Roberto C Melcangi[†]
and ***Luis M Garcia-Segura***
[†]*Author for correspondence*
University of Milan,
Department of Endocrinology
and Center of Excellence on
Neurodegenerative Diseases,
20133 Milan, Italy
Tel.: +39 025 031 8238
Fax: +39 025 031 8204
roberto.melcangi@unimi.it

Therapeutic approaches to peripheral neuropathy based on neuroactive steroids

'...a possible therapeutic approach for peripheral neuropathy might be represented by the treatment with neuroactive steroids themselves, with molecules able to induce their *in situ* synthesis or with molecules able to interact with their receptors.'

Expert Rev. Neurotherapeutics 6(8), 1121–1125 (2006)

Peripheral neuropathy is one of the most common disorders, with a population prevalence of approximately 2.4% that rises with age to 8%. This neurodegenerative event may affect the function of one or more peripheral nerves and may be either acquired or inherited. Peripheral neuropathy may be due to different causes. For instance, acquired peripheral neuropathy may occur during the aging process, after physical injury (e.g., trauma and carpal tunnel syndrome), in systemic or metabolic disorders, such as diabetes mellitus, dietary deficiencies (especially vitamin B-12), alcoholism, kidney failure, cancer, infections and autoimmune disorders (e.g., AIDS, Guillain–Barre-syndrome, Lyme disease, rheumatoid arthritis and leprosy), after exposure to toxic compounds (e.g., lead, mercury, organic solvents and arsenic) and during drug treatment (e.g., chemotherapeutic agents, anti-HIV medications, antituberculosis medications, antimicrobial drugs and lithium). Inherited forms of peripheral neuropathy are a group of disorders collectively referred to as Charcot–Marie–Tooth (CMT) disease. Moreover, it is important to highlight that when a cause cannot be identified, the condition is called idiopathic neuropathy.

Currently, there are no effective treatments that can stop or reverse this nerve damage. The most effective management is usually to identify the cause and, if possible, to eliminate it. Moreover, in many cases, the best treatment is simply pain management.

Neuroactive steroids include steroids produced by the nervous system (neurosteroids) and hormonal steroids. Neuroactive steroids exert a broad spectrum of actions both in the CNS and the peripheral nervous systems (PNS) [1]. Moreover, promising neuroprotective effects by neuroactive steroids have been obtained in experimental animal models. Neuroactive steroids, using multiple signaling mechanisms may influence many physiological parameters of rat peripheral nerves, such as synthesis of myelin proteins and of myelin membranes, Schwann cell proliferation and function of the axonal compartments. Alterations of these parameters are clearly evident in peripheral neuropathy, such as that occurring after peripheral nerve injury, during aging or in hereditary demyelinating diseases [2–4]. In the experimental models of peripheral neuropathy so far taken into consideration, neuroactive steroids exert important neuroprotective effects, and consequently could be considered as a therapeutic approach to counteract neurodegeneration.

Protective and regenerative effects of neuroactive steroids have been well characterized in experimental models of degeneration occurring after physical injury of peripheral nerves. The possible beneficial effect for the treatment

of peripheral neuropathy of two neuroactive steroids, progesterone (P) and pregnenolone (a precursor of P), was first suggested by Koenig and colleagues, who demonstrated that both steroids, 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 [5]. Successive studies showed that, in an experimental model of sciatic nerve transection, the treatment with neuroactive steroids is also able to modulate the expression of important myelin proteins, such as glycoprotein zero (P0). Indeed, treatment with P or its metabolite dihydroprogesterone (DHP) significantly increases the low P0 mRNA levels present in the distal portion from the cut [6]. These effects are also evident in rat Schwann cell cultures, indicating a direct effect of these steroids on the cells producing P0. In the same models, the gene expression of another important myelin protein, such as peripheral myelin protein 22 (PMP22) is influenced only by tetrahydroprogesterone (THP), a metabolite of DHP [6].

It is also interesting to consider that P stimulates gene expression of the transcription factor Krox-20, which plays an important role in myelination of peripheral nerves, as well as of other transcription factors present in Schwann cells [7]. In particular, it has been observed that P induces a rapid increase in the gene expression of *Krox-20*, *Krox-24*, *Egr-3* and *Fos B*, suggesting that this neuroactive steroid might also coordinate the signaling pathways involved in the initiation of myelination.

Recent observations have indicated that the best results on guided regeneration of facial nerves of rabbits is obtained with P when used impregnated in biodegradable prostheses, such as chitosan [8]. In particular, the exposure to P induces, in comparison to the vehicle, an increase in the number of Schwann cell nuclei, of nonmyelinated and myelinated nerve fibers (with their respective diameters), as well as in the g ratio of myelinated nerve fibers. Promising results have also been obtained with other neuroactive steroids. For instance, testosterone has been demonstrated to accelerate regeneration and functional recovery in rodent peripheral nerve injury models [9]. Removal of circulating androgens by castration decreases mRNA levels of P0 in the sciatic nerve, a phenomenon that is counteracted by subsequent treatment with dihydrotestosterone (DHT). However, since Schwann cells do not express the androgen receptor (AR), it has been hypothesized that the gene expression of P0 might be stimulated by androgen-dependent mechanisms acting on Schwann cells through the adjacent neuronal component, which seems to express AR [4,6].

After transection of the rat sciatic nerve, treatment with dehydroepiandrosterone (DHEA) reduces the extent of denervation atrophy as evaluated by gastrochemius muscle weight, and induced an earlier onset of axonal regeneration as confirmed by the increase of myelinated axons, of larger average fiber diameter and of greater axonal cross-sectional areas in the proximal, middle and distal sections [10]. Moreover, this neuroactive steroid is also able to enhance the functional recovery following crush injury of rat sciatic nerves. Thus, in this experimental model, DHEA induces a faster return to normal values of sciatic function index, determined by walking track analysis, and an increase of the number of myelinated fibers and of fiber diameters [11]. Similar results have been also obtained in the same experimental model in mice, using 17β-estradiol [12]. Additionally, glucocorticoids seem to be able to stimulate the transcription from P0 and PMP22 promoters in Schwann cells. For instance, dexamethasone and corticosterone are able to stimulate P0 expression and both promoters of the PMP22 gene [7]. Since P0 and PMP22 play an important physiological role in the maintenance of the multilamellar structure of peripheral myelin, these observations might suggest the possible utilization of neuroactive steroids in acquired and inherited forms of peripheral neuropathy, in which rebuilding of myelin is needed.

Neuroactive steroids are also able to counteract degenerative effects of aging in peripheral nerves [2,6]. That is very important because the aging process 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, such as myelin ballooning, splitting, infolding, reduplication and remyelination [2,6]. A reduction in the number of density myelinated fibers has been reported with aging in peripheral nerves of several animal species and this effect is particularly evident in myelinated fibers of small caliber. Indeed, more than 60% of the myelinated fibers with a diameter under 5 µm are lost in aged animals. Moreover, alterations in the size and shape of myelinated fibers also occur with aging [2,6].

'Neuroactive steroids are also able to counteract degenerative effects of aging in peripheral nerves. That is very important because the aging process induces important biochemical and morphological changes in peripheral nerves.'

Both P and DHP are able to increase the low protein levels of P0 present in the sciatic nerve of aged male rats, while THP significantly increases the protein levels of PMP22 [2,6]. Moreover, the treatment with these neuroactive steroids has clear effects on the number and shape of myelinated fibers, as well as on the frequency of myelin abnormalities. In particular, one of the most striking effects of P and its metabolites (DHP and THP) is on myelinated fibers of small caliber (<5 µm), with a significant increase in their number. 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. Moreover, the g ratio of small myelinated fibers is significantly increased by P 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 [2,6].

Another significant effect of the treatments with P, DHP and THP 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 [2,6]. In conclusion, this set of experiments indicates that these neuroactive steroids are able to reduce morphological changes associated with aging in the sciatic nerve. These effects seem to be a peculiarity of P and its derivatives, because neither T nor DHT or 3α-diol are able to influence the morphological parameters analyzed in these experiments [2,6].

Recent evidence suggests that P and DHP may prevent myelin structural abnormalities induced by experimental diabetes in rats [13]. Peripheral neuropathy is the most frequent symptomatic complication of diabetes mellitus and potentially one of the most devastating. This alteration occurs equally in Type 1 and 2 diabetes, as well as in various forms of acquired diabetes and is associated with a spectrum of changes in peripheral nerves, including myelin damage and decreased nerve conduction velocity. In streptozotocin (STZ)-treated rats, an animal model of Type 1 diabetes, similar structural abnormalities and neurophysiological changes in peripheral nerves have been observed. 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 the myelin lamellae. The most abundant myelin abnormality, as in the case of aging rats, is the presence of myelin infoldings in the axoplasm. P and DHP are able to counteract the increase in the number of fibers with myelin infoldings induced by STZ treatment in the sciatic nerve [13]. This effect is very similar to that mentioned above on the morphological alterations of myelin in the sciatic nerve of aged rats [2,6]. Therefore, neuroactive steroids, such as P and DHP may also represent promising therapeutic alternatives to counteract peripheral myelin alterations induced by diabetes.

While the experiments with neuroactive steroids in animal models reviewed here clearly indicate that these molecules have a potential therapeutic interest for the treatment of peripheral neuropathy, their clinical use may present some limitations owing to undesirable side effects associated with the hormonal actions of some of these molecules. An alternative to the use of neuroactive steroids is to target their receptors and signaling mechanisms. In this context, it is important to consider that while P and DHP are able to bind progesterone receptors (PR), THP is well known as a potent ligand of the γ-aminobutyric acid (GABA)-A receptor. For instance, it has been proposed that the effects of P and DHP on gene expression of P0 are linked to an interaction with PR, which is expressed by Schwann cells. This hypothesis is confirmed by the finding in primary cultures of Schwann cells isolated from neonatal rat sciatic nerves, mifepristone, a PR antagonist, is able to block the effects of P and DHP on the expression of protein P0. An effect of P and DHP on P0 gene expression through the PR might suggest a genomic mechanism, in which the ligand–receptor complex interacts with steroid responsive elements located in the promoter region of P0. In agreement with this hypothesis, a computer analysis has demonstrated that putative progesterone responsive elements are present on the P0 promoter. Moreover, overexpression of steroid receptor coactivator (SRC)-1 increased the response of immortalized cell lines of Schwann cells to DHP. In contrast, the effect of this neuroactive steroid was completely lost in the SRC-1-deficient cells [14].

On the contrary, the effects of THP on protein PMP22 seem to be due to an interaction of this steroid with the GABA-A receptor, whose subunits have been found in Schwann cells [14]. For instance, in primary cultures of Schwann cells the effect of THP on PMP22 is blocked by bicuculline, a classical antagonist of the GABA-A receptors. The possible involvement of the GABA-A receptor in the control of the expression of PMP22 is also supported by the observation that in rat Schwann cells, 3α-diol, another steroid able to interact with this neurotransmitter receptor, is able to increase PMP22 expression.

'An alternative to the use of neuroactive steroids is to target their receptors and signaling mechanisms. In this context, it is important to consider that while P and DHP are able to bind progesterone receptors (PR), THP is well known as a potent ligand of the γ-aminobutyric acid (GABA)-A receptor.'

The conclusion that the expression of P0 seems to be under the control of the PR, while that of PMP22 is under GABA-A receptor influence is further supported by the findings that the GABA-A receptor agonist muscimol does not increase the mRNA levels of protein P0, while it increases the expression of protein PMP22. Therefore, an alternative to the use of P, DHP and THP may be the use of molecules that target PR and/or GABA-A receptor [14]. Indeed, Sereda and colleagues have demonstrated that the treatment with an antagonist of PR (i.e., onapristone) is able to reduce the overexpression of PMP22 and to improve CMT phenotype in an experimental model of CMT1A (i.e., PMP22-transgenic rats) [3]. This opens the possibility of using selective PR modulators, perhaps in combination with GABA-A ligands, for the treatment of peripheral neuropathy. Selective estrogen receptor modulators may also offer a potential therapeutic interest, since the selective estrogen receptor modulator LY117018 has been shown to enhance functional recovery after injury of the sciatic nerve [15].

Another alternative to a therapeutic strategy with neuroactive steroids themselves, or with specific synthetic ligands of their receptors, might be the use of pharmacological agents that increase the synthesis of endogenous neuroactive steroids within the nervous system. The capability to synthesize neuroactive steroids is not only a peculiarity of the classical steroidogenic tissues, such as the gonads and adrenal gland, but may also be ascribed to the nervous system. The synthesis of pregnenolone from cholesterol by the cholesterol side-chain cleavage enzyme (P450scc) is the first enzymatic step of steroidogenesis. However, there is a previous step that is rate limiting and hormonally regulated: the transfer of cholesterol from the outer to the inner mitochondrial

membrane, where the P450scc enzyme is located. Proteins located in the mitochondrial membranes, such as the steroidogenic acute regulatory protein (StAR) and the peripheral benzodiazepine receptor (PBR), allow the cholesterol to cross the hydrophilic intermembrane space.

StAR was first characterized in murine MA-10 Leydig tumor cells as a mitochondrial protein responsible for the acute induction of steroidogenesis. StAR is formed as a 37-kDa protein, which is rapidly transported into mitochondria where it is cleaved, generating a mature 30-kDa intramitochondrial StAR protein that is inactive. In the PNS, StAR is expressed by Schwann cells [16].

PBR was initially described on peripheral tissues as a second binding site for diazepam, which binds with higher affinity to GABA-A receptors on the nervous system. Since then, many studies have demonstrated that PBR is pharmacologically and structurally distinct from the central benzodiazepine/GABA-A receptors. PBR is an 18-kDa peptide located predominantly in the mitochondrial membranes and represents a critical component of the permeability transition pore, a multiprotein complex implicated in the regulation of apoptosis. In addition, PBR has been related with the regulation of several physiological events, including the control of steroidogenesis. PBR is expressed in the nervous system, predominantly in glial cells, including Schwann cells. Different forms of neural injury and different neuropathological conditions result in the induction of the expression of PBR in the areas of the nervous system involved in the neurodegenerative events. For instance, PBR basal expression is upregulated in gliomas, in neurodegenerative disorders and in various forms of brain injury and inflammation [17]. A very similar effect occurs in the PNS. Indeed, the expression of PBR in Schwann cells is increased after nerve lesions and returns to normal levels when regeneration is completed.

Therefore, it is conceivable that molecules that target PBR and StAR may regulate the conversion of cholesterol into pregnenolone and the production of neuroactive steroids in peripheral nerves and may protect peripheral nerves from degeneration. Indeed, it has been demonstrated that a PBR ligand, SSR180575, is able to increase the survival of facial nerve motoneurons after axotomy and the regeneration of peripheral nerves [18]. Another PBR ligand, Ro5-4864, increases concentrations of pregnenolone in the rat sciatic nerve and its expression is increased in Schwann cells and in dorsal root ganglion neurons after nerve lesions and return to normal levels when regeneration is complete. The induction of PBR expression might be interpreted as an endogenous neuroprotective response to reduce the damage. In agreement, with this hypothesis, it has been shown that Ro5-4864 exerts a beneficial effect on morphological parameters of the sciatic nerve of aged male rats. Namely, the treatment significantly increased the total number of myelinated fibers and decreased the percentage of fibers with myelin decompaction. In contrast to the neuroprotective effects exerted by Ro5-4864, the PBR ligand PK-11195, which binds to a different site in the PBR structure, did not significantly affect any of the parameters analyzed [19]. Interestingly, it has been recently reported that Ro5-4864, and not PK-11195, also exerts neuroprotective effects in the CNS [20]. In addition, to increase the local synthesis of neuroprotective steroids, other functions of PBR may also be important to maintain sciatic nerve integrity. These include the regulation of oxidative processes, since PBR may modulate mitochondrial sensitivity to reactive oxygen species (ROS). ROS have been implicated in several neurodegenerative events and these become generally worsened during the aging process. For instance, it is well known that the rate and the degree of recovery after peripheral nerve lesions decline with age and it has been proposed that ROS contribute's to this delayed recovery. Consequently, Ro5–4864 may in part protect peripheral nerves by reducing sensitivity to ROS-induced damage.

In conclusion, the data reported here strongly suggest that a possible therapeutic approach for peripheral neuropathy might be represented by the treatment with neuroactive steroids themselves, with molecules able to induce their *in situ* synthesis or with molecules able to interact with their receptors. However, before considering broad clinical applications, it is still necessary for more basic research to clarify the mechanisms of action and potential risks of some of these treatments. While protective effects of neuroactive steroids for peripheral nerves are well supported by experimental data, it is still necessary to screen the neuroprotective potency of available steroid receptor selective modulators. PBR ligands and other molecules that may enhance steroidogenesis in peripheral nerves should also be systematically tested in models of peripheral neuropathy. It is also highly probable that in the near future new steroid receptor selective modulators and new PBR ligands with protective properties for peripheral nerves will be available. Compounds that regulate StAR activity may also be explored and developed. The promising potential of these molecules for the treatment of different forms of peripheral neuropathy merits a sustained research and development effort.

References

1 Melcangi RC, Panzica GC. Neuroactive steroids: old players in a new game. *Neuroscience* 138(3), 733–739 (2006).

2 Azcoitia I, Leonelli E, Magnaghi V, Veiga S, Garcia-Segura LM, Melcangi RC. Progesterone and its derivatives dihydroprogesterone and tetrahydroprogesterone reduce myelin fiber morphological abnormalities and myelin fiber loss in the sciatic nerve of aged rats. *Neurobiol. Aging* 24(6), 853–860 (2003).

3 Sereda MW, Meyer zu Horste G, Suter U, Uzma N, Nave KA. Therapeutic administration of progesterone antagonist in a model of Charcot-Marie-Tooth disease (CMT-1A). *Nat. Med.* 9(12), 1533–1537 (2003).

4 Leonelli E, Ballabio M, Consoli A, Roglio I, Magnaghi V, Melcangi RC. Neuroactive steroids: a therapeutic approach to maintain peripheral nerve integrity during neurodegenerative events. *J. Mol. Neurosci.* 28(1), 65–76 (2006).

5 Koenig HL, Schumacher M, Ferzaz B *et al*. Progesterone synthesis and myelin formation by Schwann cells. *Science* 268(5216), 1500–1503 (1995).

6 Melcangi RC, Azcoitia I, Ballabio M *et al*. Neuroactive steroids influence peripheral myelination: a promising opportunity for preventing or treating age-dependent dysfunctions of peripheral nerves. *Prog. Neurobiol.* 71(1), 57–66 (2003).

7 Schumacher M, Guennoun R, Mercier G *et al*. Progesterone synthesis and myelin formation in peripheral nerves. *Brain Res. Rev.* 37(1–3), 343–359 (2001).

8 Chavez-Delgado ME, Gomez-Pinedo U, Feria-Velasco A *et al*. Ultrastructural analysis of guided nerve regeneration using progesterone- and pregnenolone-loaded chitosan prostheses. *J. Biomed. Mater. Res. B Appl. Biomater.* 74(1), 589–600 (2005).

9 Tanzer L, Jones KJ. Neurotherapeutic action of testosterone on hamster facial nerve regeneration: temporal window of effects. *Horm. Behav.* 45(5), 339–344 (2004).

10 Ayhan S, Markal N, Siemionow K, Araneo B, Simionow M. Effect of subepineurial dehydroepiandrosterone treatment on healing of transected nerves repaired with the epineurial sleeve technique. *Microsurgery* 23(1), 49–55 (2003).

11 Gudemez E, Ozer K, Cunningham B, Siemionow K, Browne E, Siemionow M. Dehydroepiandrosterone as an enhancer of functional recovery following crush injury to rat sciatic nerve. *Microsurgery* 22(6), 234–241 (2002).

12 Islamov RR, Hendricks WA, Jones RJ, Lyall GJ, Spanier NS, Murashov AK. 17β-estradiol stimulates regeneration of sciatic nerve in female mice. *Brain Res.* 943(2), 283–286 (2002).

13 Veiga S, Leonelli E, Beelke M, Garcia-Segura LM, Melcangi RC. Neuroactive steroids prevent peripheral myelin alterations induced by diabetes. *Neurosci. Lett.* 402(1–2), 150–153 (2006).

14 Melcangi RC, Cavarretta IT, Ballabio M *et al*. Peripheral nerves: a target for the action of neuroactive steroids. *Brain Res. Rev.* 48(2), 328–338 (2005).

15 McMurray R, Islamov R, Murashov AK. Raloxifene analog LY117018 enhances the regeneration of sciatic nerve in ovariectomized female mice. *Brain Res.* 980(1), 140–145 (2003).

16 Benmessahel Y, Troadec J-D, Cadepond F *et al*. Downregulation of steroidogenic acute regulatory protein (StAR) gene expression by cyclic AMP in cultured Schwann cells. *Glia* 45(3), 213–228 (2004).

17 Papadopoulos V, Lecanu L, Brown RC, Han Z, Yao ZX. Peripheral-type benzodiazepine receptor in neurosteroid biosynthesis, neurophatology and neurological disorders. *Neuroscience* 138(3), 749–756 (2006).

18 Ferzaz B, Brault E, Bourliaud G *et al*. SSR180575 (7-chloro-N,N,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4H-pyridazino[4,5-b]indole-1-acetamide), a peripheral benzodiazepine receptor ligand, promotes neuronal survival and repair. *J. Pharmacol. Exp. Ther.* 301(3), 1067–1078 (2002).

19 Leonelli E, Yague JG, Ballabio M *et al*. Ro5–4864, a synthetic ligand of peripheral benzodiazepine receptor, reduces aging-associated myelin degeneration in the sciatic nerve of male rats. *Mech. Ageing Dev.* 126(11), 1159–1163 (2005).

20 Veiga S, Azcoitia I, Garcia-Segura LM. Ro5–4864, a peripheral benzodiazepine receptor ligand, reduces reative gliosis and protects hippocampal hilar neurons from kainic acid excitotoxicity. *J. Neurosci. Res.* 80(1), 129–137 (2005).

Affiliations

- *Roberto C Melcangi*
  University of Milan, Department of Endocrinology and Center of Excellence on Neurodegenerative Diseases, 20133 Milan, Italy
  Tel.: +34 915 854 729
  Fax: +39 025 031 8204
  roberto.melcangi@unimi.it
- *Luis M Garcia-Segura*
  Instituto Cajal, C.S.I.C., Avenida Doctor Arce 37, 28002 Madrid, Spain
  Tel.: +39 025 031 8238
  Fax: +34 915 854 754
  lmgs@cajal.csic.es