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Review

Neuroactive steroids and peripheral neuropathy

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ABSTRACT

Peripheral neuropathy, either inherited or acquired, represents a very common disorder for which effective clinical treatments are not available yet. Observations here summarized indicate that neuroactive steroids, such as progesterone, testosterone and their reduced metabolites, might represent a promising therapeutic option. Peripheral nerves are able to synthesize and metabolize neuroactive steroids and are a target for these molecules, since they express classical and non-classical steroid receptors. Neuroactive steroids modulate the expression of key transcription factors for Schwann cell function, regulate Schwann cell proliferation and promote the expression of myelin proteins involved in the maintenance of myelin multilamellar structure, such as myelin protein zero and peripheral myelin protein 22. These actions may result in the protection and regeneration of peripheral nerves affected by different forms of pathological alterations. Indeed, neuroactive steroids are able to counteract biochemical, morphological and functional alterations of peripheral nerves in different experimental models of neuropathy, including the alterations caused by aging, diabetic neuropathy and physical injury. Therefore, neuroactive steroids, pharmacological agents able to increase their local synthesis and synthetic ligands for their receptors have a promising potential for the treatment of different forms of peripheral neuropathy.

Contents

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Abbreviations: GABA, γ-amino butyric acid; AR, androgen receptor; 3α-diol, 5α-androstane-3α, 17β-diol; CMT, Charcot–Marie–Tooth; DHEA, dehydroepiandrosterone; 5α-DH PROG, dihydroprogesterone; DHT, dihydrotestosterone; DRG, dorsal root ganglia; 3α-HSD, 3α-hydroxysteroid dehydrogenase; P0, myelin protein zero; NCV, nerve conduction velocity; PBR, peripheral benzodiazepine receptor; PMP22, peripheral myelin protein 22; PNS, peripheral nervous system; PROG, progesterone; PR, progesterone receptor; 5α-R, 5α-reductase; SRC-1, steroid receptor coactivator-1; SRA, steroid receptor RNA activator; STZ, streptozotocin; 3α, 5α-TH PROG, tetrahydroprogesterone; T, testosterone; TSPO, translocator protein-18 kDa; TFs, transcription factors

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1. Introduction

Peripheral neuropathy, a process affecting the function of one (i.e., mononeuropathy) or more peripheral nerves (i.e., multi- or polyneuropathy), is one of the most common disorders with a prevalence of about 2.4% that rises with aging to 8% in the general population (England and Asbury, 2004). Peripheral neuropathy may be either inherited or acquired. Inherited forms of peripheral neuropathy are a group of disorders collectively referred to Charcot–Marie–Tooth (CMT) disease including demyelinating and axonal variants. Acquired peripheral neuropathy may occur during aging process, after physical injury (e.g., trauma, compression, entrapment, etc.), in systemic or metabolic disorders (e.g., diabetes mellitus, vitamin deficiencies, alcoholism, kidney failure, cancer, etc.), in infections and autoimmune disorders (e.g., AIDS, hepatitis, Guillain–Barré syndrome, Lyme disease, rheumatoid arthritis, leprosy, sarcoidosis, syphilis, systemic lupus erythematosus, etc.), after exposure to toxic compounds (e.g., lead, mercury, nitrous oxide, organic solvents, arsenic, etc.) and during drug treatment (e.g., chemotherapeutic, antiretroviral, anti-tuberculosis medications, antimicrobial drugs, lithium, etc.). The major clinical manifestations include negative sensory symptoms (e.g., loss tough, thermal and pain sensation, inability to determine joint position) and positive sensory symptoms (e.g., burning, pain, tingling, numbness, etc.), muscle waste and weakness and autonomic impairment (e.g., blurred vision, altered sweating, nausea or vomiting after meal, decreased ability to regulate body temperature, diarrhea or constipation, urinary hesitancy, male impotence, orthostatic hypotension). Axonal damage is more frequent than primary demyelination, but these pathological features frequently co-exist. Neurophysiological examination of peripheral nerves and muscles is routinely used in clinical practice and give detailed information about the site and type of nerve damage. Evidence of a demyelinating neuropathy increases the possibility to define the etiology and to start an effective treatment with immunemodulatory and/or immune-suppressant drugs. Conversely, there is no causative treatment for most of the axonal neuropathies. When indicated, patients can follow symptomatic treatment for pain and physical therapy.

A possible clue to identify putative targets for a more effective treatment would be the identification of relevant molecular events occurring in the peripheral nerves under physiologic and pathologic conditions. Under this perspective, recent observations have indicated that peripheral nerves are able to synthesize and metabolize neuroactive steroids. The term neuroactive steroids defines steroids acting in the nervous system and includes steroids produced by the nervous system (neurosteroids) and hormonal steroids coming from classical steroidogenic tissues (i.e., gonads and adrenal glands). Moreover, peripheral nerves express receptors for neuroactive steroids and consequently represent a target for them. Indeed, the observations so far obtained, and that will be summarized in the present review, show that neuroactive steroids are involved in the regulation of different functions of peripheral nerves, including Schwann cell proliferation and their cellular products (Azcoitia et al., 2003; Chan et al., 1998, 2000; Désarnaud et al., 1998, 2000; Guennoun et al., 2001; Lubischer and Bebinger, 1999; Magnaghi et al., 1999, 2004a,b, 2007; Melcangi et al., 1998, 1999, 2000a,b, 2001a,b, 2003a, b; Mercier et al., 2001; Rodriguez-Waitkus et al., 2003; Svenningsen and Kanje, 1999; Tanzer and Jones, 2004). Moreover, studies with experimental models demonstrated that neuroactive steroids exert also neuroprotective effects on peripheral neuropathies, suggesting new possible therapeutic strategies.

2. Synthesis, metabolism and receptors of neuroactive steroids in peripheral nerves

Several observations have demonstrated that Schwann cells express peripheral benzodiazepine receptor (PBR), now renamed as Translocator Protein-18 kDa (TSPO) (Papadopoulos et al., 2006), its endogenous ligand, octadecaneuropeptide (Lacor et al., 1996, 1999; Schumacher et al., 2001) and the steroidogenic acute regulatory protein (Benmessahel et al., 2004). These molecules participate in the transport of cholesterol from intracellular stores to the inner mitochondrial membrane where the cytochrome P450scc (i.e., the enzyme that converts cholesterol to pregnenolone) is located. Both cytochrome P450scc and 3β-hydroxysteroid dehydrogenase (i.e., the enzyme converting pregnenolone into progesterone, PROG) are present in Schwann cells (Chan et al., 1998, 2000; Coirini et al., 2003; Guennoun et al., 1997; Koenig et al., 1995; Rodriguez-Waitkus et al., 2003; Schumacher et al., 2001). Moreover, metabolism of 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) (Melcangi et al., 2001b; Yokoi et al., 1998) also occurs in peripheral nerves. In particular, PROG can be converted into dihydroprogesterone (5α-DH PROG) and subsequently into tetrahydroprogesterone (3α, 5α-TH PROG), testosterone (T) into dihydrotestosterone (DHT) and then into 5α-androstane-3α, 17β-diol (3α-diol) (Melcangi et al., 2001b).

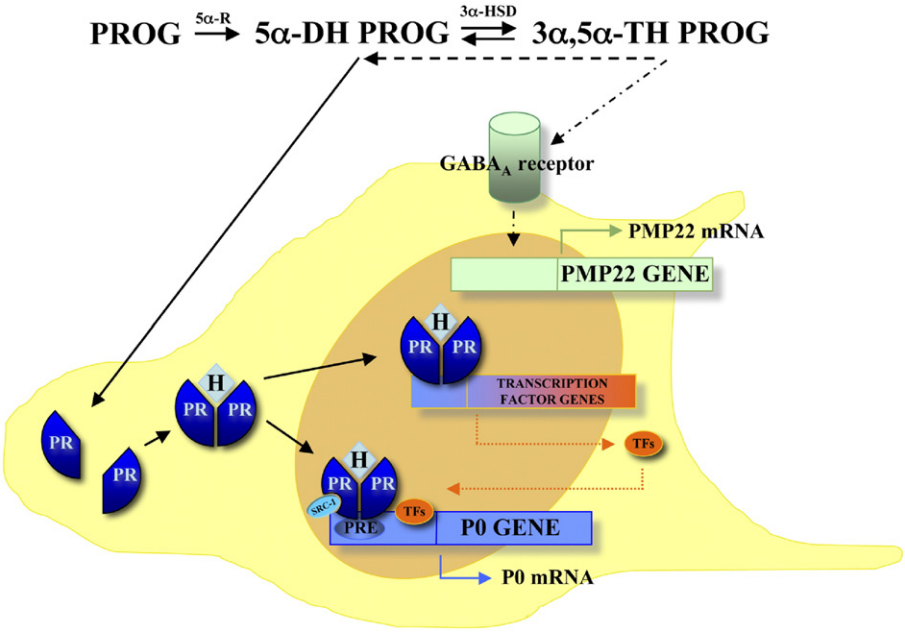

Fig. 1 – Schematic representation of the possible mechanisms of action of progesterone (PROG), dihydroprogesterone (5α-DH PROG) and tetrahydroprogesterone (3α, 5α-TH PROG) on the gene expression of P0 and PMP22 in Schwann cells. Details are provided in the text.

Peripheral nerves and Schwann cells not only synthesize and metabolize neuroactive steroids, but also express classical and non-classical steroid receptors. Namely, classical steroid receptors for PROG (PR), estrogens, glucocorticoids and mineralocorticoids (Groyer et al., 2006; Islamov et al., 2003; Jung-Testas et al., 1996; Magnaghi et al., 1999; Melcangi et al., 2001a) have been demonstrated in rat sciatic nerve and in Schwann cells. Also androgen receptor (AR) expression has been demonstrated in rat sciatic nerve, but it seems to be located in the endoneurial compartment and not in Schwann cells (Jordan et al., 2002; Magnaghi et al., 1999). 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 intracellular receptor like the sigma 1 (Al-Dahan et al., 1994; Al-Dahan and Thalmann, 1996; Falkestein and Wehling, 2000; Frye et al., 1996; Lambert et al., 2001, 2003; Maurice et al., 2001; Romieu et al., 2003; Rupprecht et al., 2001). Some of these receptors, such as GABA-A (i.e., α2, α3, β1, β2 and β3 subunits) and GABA-B (i.e., GABA-B1 and GABA-B2) receptors have been identified (Melcangi et al., 1999; Magnaghi et al., 2004a) also in peripheral nerves and Schwann cells. Moreover, rat sural nerve expresses NMDA receptor 1 subunit, glutamate receptor 1 (GluR 1) AMPA subunit and GluR 5, 6 and 7 kainate subunits (Coggeshall and Carlton, 1998; Verkhratsky and Steinhauser, 2000), and Schwann cells of mammalian peripheral vestibular system express GluR 2, 3 and 4 (Dememes et al., 1995; Verkhratsky and Steinhauser, 2000). Finally, the presence of sigma 1 receptor has been recently confirmed at the level of Schwann cells of rat sciatic nerve (Palacios et al., 2004).

3. Schwann cell responses to neuroactive steroids

It is now clear that neuroactive steroids are able to modulate the expression of myelin proteins of peripheral nervous system (PNS), such as myelin protein zero (P0) and the peripheral myelin protein 22 (PMP22). 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 modulated by the treatment with PROG and its derivatives. Namely, the expression of P0 in sciatic nerve of adult male rats, as well as that in rat Schwann cell culture is increased by the treatment with PROG, 5α-DH PROG or 3α, 5α-TH PROG, while in case of PMP22, only 3α, 5α-TH PROG is effective (Melcangi et al., 1998, 1999, 2001a,b, 2005).

Not only PROG and its neuroactive derivatives are able to influence the synthesis of myelin proteins, but also neuroactive derivatives of T are effective. For instance, in adult male rats, castration decreases the expression of P0 in the sciatic nerve (Magnaghi et al., 1999, 2004b) and the subsequent treatment with DHT or 3α-diol is able to restore the levels of the messenger of this myelin protein (Magnaghi et al., 1999, 2004b). Castration also decreases the mRNA levels of PMP22 in sciatic nerve, but in this case only 3α-diol is able to counteract this effect (Magnaghi et al., 2004b). A very similar pattern of effects is also evident in cultures of rat Schwann cells. In this experimental model, DHT increases P0 mRNA levels (Magnaghi et al., 1999), while the treatment with 3α-diol increases PMP22 mRNA levels (Melcangi et al., 2000a).

Several mechanisms seem to be involved in the effects of neuroactive steroids on the expression of myelin proteins (Fig. 1). Thus, the expression of P0 seems to be under the control of

classical receptors, such as PR and AR, while a role for a non-classical steroid receptor, like GABA-A receptor may be hypothesized in case of PMP22 (Melcangi et al., 2005). PR involvement on the expression of P0 may be confirmed by the finding that in cultured rat Schwann cells an antagonist of this steroid receptor, such as mifepristone, is able to block the stimulatory effect exerted by PROG or 5α-DH PROG (i.e., classical ligands of PR). Interestingly, this antagonist is also effective in blocking the effect of 3α, 5α-TH PROG (i.e., a neuroactive steroid which is able to interact with GABA-A receptor) on P0 (Magnaghi et al., 2001). Indeed, the activity of the 3α-HSD is bi-directional (Melcangi et al., 2001b) and consequently 3α, 5α-TH PROG might be retro-converted into 5α-DH PROG exerting its effect on P0 via an activation of PR. A role for the classical PR is also supported by *in vivo* observations. For instance, treatment with mifepristone since the first day of life decreases 20 days later the expression of P0 (Melcangi et al., 2003a). Activation of a classical steroid receptor, such as PR, clearly suggests that the effect of PROG derivatives on P0 expression is due to a classical steroid genomic effect. This hypothesis is supported by the finding that a coactivator, such as steroid receptor coactivator-1 (SRC-1) participates in the regulation of P0 gene expression by 5α-DH PROG. Indeed, as demonstrated in an immortalized cell line of Schwann cell (i.e., MSC80 cells) stably transfected to over- or down-express SRC-1, the effect of 5α-DH PROG on P0 expression was increased or completely lost respectively (Cavarretta et al., 2004). Moreover, a further support to the hypothesis that P0 is under the control of a classical steroid genomic mechanism is that putative progesterone responsive elements are present on P0 gene (Magnaghi et al., 1999).

A role for AR in controlling expression of P0 may be also hypothesized. Indeed, *in vivo* treatment with an antagonist of this steroid receptor (i.e., flutamide) decreases the synthesis of P0 in rat sciatic nerve (Magnaghi et al., 2004b). Interestingly, 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, where PR antagonist is only able to decrease the synthesis of P0 at postnatal day 20 (Melcangi et al., 2003a). A possible hypothesis could be 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.

As mentioned above, the expression of PMP22 seems to be under the control of GABA-A receptor. In fact, experiments performed in Schwann cell cultures utilizing agonists or antagonists of GABA-A receptor have indicated that bicuculline (i.e., a specific antagonist of this receptor) completely abolishes the stimulatory effect exerted by 3α, 5α-TH PROG on PMP22, while muscimol (i.e., an agonist of GABA-A receptor) exerts a stimulatory effect on PMP22, which is comparable to that exerted by 3α, 5α-TH PROG (Magnaghi et al., 2001). The specificity of the effect of 3α, 5α-TH PROG on the GABA-A receptor is also supported by the finding that isopregnanolone, which does not interact with GABA-A receptor, is unable to modify the expression of this myelin protein. Moreover, the finding that among T derivatives so far considered, only 3α-diol (i.e., a neuroactive steroid which is able to interact with GABA-A receptor) significantly increases PMP22 mRNA levels (Magnaghi et al., 2004b; Melcangi et al., 2000a) gives further support to the role of GABA-A receptor in controlling PMP22 expression.

Recent data, however, indicate that expression of P0 and PMP22 is not only merely under the control of classical (i.e., PR and AR) and non-classical steroid (GABA-A receptor) receptors respectively, but sex is another variable. As we have recently demonstrated, at least in culture of rat Schwann cells, the effects of PROG and its derivatives on the expression of myelin proteins are sexually dimorphic (Magnaghi et al., 2006). Namely, PROG or 5α-DH PROG treatment induces a stimulatory effect on P0 mRNA levels in Schwann cell cultures from male rats but not in those from females. In contrast, treatment with 3α, 5α-TH PROG increases gene expression of P0 in Schwann cells from female rats and not in cells from males. A similar sex-difference is also evident for PMP22. The expression of this myelin protein is stimulated by PROG in cultures from males and by 3α, 5α-TH PROG in cultures from females (Magnaghi et al., 2006).

Interestingly, neuroactive steroids not only regulate the expression of myelin proteins but also that of transcription factors (TFs) with a key role in Schwann cell physiology and in their myelinating program. For instance, data obtained in culture of rat Schwann cells (Guennoun et al., 2001; Mercier et al., 2001) have indicated that PROG stimulates the gene expression of Krox-20, Krox-24, Egr-3 and FosB. Moreover, we have recently demonstrated that not only PROG, but also its derivatives affect the synthesis of TFs, such as Krox-20 and Sox-10. Indeed, in culture of rat Schwann cells the expression of Krox-20 is stimulated by the treatment with 5α-DH PROG or 3α, 5α-TH PROG, while that of Sox-10 is only stimulated by 5α-DH PROG (Magnaghi et al., 2007).

These observations, together with the concept that PROG derivatives are also able to influence directly the expression of myelin proteins suggest that they might coordinate Schwann cell myelinating program utilizing different intracellular pathways.

Neuroactive steroids not only influence the expression of myelin proteins by Schwann cells but they also affect their proliferation. A stimulatory effect of PROG on proliferation of Schwann cells has been detected *in vitro* (Svenningsen and Kanje, 1999; Bartolami et al., 2003). Interestingly, also this effect of PROG seems to be dependent on the sex of the animals, since it has been demonstrated that PROG increases Schwann cell proliferation in cultures of segments of rat sciatic nerve from females, but is ineffective in cultures from males (Svenningsen and Kanje, 1999). An effect of androgens on Schwann cell proliferation is also evident. Namely, the number of terminal Schwann cells unsheathing the synaptic junction between motor nerve endings and muscles decreases after castration and this effect is counteracted by T replacement (Lubischer and Bebinger, 1999).

Interestingly, not only neuroactive steroids themselves, but also steroid coactivators, which as previously mentioned participate in the effects exerted by neuroactive steroids on myelin proteins, are able to affect cell proliferation. We have demonstrated that cell proliferation in an immortalized line of Schwann cells (i.e., MSC80 cells) overexpressing SRC-1 is slower than in cells in which the coactivator expression is down regulated (Melcangi et al., 2005). In contrast, over-expression of another coactivator, such as steroid receptor

RNA activator (SRA), induces an increase in the proliferation of MSC80 cells (Melcangi et al., 2005).

The effects of neuroactive steroids mentioned above on the expression of myelin proteins and Schwann cell proliferation have important repercussions for myelin formation and the maintenance of myelin structure. Effects of neuroactive steroids on the expression of myelin proteins such as P0 and PMP22 are particularly relevant since these two proteins play an important role for the maintenance of the multilamellar structure of PNS myelin (D'Urso et al., 1990). P0, a member of the immunoglobulin gene superfamily (IgCAM), accounts for more than half of the total peripheral myelin proteins (Ishaque et al., 1980), and it is predominantly confined to the compact portion of the mature myelin. The importance of P0 for stabilizing compact myelin is illustrated by the severe phenotype of P0-negative mice generated by homologous recombination (Giese et al., 1992; Martini et al., 1995; Zielasek et al., 1996), with pathological alterations affecting both myelin and axonal compartments, similar to those occurring in some dominantly inherited human peripheral neuropathies (e.g., Charcot–Marie–Tooth type 1b, CMT1B and Déjérine-Sottas syndrome, DSS). PMP22, like P0, is localized in the compact myelin and represents 2–5% of peripheral myelin proteins in rodents and humans (Pareek et al., 1993). PMP22 may form complexes in the myelin membranes with P0 (D'Urso et al., 1990, 1999), and their interactions may participate in holding adjacent Schwann cell membranes together, stabilizing myelin compaction.

Several alterations of the PMP22 gene have been associated with a set of hereditary peripheral neuropathies in humans (CMT 1A; DSS; hereditary neuropathy with liability to pressure palsies, HNPP) and with the Trembler and Trembler J alleles in mice (Naef and Suter, 1998; Suter and Scherer, 2003).

In agreement with the effect exerted on the proteins of peripheral myelin, PROG is also able to stimulate the myelin synthesis itself. For instance, PROG is able to accelerate the time of initiation and to enhance the rate of myelin synthesis in Schwann cells co-cultured with dorsal root ganglia (DRG) neurons (Chan et al., 1998, 2000).

Moreover, recent observations have indicated that also axonal compartment of PNS neurons may be considered a target for the action of neuroactive steroids. Thus, PROG affects the expression of neuronal genes that may promote myelination process by Schwann cells. For instance, in co-culture of Schwann cells and DRG neurons two genes, like a small Ras-like GTP-binding protein (Rap 1b) and phosphoribosyl diphosphate synthase-associated protein, which are induced in co-cultures during myelin synthesis, are also induced by PROG treatment (Chan et al., 2000; Rodriguez-Waitkus et al., 2003). Moreover, we have observed that the blockade of PR results in axonal impairment in the sciatic nerve of male rats. Indeed, morphological analysis of sciatic nerves of animals treated with mifepristone during development indicates a reduced axon diameter compared to myelin thickness and an increased neurofilament density (Melcangi et al., 2003a).

4. Neuroactive steroids as protective agents in peripheral nervous system

Neuroactive steroids may act as protective agents in several experimental models of neurodegenerative disorders of central nervous system (Aguado-Llera et al., 2006; Ciriza et al., 2004; Griffin et al., 2004; Lapchak and Araujo, 2001; McCullogh and Hurn, 2003; Schumacher et al., 2003). As mentioned above, neuroactive steroids may influence also many parameters of PNS, and consequently the efficacy of these molecules has been tested in different experimental models of peripheral neuropathy.

4.1. *Aging process*

Aging induces important biochemical and morphological changes in peripheral nerves. For instance, aging is associated with a decrease in the synthesis of P0 and PMP22, large myelinated fibers undergo atrophy, while myelin sheaths increase in thickness and show various irregularities, like myelin ballooning, splitting, infolding, reduplication and remyelination (Azcoitia et al., 2003; Melcangi et al., 2003b). Neuroactive steroids, such as PROG and its derivatives, are able to stimulate the expression of P0 and PMP22 in the sciatic nerve of aged rats. Thus, the treatment with PROG or 5α-DH PROG increases the low levels of P0 present in aged male rat, while 3α, 5α-TH PROG significantly increases those of PMP22 (Melcangi et al., 1998, 1999, 2000b, 2003b).

PROG, 5α-DH PROG or 3α, 5α-TH PROG also has clear effects on the number and shape of myelinated fibers as well as on the frequency of myelin abnormalities (Azcoitia et al., 2003; Melcangi et al., 2003b). In particular, the most striking effect of these neuroactive steroids is on myelinated fibers of small caliber (<5 μm), with a significant increase in their number. The *g* ratio (i.e., the ratio between the axonal and the entire fiber diameter) of small myelinated fibers is also 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 significant 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 (i.e., a reduction in the frequency of axons with myelin infoldings) and in the proportion of fibers with irregular shapes (Azcoitia et al., 2003; Melcangi et al., 2003b). All these effects seem to be a peculiarity of PROG and its derivatives, because neither T nor DHT or 3α-diol is able to influence the morphological parameters analyzed in these experiments (Azcoitia et al., 2003; Melcangi et al., 2003b).

4.2. *Physical injury*

Peripheral nerves are frequently exposed to physical damage, i.e., crushing, cutting, entrapment with nerve compression. A possible classification of this kind of peripheral neuropathies is based on the severity of nerve injury. Mild physical injury can induce a partial injury, which does not involve loss of nerve continuity and where local ion-induced conduction block and subtle alteration in myelin structure occur. More severe injury can lead to a complete interruption of the nerve axon and myelin, characterized, however, by a preservation of surrounding mesenchymal structures, including perineurium and epineurium. At this stage, a calcium-mediated process known as Wallerian degeneration distal to the injury site, characterized by physical fragmentation of axons and myelin,

activation of Schwann cells and macrophage, occurs. The most severe injury causes disconnection of nerves, characterized by complete functional loss, scar formation and loss of mesenchymal guide that is important to direct in a properly way the axonal regrowth.

Protective and regenerative effects of neuroactive steroids have been well characterized in experimental models of degeneration occurring after physical injury of peripheral nerves. For instance, neuroactive steroids may increase gene expression of P0 after nerve transection. Namely, PROG and 5α-DH PROG significantly increase the low messenger levels of this myelin protein in the distal portion from the cut of the sciatic nerve (Melcangi et al., 2000a). Moreover, PROG and pregnenolone (a precursor of PROG), when given locally, are able to counteract the decrease of the amounts of myelin membranes induced by a cryolesion in the sciatic nerve of the mouse (Koenig et al., 1995). Furthermore, the best result on guided regeneration of facial nerve of rabbit is obtained with PROG when used impregnated in biodegradable prostheses, such as chitosan (Chavez-Delgado et al., 2005). In this experimental model, PROG induces an increase in the number of Schwann cell nuclei, of nonmyelinated and myelinated nerve fibers (with increase also in their diameters), as well as in the *g*-ratio of myelinated nerve fibers.

Promising results have been also obtained with other neuroactive steroids. For instance, in several rodent peripheral nerve injury models (e.g., hamster facial motoneuron, rat sciatic motoneuron, rat pudendal motoneuron, etc.) T and its derivative, DHT, accelerate regeneration and functional recovery of nerves (Huppenbauer et al., 2005; Jones et al., 2001; Tanzer and Jones, 1997, 2004; Yu, 1982; Vita et al., 1983). Moreover, dehydroepiandrosterone (DHEA) is protective after rat sciatic nerve transection, reducing the extent of denervation atrophy and inducing an earlier onset of axonal regeneration (Ayhan et al., 2003), and after crush injury of rat sciatic nerve inducing a faster return to normal values of sciatic function index and increasing the number of myelinated fibers and of fiber diameters (Gudemez et al., 2002). Similar results have been also obtained in the same experimental model in mice, using 17β-estradiol (Islamov et al., 2002).

4.3. *Diabetic neuropathy*

More than 50% of diabetic patients show different types of peripheral nerve impairment, ranging from mononeuropathy, plexopathy, mononeuritis multiplex and distal, symmetric sensory-motor polyneuropathy. The most common distal polyneuropathy is associated with a spectrum of functional and structural changes in peripheral nerves, like a slowing in nerve conduction velocity (NCV), axonal degeneration, paranodal demyelination and loss of myelinated fibers (Sugimoto et al., 2000; Vinik et al., 2000). Decreased Na^+,K^+-ATPase activity in peripheral nerves and reduced intra-epidermal nerve fiber density associated with impaired nociceptive threshold are well characterized both in humans and in different experimental models of diabetic neuropathy, like for instance the streptozotocin (STZ)-treated rats (Berry, 1997; Bianchi et al., 2004; Biessels et al., 1999; Eckersley, 2002; Greene et al., 1989; Lauria et al., 2003, 2005; Periquet et al., 1999; Yagihashi, 1997). Moreover, we have recently demonstrated

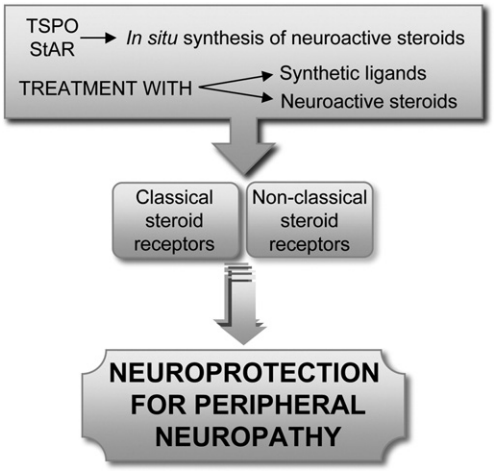

Fig. 2 – Possible strategies to obtain neuroprotection for peripheral neuropathies.

that in this experimental model also the gene expression of P0 and PMP22 is affected (Leonelli et al., 2007). STZ induced diabetes produces several morphological alterations in the myelinated fibers of the peripheral nerves. These alterations include myelin invaginations in the axoplasm (infoldings) and myelin evaginations in the Schwann cell cytoplasm (outfoldings) as well as alterations in myelin compaction such as abnormally wide incisures and abnormal separation of myelin lamellae. Similarly to what observed in aged rats, the most abundant myelin abnormality observed in STZ-rat is the presence of myelin infoldings in the axoplasm (Veiga et al., 2006).

Recent observations obtained in our laboratory have indicated that neuroactive steroids, such as PROG and its derivatives exert important protective effects in STZ-rat. Namely, we observed that PROG and 5α-DH PROG are able to counteract the increase in the number of fibers with myelin infoldings induced by STZ treatment in the sciatic nerve (Veiga et al., 2006). This effect is very similar to that mentioned above on the morphological alterations of myelin in the sciatic nerve of aged rats (Azcoitia et al., 2003; Melcangi et al., 2003b).

Neuroactive steroids are also able to influence biochemical and functional parameters of peripheral nerves. For instance, DHEA prevents vascular and neuronal dysfunction in the sciatic nerve of STZ-rats (Yorek et al., 2002). We have recently demonstrated that chronic steroid treatment with PROG, or with its derivatives, 5α-DH PROG and 3α, 5α-TH PROG, counteracts the impairment of NCV and thermal threshold, restoring skin innervation density and myelin protein mRNA levels (i.e., P0 and PMP22), and improving of Na^+,K^+-ATPase activity (Leonelli et al., 2007).

5. Concluding remarks

Observations here summarized suggest that peripheral nerves are target for functional and protective effects of neuroactive steroids. The finding that these molecules or synthetic ligands of their receptors might represent potential therapeutic tools for peripheral neuropathies is extremely interesting because,

as reported in this review, in many situations there are no effective treatments that can stop or reverse peripheral nerve damage. Moreover, an intriguing therapeutic strategy could be also to consider the use of pharmacological agents able to increase the synthesis of neuroactive steroids directly in the nervous system (Fig. 2). Indeed, recent observations have indicated that ligands of TSPO, which increase the synthesis of neuroactive steroids, exert also protective effects on peripheral nerves. For instance, a ligand like SSR180575 is able to increase the survival of facial nerve motoneurons after axotomy and the regeneration of peripheral nerves (Ferzaz et al., 2002). Moreover, another ligand like Ro5-4864 exerts a beneficial effect on morphological parameters of the sciatic nerve of aged male rats significantly increasing the total number of myelinated fibers and decreasing the percentage of fibers with myelin decompaction (Leonelli et al., 2005). Finally, the finding that neuroactive steroids directly target several autonomic functions, which are also affected in peripheral neuropathy, may open new interesting therapeutic perspectives. For instance, neuroactive steroids such as T, affect autonomic responses related to body temperature (Notelovitz, 2004) and male erectile physiology (Traish and Guay, 2006) and PROG metabolites affect orthostatic hypotension (Heesch and Foley, 2001). Neuroactive steroids may also regulate autonomic nervous system by actions on the CNS, such as the modulation of serotonergic systems of the hindbrain (Bethea et al., 2002) or opioid peptide neurons in the hypothalamus (Gu and Simerly, 1994). In addition, PROG inhibits acetylcholine-mediated responses of muscarinic receptors, which are essential for autonomic nervous system function (Horishita et al., 2005). Altogether, these observations indicate that the promising potential of these molecules for the treatment of different forms of peripheral neuropathy merits in the near future a sustained research and development effort.

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