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Effects of neuroactive steroids on myelin of peripheral nervous system☆

R.C. Melcangi*, M. Ballabio, I. Cavarretta, L.C. Gonzalez, E. Leonelli, S. Veiga, L. Martini, V. Magnaghi

Department of Endocrinology and Center of Excellence on Neurodegenerative Diseases, University of Milan, Via Balzaretti 9, 20133 Milan, Italy

Abstract

Peripheral nervous system (PNS) possess both classical (e.g. progesterone receptor, PR, androgen receptor, AR) and non-classical (e.g. $GABA_A$ receptor) steroid receptors and consequently may represent a target for the action of neuroactive steroids. Our data have indicated that neuroactive steroids, like for instance, progesterone, dihydroprogesterone, tetrahydroprogesterone, dihydrotestosterone and 3α-diol, stimulate both in vivo and in vitro (Schwann cell cultures), the expression of two important proteins of the myelin of peripheral nerves, the glycoprotein Po (Po) and the peripheral myelin protein 22 (PMP22). It is important to highlight that the mechanisms by which neuroactive steroids exert their effects on the expression of Po and PMP22 involve different kind of receptors depending on the steroid and on the myelin protein considered. In particular, at least in culture of Schwann cells, the expression of Po seems to be under the control of PR, while that of PMP22 needs the $GABA_A$ receptor.

Because Po and PMP22 play an important physiological role for the maintenance of the multilamellar structure of the myelin of the PNS, the present observations might suggest the utilization of neuroactive steroids as new therapeutically approaches for the rebuilding of the peripheral myelin.

Keywords: Neuroactive steroids; Myelin; Schwann cells

1. Introduction

Recent observations have indicated that, not only central but also peripheral nervous system (PNS), is able to synthesize steroid hormones, the so-called neurosteroids, and to metabolize them, or hormonal steroids coming from the periphery, in metabolites called neuroactive steroids (see for review [1–3]). Steroidogenesis and formation of neuroactive steroids seem to take place prevalently in Schwann cells (i.e. cells forming peripheral myelin). In particular, Schwann cells are able to metabolize 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) (see for review [1–3]). This enzymatic complex is very versatile, since every steroid possessing the delta 4-3keto configuration may be first 5α-reduced and subsequently 3α-hydroxylated. For instance, testosterone (T) can be converted into dihydrotestosterone (DHT) and then into 5α-androstane-3α, 17β-diol (3α-diol), P into dihydroprogesterone (DHP) and subsequently into tetrahydroprogesterone (THP). Moreover, Schwann cells are not only able to synthesize and metabolize steroids but may also be considered a possible target for the actions of these molecules (see for review [4]). It has been clearly demonstrated that Schwann cells possess classical intracellular receptors for many families of steroids. For instance, by our and other laboratories has been demonstrated that Schwann cell express messenger for progesterone receptor (PR) and protein itself [5–8], as well as estrogen receptor (ER) [5,6]. On the contrary, the mRNA coding for androgen receptor (AR) seems not to be present in cultures of Schwann cells but only in the sciatic nerve of adult male rats taken in toto [7,8].

Moreover, Schwann cells express also non-classical steroid receptors, like for instance, the $GABA_A$ receptor [9], and consequently may also respond to steroids, which like THP, are able to actively interact with this neurotransmitter receptor (see for review [2,3]).

On the basis of these findings the possible effects of neuroactive steroids on PNS have been recently taken in consideration. This paper will summarize our recent observations

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* Corresponding author. Tel.: +39-02-50318238; fax: +39-02-50318204.

E-mail address: roberto.melcangi@unimi.it (R.C. Melcangi).

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indicating that neuroactive steroids are able to influence the expression of myelin proteins directly in Schwann cells and their specific products (e.g. myelin membranes).

2. Effects of neuroactive steroids on myelin proteins

2.1. In vivo observations

Glycoprotein Po (Po) and peripheral myelin protein 22 (PMP22) are two important components of peripheral myelin which are expressed by Schwann cells (see for review [10–12]). These two myelin proteins play an important physiological role for the maintenance of the multilamellar structure of the myelin of the PNS (see for review [10–12]). In the last few years we have obtained several observations indicating that neuroactive steroids stimulate the expression of Po and PMP22.

In particular, we have treated adult male rats for a month with eight subcutaneous injections of 1 mg of P, DHP or THP, and 24 h after the last treatment, mRNA levels of Po and PMP22 in the sciatic nerve have been evaluated by Northern blot analysis. Injections were administered every four days. The results obtained have indicated that P, DHP and THP are able to increase Po gene expression; however, DHP is significantly more effective than the other two steroids [9]. At variance to what observed in the case of Po, when we have analyzed the effect of these neuroactive steroids on PMP22 we have observed that only THP is able to stimulate the messenger level of this myelin protein [9]. At this point, we have evaluated whether these neuroactive steroids might be also useful after peripheral injury. The complete transection of the nerve, in which a segment of the nerve has been damaged or removed so that the proximal and the distal stumps are kept separated by a gap, probably represents the most severe challenge to nerve regeneration. Moreover, there is a growing body of evidence indicating that bridging a nerve gap with a tubular prosthesis (the so-called entubulation repair) represents an efficient technique to facilitate the repair of transected nerves [13,14]. To this purpose, we have utilized the transection and entubulation technique to repair the sciatic nerve of adult male rat; using this technique, we have analyzed the effects of neuroactive steroids on the gene expression of Po [15]. The data obtained have indicated that, after an experimental period of two weeks, only P and DHP are able to increase the low Po mRNA levels present in the distal portion from the cut of the sciatic nerve [15].

The finding that, in this experimental model, THP is not able to stimulate Po gene expression is rather surprising, since, as mentioned before, this steroids is effective in stimulating the messenger levels of Po present in the intact sciatic nerve of adult male rats [9]. We have already mentioned that THP is able to directly interact with the $GABA_A$ receptor (see for review [2,3]) consequently we have hypothesized that a possible decrease of $GABA_A$ receptor might be present in the transected sciatic nerve [15]. In support to this hypothesis, it is also important to mention the results we have obtained in this experimental model on PMP22 mRNA levels (i.e. a myelin protein stimulated in the intact sciatic nerve only by THP). After nerve transection we have analyzed in the distal portion from the cut of the sciatic nerve the mRNA levels of PMP22 and we have observed that, not only P and DHP, but also THP is ineffective in increasing the gene expression of this myelin protein [15].

On the basis of the observations indicating the effects of P and/or its 5α-reduced metabolites (i.e. DHP and THP) in the control of Po and PMP22 mRNA levels, we have taken into consideration the possibility that other neuroactive steroids might exert similar effects in adult animals [7]. To this purpose, we have first analyzed whether castration might influence the gene expression of Po in the sciatic nerve of adult male rats. The results obtained have indicated that castration clearly decreases (about 40%) Po mRNA levels in the sciatic nerve [7]. Subsequent experiments have been performed to investigate whether one month-treatment with T or DHT to castrated animals might counteract the drop of the levels of Po induced, in the sciatic nerve, by this endocrinological manipulation. The results obtained indicate that only the treatment with DHT, is able to increase Po mRNA levels [7]. The lack of effect of T in these experiments is not surprising, since the activity of the 5α-R, which converts T into DHT, is significantly decreased in the sciatic nerve following castration [7]. Consequently, it is possible that very low levels of DHT could be locally formed in castrated male rats treated with T.

2.2. In vitro observations

At this point, experiments have been performed in order to evaluate whether these neuroactive steroids might act directly on rat Schwann cells in culture. In an interesting agreement with the in vivo results just reported on the sciatic nerve of adult male rats, P, DHP and THP exerted a stimulatory effect on the gene expression of Po in Schwann cells [9,16].

The fact that P stimulates Po expression in Schwann cell cultures [16] has been confirmed by Desarnaud et al. [17] using a different experimental set up (Schwann cells transiently transfected with a reporter construct in which the expression of the luciferase is controlled by the promoter region of the Po gene).

In agreement with the in vivo results on the sciatic nerve of adult male rats, also in culture of Schwann cells the mRNA levels of PMP22 are only stimulated by THP [9]. At variance with our data, Desarnaud et al. [17] have found that P is able to stimulate the gene expression of PMP22, acting on promoter 1, but not on promoter 2 of the corresponding gene. However, since as mentioned above, these experiments have been performed utilizing Schwann cells transiently transfected with a reporter construct, this discrepancy is

probably due to the different experimental models applied. Moreover, it is also important to note that in a very similar experimental model Sabéran-Djoneidi et al. [18] have observed that P alone is not sufficient to promote PMP22 expression.

At this point we have evaluated the possible effect of androgens in the Schwann cells. In this context, it is important to highlight that, in order to explain the in vivo effect exerted by DHT on the gene expression of Po, we have hypothesized that the mRNA levels of this myelin protein are stimulated by androgen-dependent mechanisms acting on Schwann cells in an indirect fashion. In particular, we have proposed that androgens might act through the neuronal component, which does contain AR [7]. This interpretation, however, could be slightly modified on the basis of the results obtained in vitro utilizing rat Schwann cell cultures. Also in this case the treatment with T or 3α-diol was ineffective, while that with DHT was able to stimulate Po gene expression [7]; this is obviously in agreement with our in vivo observations [7]. However, since as mentioned above, AR mRNA is not present in Schwann cells, additional studies were necessary to try to explain the mode of action of androgens. To this purpose, we have tested the hypothesis that DHT might be able to activate Po gene expression by acting through a steroid receptor other than the AR. Since, as mentioned above, DHP, a steroid which interacts with the PR, may activate Po gene expression [9,16,19], we have postulated that DHT might interact with the PR, and activate progesterone responsive elements (PRE). The 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, at variance to that observed with T, is able to exert a transcriptional activity via the human PR [7].

Altogether, these observations indicate that the mechanisms through which androgens exert their effects on Po gene expression may be complex in nature, since androgens might operate not only via the AR but also via the PR.

Recently, we have also evaluated whether, like in the case of Po, also the messenger levels of PMP22, might be modulated by androgen treatment. To this purpose, we have analyzed the effect of T, DHT or 3α-diol on the mRNA levels of PMP22 in Schwann cell cultures. The data obtained have indicated that, while T and DHT prove to be ineffective, 3α-diol was able to significantly increase the gene expression of PMP22 [15].

The fact that 3α-diol is the only derivative of T able to stimulate PMP22 gene expression in Schwann cell cultures appears rather interesting, because of its similarity with the efficacy of THP on the same protein [9]. It is important to recall that, recently it has been proposed that 3α-diol, which does not bind to the AR, might interact with $GABA_A$ receptor (see for review [20]). Consequently these observations further support the concept that the PMP22 mRNA levels are stimulated in Schwann cells via the $GABA_A$ receptor.

3. Po and PMP22 expression is stimulated by neuroactive steroids via different receptors

The data so far obtained indicate that neuroactive derivatives of T and P are able to stimulate the mRNA levels of the two most important myelin proteins of the PNS, the Po and the PMP22. However, as mentioned above these molecules interact with different receptors, consequently, we have hypothesized that the mechanisms by which neuroactive steroids exert their effects on the expression of Po and PMP22 involve different kind of receptors depending on the steroid and on the myelin protein considered. For instance, in case of Po it is possible that the effects of P and DHP might directly involve the PR, which as we have demonstrated, is present both in the sciatic nerve and in Schwann cells [7,8]. Moreover, since we have observed that DHP is more effective than P, and that the 5α-R is present in the sciatic nerve and in Schwann cells [7,16,21–23] it is also possible to postulate that P acts mainly following transformation into DHP. Furthermore, since the activity of the 3α-HSD is bi-directional (see for review [2,3]), it is possible to assume that the efficacy of THP may result from a retro-conversion of this steroid into DHP (see for review [2,3]). However, THP is a potent ligand of $GABA_A$ receptor (see for review [2,3]), which is present both in sciatic nerve and in Schwann cell cultures [9], consequently it is also possible to hypothesize a direct effect of THP via the interaction with this neurotransmitter receptor.

In order to distinguish whether the stimulatory effects exerted by P and its derivatives on Po gene expression are due to an interaction to PR or to $GABA_A$ receptor or to a mixed action, we have exposed rat Schwann cells in culture to specific agonists or antagonists of these receptors [8]. The data obtained after exposure of Schwann cells to mifepristone, also known as RU 38486 (i.e. an antagonist of PR) have indicated that its presence is able to abolish the stimulatory effect exerted by P or DHP on the expression of Po, confirming an involvement of PR. Moreover, the antagonist is also able to block the effect of THP (i.e. a steroid which does not bind to the PR), suggesting that the activity of THP is mainly due to its retro-conversion into DHP, rather than to a direct interaction with the $GABA_A$ receptor. This hypothesis is also supported by the fact that a $GABA_A$ agonist, like the muscimol, does not mimic the effect of THP.

An effect of P and its neuroactive derivatives on Po gene expression, through the PR may indicate a classical steroid mechanism. That might be further supported by the results of a computer analysis we have performed, which has permitted to identify some putative PRE on the Po promoter [7]. In particular, two sequences, localized at nt-491 (AGAACA-3n-GACACC) and nt-971 (TACCTT-3n-TGTTCC) of the Po promoter, present a 41% homology with the glucocorticoid/PRE consensus sequence, GGTACA-3n-TGTTCT [24]. Moreover, the sequence localized at nt-971 region presents, respectively, a 75 and a 41% homology with two PRE identified by Savouret et al. [25] on the PR gene; these

have been named “medium” (TTGCAT-3n-TGTTCC) and “strong” (GGAACA-3n-AGTTCC) on the basis of their capacity to bind the PR.

As previously mentioned P and DHP are ineffective in modulating the gene expression of PMP22, while THP is highly effective [9]. It was consequently obvious to analyze whether the activity of this steroid on PMP22 gene expression might be mimicked by $GABA_A$ agonists and blocked by $GABA_A$ antagonists [8]. We have demonstrated that the stimulatory effect of THP on PMP22 mRNA levels in Schwann cell in culture is completely abolished by the simultaneous presence of bicuculline, a specific antagonist of $GABA_A$ receptor. Moreover, in Schwann cell cultures exposed to the $GABA_A$ agonist muscimol a clear stimulatory effect on the messenger levels of this myelin protein was observed; this was comparable to that exerted by THP. These data suggest that THP modulates the gene expression of PMP22 via the interaction with the $GABA_A$ receptor. The specificity of the effect of THP on the $GABA_A$ receptor is also clear by the fact that the inactive isomer of this steroid, the isopregnanolone, which does not interact with $GABA_A$ receptor [26], does not significantly modify the mRNA levels of PMP22 in Schwann cell cultures [8].

Altogether, the present findings strongly indicate that classical and non-classical steroid receptors are involved in a different ways in the control of the mRNA levels of Po and PMP22; in particular, Po seems to be under the control of PR, while PMP22 depends on the $GABA_A$ receptor.

4. Conclusions

The observations here summarized indicate that neuroactive steroids in several in vivo (e.g. intact and transected sciatic nerve of male rats) and in vitro (rat Schwann cell cultures) experimental models are able to stimulate the gene expression of two important proteins of peripheral myelin (i.e. Po and PMP22). Moreover, the present results suggest that the mode of action of P and its physiological derivatives on the gene expression of Po and PMP22 involves different families of receptors depending on the protein considered. Po and PMP22 play an important physiological role for the maintenance of the multilamellar structure of the myelin of the peripheral nervous system, consequently these observations might suggest the possible utilization of neuroactive steroids after peripheral injury, during aging or in particular demyelinating diseases (e.g. Charcot-Marie-Tooth type 1a and 1b, Déjérine-Sottas syndrome, etc.) in which the rebuilding of myelin is needed. Data have been recently obtained in support to this hypothesis. For instance, P is able to accelerate the time of initiation, and to enhance the rate of myelin synthesis, in the Schwann cells co-cultured with dorsal root ganglia neurons [27,28]. Moreover, in vivo observations indicate that this neuroactive steroid, when given locally, is able to counteract the decrease of the amounts of myelin membranes induced by a cryolesion in the sciatic nerve of the mouse (see for review [1]). Finally, we have recently demonstrated that not only P, but also DHP and THP, are able to reduce aging-associated morphological abnormalities of myelin and aging-associated myelin fiber loss in the sciatic nerve [29].

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