0) and the peripheral myelin protein 22 (PMP22). The reason for that is the crucial physiological role that these two proteins play in the maintenance of the multilamellar structure of PNS myelin [8,20–22,25,26,51,52,80,83,89,95]. In particular, P0 is a member of the immunoglobulin gene superfamily (IgCAM) and represents between 50% and 70% of the total proteins. This protein is a 28-kDa integral membrane glycoprotein which is predominantly confined to the compact portion of the mature myelin. P0 is a specific product of the Schwann, and is consequently absent in the CNS. This protein may be glycosylated, phosphorylated, sulfated and acylated [22,25,26,83,89,95]. The putative role for this myelin protein is to function as a membrane adhesion molecule and to promote and maintain the very tight compaction of the myelin structure by homophilic interactions. In this action, another important role is played by PMP22. Namely, P0 and PMP22 may form complexes in the myelin membranes [20,21], and their interactions probably participate in holding adjacent Schwann cell membranes together, stabilizing myelin compaction. PMP22 represents 2–5% of peripheral myelin proteins in rodents and humans [8,26,83], and like P0 is largely synthesized by Schwann cells. However, at variance to P0, which is nerve-specific, PMP22 is expressed also in other tissues, including the lung, gut, and heart [8,26,83].

The importance of P0 and PMP22 for stabilizing compact myelin is illustrated by the finding that their gene mutations cause a set of hereditary peripheral neuropathies in humans with axonal and demyelinating characteristics (e.g., Charcot-Marie-Tooth type 1A 1B, and 2, CMT1A, CMT1B, CMT2; Déjérine-Sottas syndrome, DSS; hereditary neuropathy with liability to pressure palsies, HNPP; congenital hypomyelinating neuropathy, CHN). For instance, the deletion, duplication and various missense mutations of the *PMP22* cause HNPP, CMT1A, and CMT1-like syndrome, respectively, or even DSS/CHN [92,97]. Different mutations of *P0* (e.g., missense, nonsense and frameshift mutations) cause CMT1B, DSS/CHN, CMT2-like, HNPP-like [29,59,94,97,104].

Both in vivo (i.e., in the rat sciatic nerve) and in vitro (i.e., in cultures of rat Schwann cells), the synthesis of these two important myelin proteins is affected by the treatment with PROG and its derivatives. For instance, in vivo treatments with PROG, 5α-DH PROG or 3α, 5α-TH PROG are able to increase the mRNA levels of P0 in the sciatic nerve of adult male rats [65,73,74]. In the same experimental model, the mRNA levels of PMP22 are significantly increased only by the treatment with 3α, 5α-TH PROG [65,73,74]. Some of these neuroactive steroids are also able to stimulate the expression of these two myelin proteins in aged rats. This is very important since aging is associated with a decrease in the synthesis of P0 and PMP22 [64,65,68,72]. Indeed, treatment with PROG or 5α-DH PROG is able to increase the low protein levels of P0 present in the sciatic nerve of aged male rat, while 3α, 5α-TH PROG significantly increases the protein levels of PMP22 [64,65,68,72]. Furthermore, PROG and its derivatives may also increase the gene expression of P0 after peripheral nerve injury. Namely, the treatment with PROG or 5α-DH PROG significantly increases P0 mRNA levels in the distal portion from the cut of the sciatic nerve [67]. The effects of PROG, 5α-DH PROG and 3α, 5α-TH PROG on P0 and/or PMP22 gene expression are also detected in rat Schwann cells in culture. In agreement with the in vivo results on the intact sciatic nerve of adult male rats, PROG, 5α-DH PROG and 3α, 5α-TH PROG in the case of P0, and 3α, 5α-TH PROG in the case of PMP22, exert a stimulatory effect on the gene expression of these myelin proteins [56,64,65,69,70].

Not only PROG and its neuroactive derivatives are able to influence the synthesis of myelin proteins, but also the neuroactive derivatives of T are effective. We have demonstrated that, in adult male rats, castration decreases the expression of P0 and PMP22 in the sciatic nerve [55,58]. The subsequent treatment with DHT or 3α-diol is able to restore the levels of the messenger of P0 [55,58]. On the contrary, in case of PMP22, only 3α-diol induces, in the sciatic nerve of castrated male rat, a significant increase of the synthesis of this myelin protein [58]. The effects of neuroactive derivatives of T are also evident in cultures of rat Schwann cells. In this experimental model, DHT

increases P0 mRNA levels [55], while the treatment with 3α-diol increases PMP22 mRNA levels [67].

5. The expression of P0 is under the control of classical steroid receptors while the control of PMP22 needs non-classical steroid receptors

The data so far mentioned indicate a clear role of PROG and T neuroactive derivatives (i.e., 5α-DH PROG and 3α, 5α-TH PROG in the case of PROG and DHT and 3α-diol in the case of T) in stimulating the expression of P0 and PMP22. However, these data also suggest that several mechanisms may be involved in the effects of neuroactive steroids on the expression of myelin proteins. In fact, in this context, it is important to consider that 5α-DH PROG and DHT bind to classical steroid receptors, PR and AR, respectively. In contrast, 3α, 5α-TH PROG and 3α-diol are able to bind to a non-classical steroid receptor, like $GABA_A$ receptor. On this basis, it is possible to assume that in case of P0 the effects of PROG and 5α-DH PROG might directly involve the PR, which is present both in the sciatic nerve and in Schwann cells [40,55,56]. Moreover, we have also observed that 5α-DH PROG is more potent than PROG in stimulating P0 mRNA levels [65,70,73]. This suggests that the conversion of PROG into 5α-DH PROG, by 5α-R present in the sciatic nerve and in Schwann cells [66,70,73], is a necessary step. Consequently, these observations might suggest a role of a classical steroid receptor, like PR, in the control of P0 expression. However, also 3α, 5α-TH PROG is effective in stimulating mRNA levels of this myelin protein [65,66,70,73], and, as mentioned above, this neuroactive steroid is a well-known ligand of the $GABA_A$ receptor [47,48], which is present in sciatic nerve and in Schwann cells [65]. On the other hand, since the activity of the 3α-HSD is bi-directional [66,70], 3α, 5α-TH PROG might be retro-converted into 5α-DH PROG, and consequently may exert its effect on P0 via an activation of PR (Fig. 1).

Consequently, in order to better characterize the mode of actions of PROG and its 5α- and 3α, 5α-derivatives on the expression of P0, we have utilized agonists and antagonists of PR and $GABA_A$ receptors and we have evaluated the role of these classical and non-classical steroid receptors in the control of the gene expression of P0. We have found that an antagonist of PR (i.e., mifepristone) is able to block the stimulatory effects exerted by PROG or 5α-DH PROG on P0 expression in cultured rat Schwann cells [56]. Interestingly, the effect of 3α, 5α-TH PROG on this myelin protein is also due to an activation of PR, since it is abolished by the presence of the PR antagonist [56]. Moreover, it is also possible to exclude a role for $GABA_A$ receptor in stimulating P0, since muscimol (i.e., an agonist of $GABA_A$ receptor) does not stimulate P0 expression [56]. All these observations suggest that P0 is under the control of PR.

A role for the classical PR is also supported by recent observations that we have obtained in vivo [71]. In these experiments, rats were treated with mifepristone at birth, repeating the treatment every 2 days. Then, the messenger and protein levels of P0 were evaluated in the sciatic nerve at postnatal days 20 and 30, as well as in animals treated during the first 30 days of postnatal life and then sacrificed at postnatal day 90. The PR antagonist was able to decrease P0 messenger and protein levels in the sciatic nerve of 20-day-old rats. However, the data obtained indicate that the expression of P0 is only transiently controlled by PR, since the inhibitory effect exerted by mifepristone was not evident

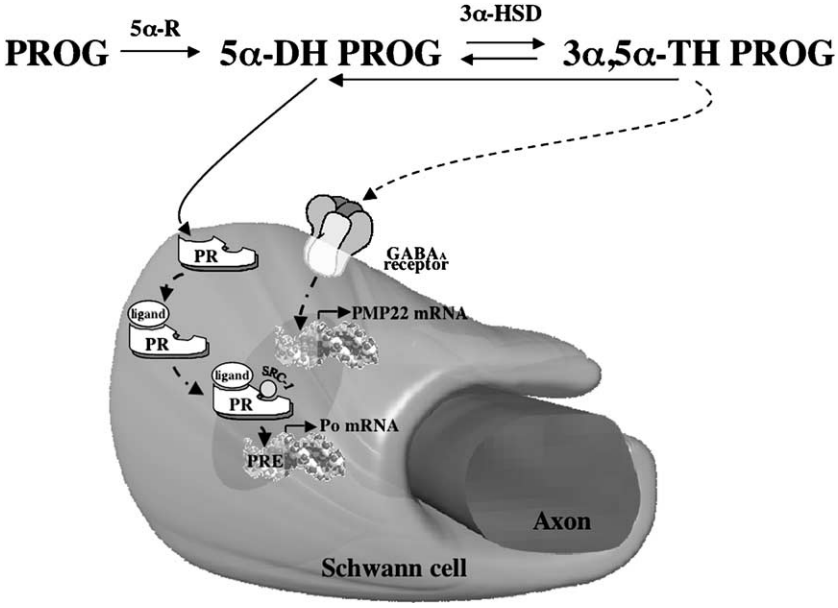

Fig. 1. Schematic representation of the possible mechanisms of the action of progesterone (PROG), dihydroprogesterone (5α-DH PROG) and tetrahydroprogesterone (3α, 5α-TH PROG) on the gene expression of P0 and PMP22. Details are provided in the text.

in 30- and 90-day-old rats, suggesting that compensatory effects might occur [71].

The PR mediated effects of PROG, 5α-DH PROG, and also of 3α, 5α-TH PROG, after retro-conversion to 5α-DH PROG, clearly suggest a classical steroid genomic effect. The classical genomic mechanism of steroids involves the activation of the steroid receptor by the ligand, followed by the dimerization and binding of the receptor to specific DNA sequences in or near the promoter of steroid-responsive genes. Then, general transcription factors are recruited into the preinitiation complex. However, interactions between basal transcription factors and steroid receptors, although necessary, are not sufficient for an efficient hormone-dependent transcriptional control. As a matter of fact, a third category of factors, the coregulators, is required for fine and full regulation of transcription.

Among coregulators, the coactivators work by bridging steroid receptors with basal transcription factors. This bridging stabilizes the preinitiation complex and consequently enhances transcription rate [3,30,63]. Some coactivators also have histone acetyl transferase activity, which contributes to the activation of transcription by facilitating the access of the transcriptional machinery to the promoter of target genes [36,96].

Several coactivators associated with steroid receptors, including PR, have been cloned so far. Among these, Steroid Receptor Coactivator-1 (SRC-1 or NCoA-1) is one of the most studied and well characterized [53,63]. The presence of this coactivator is rather ubiquitous although some tissue and gender specificity has been shown in its expression level [79]. These differences in expression may be responsible, at least in part, for the hormonal responses heterogeneity observed in different tissues. SRC-1 has been found in the motoneurons of the spinal nucleus of the bulbocavernosus of male rats [60]. However, there are no data in the literature concerning the potential biological function of SRC-1 in the PNS. For this purpose, we have recently investigated the possible role of this coactivator in the modulation of glycoprotein P0 gene expression [9]. After the demonstration that rat Schwann cell cultures and an immortalized cell line of Schwann cell (i.e., MSC80 cells) express SRC-1 mRNA, we have assessed whether the effect exerted by 5α-DH PROG on P0 mRNA levels is associated with altered levels of SRC-1. To this aim, MCS80 cells were stably transfected to over- or down-express SRC-1. Cells were then treated with 5α-DH PROG and P0 mRNA levels were assessed. The overexpression of SRC-1 increased the response of MSC80 cells to 5α-DH PROG. In contrast, the effect of the neuroactive steroid was completely lost in the SRC-1 deficient cells [9]. Moreover, in agreement to what has been observed in other experimental models [7,44,79,93,103], the expression of SRC-1 was affected by steroid treatment. Namely, in MSC80 parental cells treated with 5α-DH PROG, an increase of SRC-1 mRNA expression occurs [9]. Consistent with this, 5α-DH PROG loses its capability to further increase SRC-1 mRNA levels in MSC80 cells overexpressing the coactivator but it is able to induce the coactivator expression when added to SRC-1 deficient cells [9].

Altogether these data demonstrate that SRC-1 participates in the regulation of P0 gene expression by 5α-DH PROG (Fig. 1). This is a further confirmation that the expression of the myelin protein P0 is under the control of a classical steroid genomic mechanism. Moreover, a further support to this hypothesis is the finding that putative progesterone responsive elements (PRE) are present on P0 promoter [55].

Furthermore, as mentioned in the previous section also DHT is able to control P0 expression and, also in this case, the effect is mediated via an activation of a classical steroid receptor (i.e., AR). Indeed, in vivo treatment with an antagonist of this steroid receptor (i.e., flutamide) decreases the synthesis of P0 in rat sciatic nerve [58]. Interestingly, the inhibition of AR influences P0 synthesis in adult age only. This age-linked effect is different from what we have observed after the in vivo treatment with mifepristone [71]. As mentioned above, this PR antagonist is only able to decrease the synthesis of P0 at postnatal day 20. Consequently, the two steroid receptors so far considered (i.e., PR and AR), and their ligands, 5α-DH PROG and DHT, may exert their major effects on P0 synthesis in two different stages of life (i.e., PR during development and AR in adult age). A further extension of this hypothesis is that PROG derivatives may be necessary for inducing P0 synthesis during the first steps of the myelination process, while the subsequent intervention of T derivatives will participate in the maintenance of this process.

However, it is important to highlight that, at variance with what occurs in the case of PR [40,55,56], Schwann cells do not seem to express AR [39,55]. Consequently, while the effects of 5α-DH PROG on the synthesis of P0 are probably due to a direct action on Schwann cells, in the case of DHT the effects on the synthesis of P0 might only be ascribed to an indirect effect through the adjacent neuronal component or via the endoneurial compartment (i.e., fibroblasts), which express AR [39,55] (Fig. 2). On the other hand, additional mechanisms might be also present. For instance, we have also tested the hypothesis that DHT might be able to activate P0 gene expression by acting through a steroid receptor other than the AR. In particular, we have postulated that DHT might interact with the PR and activate progesterone responsive elements (PRE). Data obtained indicate that, in a human neuroblastoma cell line (SK-N-MC) co-transfected with the hPR_B and with a reporter plasmid containing a PRE, DHT is able to exert a transcriptional activity via the human PR [55]. Consequently, this observation suggests that the mechanisms through which DHT exerts its effects on P0 gene expression may be complex in nature, since it might operate not only via the AR but also via the PR (Fig. 2).

The situation is different in case of PMP22, since its expression is only affected by 3α, 5α-TH PROG. Con-

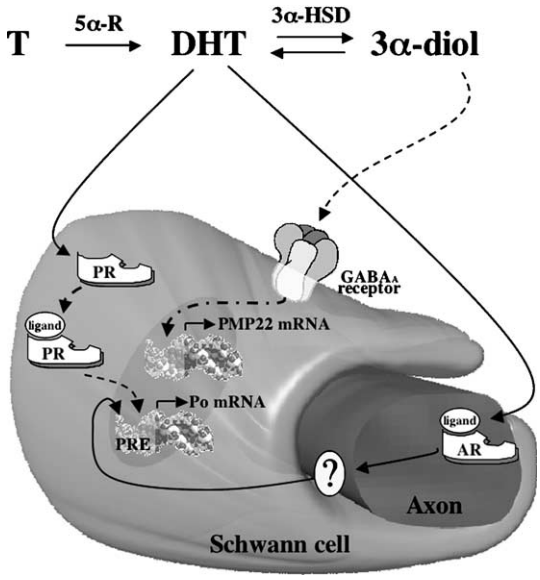

Fig. 2. Schematic representation of the possible mechanisms of action of testosterone (T), dihydrotestosterone (DHT) and 5α-androstane-3α, 17β-diol (3α-diol) on the gene expression of P0 and PMP22. Details are provided in the text.

sequently, only an effect via the $GABA_A$ receptor might be considered (Fig. 1). Experiments performed in Schwann cell cultures utilizing agonists or antagonists of $GABA_A$ receptor have confirmed this hypothesis [56]. In particular, we have observed that the stimulatory effect exerted by 3α, 5α-TH PROG on PMP22 is completely abolished by the simultaneous presence of a specific antagonist of $GABA_A$ receptor (i.e., bicuculline). Moreover, muscimol exerts a stimulatory effect on PMP22, which is comparable to that exerted by 3α, 5α-TH PROG. Finally, the specificity of the effect of 3α, 5α-TH PROG on the $GABA_A$ receptor is also clearly supported by the finding that isopregnanolone, an inactive isomer of 3α, 5α-TH PROG that does not interact with $GABA_A$ receptor, does not significantly modify the mRNA levels of PMP22 in Schwann cell cultures [56].

As mentioned in the previous section, among T derivatives so far considered, only 3α-diol is able to significantly increase PMP22 mRNA levels [58,67] (Fig. 2). Since 3α-diol does not bind to the AR but interacts with $GABA_A$ receptor [24,28], these findings further support the concept that PMP22 expression is under the control of $GABA_A$ receptor.

6. Effects of neuroactive steroids on Schwann cell proliferation

Neuroactive steroids are not only able to influence the expression of myelin proteins by Schwann cells but they also affect the proliferation of this kind of cells. For instance, the effects of PROG and estrogens on the proliferation of Schwann cells have been analyzed in cultures of segments of the rat sciatic nerve obtained from adult or newborn male and female rats [98]. In these experiments, it has been observed that these two neuroactive steroids are able to enhance [^{3}H] thymidine incorporation into the Schwann cells, and that this effect is dependent on the sex and the age of the animals. In fact, estrogens are effective on Schwann cell proliferation in segments from adult male and newborn rats, but do not exert any effect on segments from adult female rats; PROG, on the contrary, increases Schwann cell proliferation in segments obtained from adult female and newborn rats. The effects of estrogens and PROG were blocked by their respective receptor antagonists [98]. Interestingly, we have recently observed that also altered levels of steroid coactivators are able to affect cell proliferation. As shown in Fig. 3, we have observed that proliferation in MSC80 cells overexpressing SRC-1 (MSC80/SRC-1+) is slower than in the cells with down regulated coactivator expression (MSC80/SRC-1−). The situation is different when we have analyzed the effect of Steroid Receptor RNA Activator (SRA). This coactivator acts as an RNA transcript by regulating eukaryotic gene expression mediated by steroid receptors [49,63]. We presently demonstrate that SRA, like SRC-1, is present in Schwann cells as well as in MSC80 cells (Fig. 4), and is able to affect MSC80 cell proliferation. However, at variance to what occurs in the case of SRC-1 (Fig. 3), the overexpression of SRA (MSC80/SRA+) induces an increase of MSC80 cell proliferation (Fig. 5). In this context, it might be interesting to note that a similar effect occurs in a transgenic-mouse model that uses the mouse mammary tumor virus long terminal repeat to direct the expression of human SRA to the mammary glands. In this experimental model, the overexpression of SRA strongly increases cell proliferation [49].

Interestingly, not only the activation of classical steroid receptors but also that of non-classical steroid receptors is able to affect the proliferation of Schwann cells. For instance, we have recently demonstrated that in rat Schwann cell

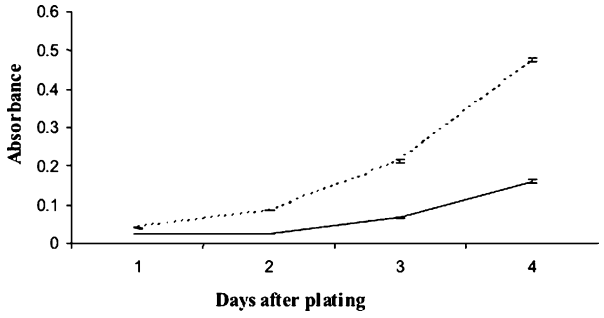

Fig. 3. Growth curve of MSC80 cells in the presence of altered levels of SRC-1. MSC80 cells were stably transfected with the sense or with the antisense pCR3.1hSRC-1a plasmid in order to obtain over- (MSC80/SRC-1+) or down-expression (MSC80/SRC-1−) of the coactivator. MSC80/SRC-1+ (continuous line) and MSC80/SRC-1− (dotted line) cells were seeded, at a low density, in 6 cm Petri dishes under normal growing conditions. After 24 h cell growth was estimated by MTT colorimetric assay. The assay was performed daily for 4 days. The absolute values of absorbance reported in the plot represent the mean values of triplicates ± SE.

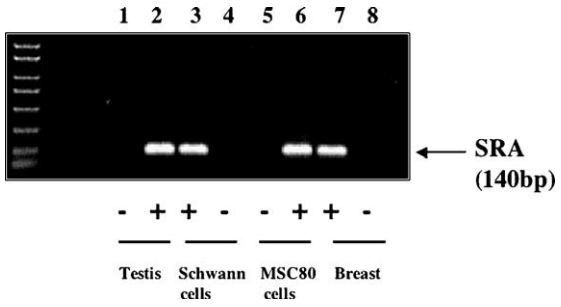

Fig. 4. SRA expression in peripheral glial cells. RT-PCR was performed on total RNA extracted from testis, Schwann cells, MSC80 cells and breast in the presence (lanes 2, 3, 6 and 7 respectively) or absence (lanes 1, 4, 5 and 8 respectively) of reverse transcriptase.

cultures the treatment with an agonist of $GABA_B$ receptor, like baclofen, is able to decrease cell proliferation [57].

7. Neuroactive steroids influence the morphology of myelin sheaths and axons

The aging process induces important morphological changes in peripheral nerves. For instance, large myelinated fibers undergo atrophy, while myelin sheaths increase in thickness and show various irregularities, like myelin ballooning, splitting, infolding, reduplication and remyelination [4,10,37,68,72,100,101]. A reduction in the number of density of myelinated fibers has been reported with aging in the peripheral nerves of several animal species and this effect is particularly evident in myelinated fibers of small caliber. Thus, more than 60% of the myelinated fibers with a diameter under 5 μm are lost in aged animals [4,72].

Alterations in the size and shape of myelinated fibers also occur with aging. The majority (>80%) of myelinated fibers, in the sciatic nerve of young rats, show a circular or ovoidal profile in cross-sections. However, lobulated, triangular and crescent-shaped profiles are also observed [4,72]. The irregular shapes are increased with aging. Thus, less than 50% of the myelinated fibers in old animals had a circular or ovoidal profile [4,72].

On the basis of the effects exerted by neuroactive steroids on P0 and PMP22 expression, we have recently evaluated whether pharmacological treatments with neuroactive steroids may counteract aging-associated morphological alterations of peripheral nerves [4,72]. To this purpose, we have analyzed the possible effect of PROG, 5α-DH PROG and 3α, 5α-TH PROG and of T and its derivatives, DHT and 3α-diol, on different morphological parameters of the myelin compartment of the sciatic nerve of 22–24 month-old male rats. Data obtained by treatment with PROG, 5α-DH PROG or 3α, 5α-TH PROG indicate that these neuroactive steroids have clear effects on the number and shape of myelinated fibers as well as on the frequency of myelin abnormalities [4,72]. These effects seem to be a peculiarity of PROG and its derivatives, since neither T nor DHT or 3α-diol is able to influence the morphological parameters analyzed in these experiments [4,72].

One of the most striking effects of PROG, 5α-DH PROG and 3α, 5α-TH PROG is on myelinated fibers of small caliber (<5 μm), with a significant increase in their number. In contrast, the number of myelinated fibers of a size larger than 5 μm is not significantly affected by the treatment with these neuroactive steroids. The increase in the number of small myelinated fibers after neuroactive steroid treatment is accompanied by a decrease of similar magnitude in the number of unmyelinated axons and, in particular, to large (>3 μm) unmyelinated axons. Namely, sciatic nerves from rats treated with PROG, or its derivatives, show a significant decrease in the number of large unmyelinated axons compared to animals treated with vehicle. Furthermore, the *g* ratio (i.e., the quotient between the axon perimeter and the fiber perimeter) of small myelinated fibers is significantly increased by PROG or its derivatives. This suggests that the increase in the number of myelinated fibers reflects an increased remyelination of small fibers in aged sciatic nerves.

Another marked effect of the treatments with PROG, 5α-DH PROG and 3α, 5α-TH PROG is the reduction in the frequency of axons with myelin abnormalities [4,72]. This effect is mainly due to a reduction in the frequency of axons with myelin infoldings. Moreover, PROG reduces the proportion of fibers with irregular shapes. As mentioned before, myelin abnormalities and irregular fiber profiles are typical markers of the aging process in peripheral nerves [10,100,101]. Therefore, our data indicate that PROG, 5α-DH PROG and 3α, 5α-TH PROG are able to reduce morphological changes associated with aging in the sciatic nerve [4,72].

However, our recent observations have indicated that not only myelin but also the axonal compartment may be considered a target for the action of neuroactive steroids. In particular, by light microscopy, we have observed that the

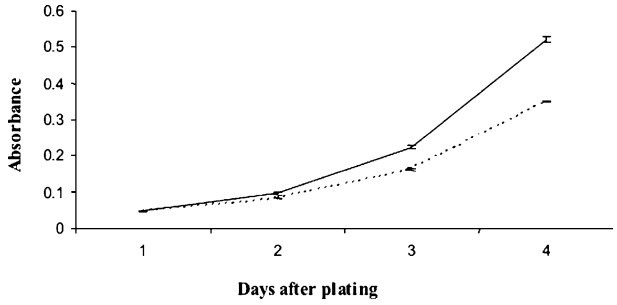

Fig. 5. Growth curve of MSC80 cells in the presence of altered levels of SRA. MSC80 cells were stably transfected with the sense or with the antisense pCR3.1SRA plasmid in order to obtain over- (MSC80/SRA+) or down-expression (MSC80/SRA−) of the coactivator. MSC80/SRA+ (continuous line) and MSC80/SRA− (dotted line) cells were seeded, at a low density, in 6 cm Petri dishes under normal growing conditions. After 24 h cell growth was estimated by MTT colorimetric assay. The assay was performed daily for 4 days. The absolute values of absorbance reported in the plot represent the mean values of triplicates ± SE.

sciatic nerve of rats treated since the first day of life with mifepristone shows, at 30 days of life, an overall reduction in fiber diameter and an increased number of axons with a diameter lower to that it would correspond for their myelin thickness [71]. This effect is also evident in animals treated with the antagonist during the first 30 days of postnatal life and then sacrificed at 90 days. A further confirmation of this effect is also coming by morphometric analysis, which indicates that the fiber diameter is slightly reduced in animals treated with mifepristone compared to controls. Surprisingly, the reduction in fiber diameter seems to be related to a reduction in axon diameter rather than to a thinning of the myelin sheath. Indeed, axon diameter is lower in animals treated with antagonist than in control at 30 and 90 postnatal days, whereas myelin thickness is similar or even higher in treated animals compared to controls. Furthermore, also the analysis of the *g* ratio confirms this interpretation. Indeed, this morphological parameter shows a tendency to the reduction in both groups of rats treated with mifepristone compared to untreated animals, meaning that the axon is affected by the treatment more than the myelin thickness. Finally, also morphological examination by electron microscopy, and in particular the analysis of neurofilament density, shows an axonal impairment. Morphological analysis of sciatic nerves of animals treated with mifepristone during the first month of life indicates a reduced axon diameter compared to myelin thickness and an increased neurofilament density [71]. A further confirmation of the effect of neuroactive steroids on axons might come also via their neurotherapeutic effects on peripheral nerve regeneration. For instance, in rodent peripheral nerve injury models, testosterone accelerates regeneration and functional recovery [38,99]. In conclusion, the present observations strongly suggest that the possible target of neuroactive steroids is not only the myelin compartment but also the axon.

8. Conclusions

The information reviewed here demonstrates that neuroactive steroids, through interaction with classical and non-classical steroid receptors present at the level of peripheral nerve, and particularly in Schwann cells, may influence the expression of myelin proteins (i.e., P0 and PMP22) and peripheral myelination. Altered levels of these myelin proteins, associated with morphological changes of myelin membranes, are clearly evident in physiological or pathological situations, like for instance after peripheral nerve injury, during aging or in hereditary demyelinating diseases (e.g., CMT1A, CMT1B, HNPP and DSS) [26,29,59,64,65,67,68,72,92,94,97,104]. Indeed, neuroactive steroids exert important neuroprotective effects after peripheral nerve injury and during aging [38,64,65,67,72,99]. These effects are very important since they suggest that these molecules might represent a therapeutic approach to counteract neurodegeneration not only in central nervous system, as extensively already demonstrated [16,27,31,34,50,62,77,84,91,105], but also at the level of peripheral nerves. In this context, it is important to remember that very recent results obtained by Sereda and co-workers [92] have suggested that PR is a promising pharmacological target for the therapy of CMT1A. Namely, in a model of CMT1A (i.e., PMP22-transgenic rats), they have demonstrated that the treatment with an antagonist of PR is able to reduce the overexpression of PMP22 and to improve CMT phenotype [92]. These observations may open new perspectives for the treatment of this pathology. However, as mentioned in the present review, data obtained in our laboratory indicate that PMP22 is preferentially stimulated via an interaction with $GABA_A$ receptor [56,65,70,73]. Consequently, in the future it will be very important to compare the outcome of the block of $GABA_A$ receptor in the CMT rats with the data obtained with the block of PR, and to explore also the possible additive effects of these two therapeutic strategies. Furthermore, we have recently demonstrated that PMP22 is not only under the control of the $GABA_A$ receptor but also of the $GABA_B$ receptor, which is present in Schwann cells [57]. Indeed, its activation via treatment with a $GABA_B$ agonist (i.e., baclofen) influences the expression of several myelin proteins, but the more striking effect is on PMP22 expression. However, at variance to what occurs in case of $GABA_A$, a decrease of this myelin protein occurs in rat Schwann cell cultures after treatment with baclofen [57]. This finding seems to be extremely interesting since it might suggest that depending on the receptor involved (i.e., $GABA_A$ or $GABA_B$), GABA itself, or neuroactive active steroids which are able to modulate these non-classical steroid receptors, may increase or decrease the synthesis of PMP22. Finally, the observations reviewed here also suggest that neuroactive steroids are not only able to influence the myelin compartment but also the axon, a compartment that is also affected during aging [100,101] or in demyelinating diseases like CMT [97]. In conclusion, neuroactive steroids are molecules with a broad spectrum of trophic actions in PNS, and they, or their synthetic receptor ligands, may be proposed as potential therapeutic agents to preserve the integrity of peripheral nerves during neurodenegerative events.

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