Membrane progesterone receptors (mPRs/PAQRs) in Schwann cells represent a promising target for the promotion of neuroregeneration

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Schwann cells and neuroregeneration: Peripheral nerve injury is a common cause of morbidity, which affects millions of people worldwide. The peripheral nervous system, differently from the central nervous system, has an intrinsic ability to regenerate after injury. However, in most cases the regenerative outcome is not completely satisfactory, in particular for long-gap peripheral nerve injuries in which the microsurgical approach is not possible. In these cases, the current research effort is mostly aimed at the identification of pharmacological and/or cell therapy approaches that, coupled with the use of biomaterial conduits, provide scaffold, mechanical support and guidance to the regeneration process, and can increase regeneration speed and efficiency (Faroni et al., 2015).

Schwann cells, the main glial cells of the peripheral nervous system (Castelnovo et al., 2017), play a well-established role in the promotion of nerve regeneration. After nerve injury, mature Schwann cells can trans-differentiate from the myelinating or non-myelinating (Remak) differentiation state, into a phenotype known as repair Schwann cells that promotes nerve regeneration (Jessen and Arthur-Farraj, 2019). When they assume this phenotype, Schwann cells become proliferative and migratory and express specific differentiation markers (e.g., Olig1 and Shh). Moreover, they show some stemness features, undergoing a partial epithelial to mesenchymal transition process. The different characteristics of repair Schwann cells and the physiological changes mature Schwann cells undergo during this transition are described in detail in a recent review (Jessen and Arthur-Farraj, 2019). In this state, they contribute to debris removal, in a process known as Wallerian degeneration, involving macrophage recruitment. At the same time, they secrete stimulating factors that promote nerve regeneration (Faroni et al., 2015). Schwann cells can therefore be exploited as an experimental tool to promote the previously mentioned cell therapy approach to promote nerve regeneration. However, the cell therapy experimental approach often relies on the use of stem cells of different origins, including neural stem cells, embryonic stem cells, induced pluripotent stem cells and adult mesenchymal stem cells. These cells have the advantage of having a greater expansion capability *in vitro* compared to human primary Schwann cells, and do not require the sacrifice of a healthy nerve from the patient. These stem cells can be differentiated into a Schwann cell-like phenotype, which can recapitulate some characteristics of primary Schwann cells. These differentiated stem cells were shown to increase nerve regeneration both *in vitro* and *in vivo* in rodent experimental models of nerve injury (Faroni et al., 2015).

Schwann cells are also present in the central nervous system under certain pathological conditions, such as multiple sclerosis and spinal cord injury, raising the possibility they may also play a neuroregenerative role in the central nervous system. These cells can either originate in the peripheral nervous system and then migrate into the spinal cord moving along vascular networks, or derive from oligodendrocyte precursor cells, following spinal cord demyelination or injury (Garcia-Diaz and Baron-Van Evercooren, 2020). Therefore, they may also be a useful tool for the treatment of spinal cord injury. However, the main setback in exploiting Schwann cells in this context is their limited ability to proliferate inside the central nervous system due to the presence of inhibitors produced by astrocytes and the presence of central myelin (Garcia-Diaz and Baron-Van Evercooren, 2020).

Role of progestogens and membrane progesterone receptors (mPRs) in the promotion of nerve regeneration: There is evidence that neuroactive steroids, and in particular progesterone and its active metabolites dihydroprogesterone and allopregnanolone, play an important physiological role in the nervous system, and in particular in Schwann cells (Castelnovo et al., 2017). These progestogens exert their actions through multiple receptor mechanisms in the nervous system, including the classic intracellular progesterone receptor (PR), membrane progesterone receptors (mPRs) and the neurotransmitter gamma-aminobutyric acid type A (GABA-A) receptor. Some results have suggested that progestogens modulate Schwann cell physiology during regeneration, with most of the effects reportedly mediated by allopregnanolone's allosteric action through the GABA-A receptor. Allopregnanolone was shown to increase Schwann cell proliferation in primary rat Schwann cells (Perego et al., 2012; Melfi et al., 2017), and this action was proposed to be GABA-A receptor mediated since it was replicated by muscimol and blocked by bicuculline, a specific GABA-A receptor agonist and antagonist, respectively (Perego et al., 2012). Allopregnanolone also changed Schwann cell morphology, motility and myelination, which are crucial processes for nerve development, maturation and regeneration (Melfi et al., 2017). These effects were mediated by Src/FAK activation, likely involving GABA-A receptor-dependent mechanisms (Melfi et al., 2017), although other signaling pathways may also be involved (Thomas and Pang, 2012). It is important to note that these progestogens exert neuroprotective actions in different experimental models of nerve damage, for example, nerve transection, cryolesion, crush, guided regeneration of the facial nerve, and docetaxel-induced peripheral neurotoxicity (Giatti et al., 2020), supporting neuroprotective and pro-regenerative roles for this class of neurosteroids in the nervous system.

The mPRs are also potential intermediaries of progestogen neurosteroid actions in the nervous system, since allopregnanolone also activates mPRs and exerts some neuroprotective actions through these receptors in neuronal cells (Thomas and Pang, 2012; Pang et al., 2013). Therefore, in two recent studies, we investigated the role of progestogens and mPRs in Schwann cell physiology. The mPRs belong to the progestin and AdipoQ receptor (PAQR) family and comprise five isoforms: mPRα (PAQR7), mPRβ (PAQR8), mPRγ (PAQR5), mPRδ (PAQR6) and mPRε (PAQR9) (Thomas and Pang, 2012). These membrane receptors are coupled to G proteins; mPRα, β and γ and are coupled to inhibitory G proteins (Gi), while mPRδ and ε are coupled to stimulatory G proteins (Gs) (Pang et al., 2013). We found that mPRs are present and active in both a Schwann cell line model and in primary rat Schwann cells, where they promote cell migration, proliferation and differentiation (Castelnovo et al., 2019, 2020). Indeed, mPR activation with the specific mPR agonist, Organon OD 02-0, changes Schwann cell morphology, making them longer, consistent with previous reports of morphological changes following nerve injury *in vivo* (Jessen and Arthur-Farraj, 2019). The molecular mechanism may involve Akt activation and a decrease in cyclic AMP (cAMP) levels, the latter likely due to Gi protein activation and reduced adenylate cyclase activity (Castelnovo et al., 2019, 2020). The modulation of cAMP levels has been reported by different authors to promote either proliferation or differentiation towards a myelinating state, in which proliferation is reduced. It is possible that this discrepancy may be due to the ability of cAMP to mediate a different response based on the various intracellular pathways activated in Schwann cells (Monje, 2015). The cAMP regulatory mechanism is complex and requires further studies to be elucidated.

Considering that migration, proliferation and morphological changes are all characteristics attributable to repair Schwann cells (Faroni et al., 2015; Jessen and Arthur-Farraj, 2019; Castelnovo et al., 2020), these studies corroborate the hypothesis that progestogens may play a role in the promotion of nerve regeneration and suggest their actions are mediated through mPRs. Moreover, mPR activation induced rapid changes in the expression levels of some Schwann cell differentiation markers (Castelnovo et al., 2020). Both markers of myelinating (Sox10 and Krox20) and non-myelinating (GFAP and p75-NTR) Schwann cells were down-regulated by mPR activation. Two specific markers of repairing Schwann cells, Olig1 and Shh, were modulated too. Olig1 was increased, in line with previous results, while Shh was down regulated, differently from what observed *in vivo* (Jessen and Arthur-Farraj, 2019). This discrepancy may be due to *in vitro-in vivo* differences and deserves further investigation. Altogether, these findings suggest that mPRs play a novel role in the promotion of a pro-regenerative phenotype through their direct control of Schwann cell morphology and functions (Castelnovo et al., 2019, 2020).

Recently, it was shown that some effects of neurosteroids on GABAergic inhibition are metabotropic and not dependent on the allosteric binding to the GABA-A receptor, but on mPR-mediated phosphorylation of

the GABA-A receptor (Parakala et al., 2019). Therefore, it can be hypothesized that progestogen effects on Schwann cell migration, proliferation and morphology may involve a common mechanism, possibly involving GABA-A phosphorylation following mPR activation. This mechanism of GABA-A cross-activation is very novel and will need to be furtherly investigated.

Can mPRs be relevant in promoting spinal cord regeneration? As previously mentioned, progestogens could potentially also play a role in neuroprotection and promotion of nerve regeneration in the central nervous system because progestogen levels are increased in rodent models following spinal cord transection, stroke and ischemia (Giatti et al., 2020). An interesting finding in our recent studies is that mPRs can modulate the expression of the myelin associated glycoprotein (MAG) (Castelnovo et al., 2019, 2020), which is an important myelin protein involved in neuron-glia interactions and myelin compaction, both in the peripheral and in the central nervous systems. Recently, MAG was shown to inhibit Schwann cell migration and induce cell death through a mechanism involving p75-NTR cleavage (Chaudhry et al., 2017). Moreover, it was possible to increase *in vivo* Schwann cell migration and survival by preventing MAG-mediated p75-NTR cleavage (Chaudhry et al., 2017). Notably, the findings by Chaudhry and colleagues indicate the potential use of Schwann cells to promote nerve regeneration in the central nervous system for spinal cord regeneration. As previously mentioned, poor migration of Schwann cells transplanted in the central nervous system is an important limitation hindering the implementation of this strategy to promote spinal cord regeneration (Garcia-Diaz and Baron-Van Evercooren, 2020). Using primary rat Schwann cells, we demonstrated that mPR activation reduced MAG gene and protein expression, alongside the previously described effects on cell migration, and also regulated *p75-NTR* gene expression (Castelnovo et al., 2020). Considering these findings, mPR activation may be a promising target for regeneration, in peripheral nerves and potentially also the spinal cord, although this possibility is for now only theorical and needs to be investigated experimentally. Furthermore, beside the possible pro-regenerative action of mPR in the spinal cord through the stimulation of Schwann cells proliferation and myelination, the possibility that mPR activation may exert direct neuroprotective effects within the spinal cord should be considered, since mPRs can exert neuroprotective effects *in vitro* (Thomas and Pang, 2012).

Concluding remarks: The described pro-regenerative effects of progestogen treatment, and in particular of mPR activation, in Schwann cells are schematized in **Figure 1**. Progestogens may modulate several signaling pathways, including the activation of Akt and Src pathways and the decrease in activity of adenylate cyclase. They can also modulate the expression of MAG and of several Schwann cell differentiation factors and increase cell proliferation and migration. The mPRs can also modify Schwann cell morphology, in a way compatible with a transition towards the repair phenotype. Most of the outcomes were shown to follow mPR activation, others may depend on GABA-A trans-activation by mPRs. Altogether, these findings suggest mPR modulation as an interesting target for the regulation of Schwann cell biology in a way that appears to be beneficial for the promotion of nerve regeneration. In the future, it is worthwhile to focus on better understanding the intracellular mechanisms of mPR action, defining the role played by the different pathways downstream of mPR activation. Interestingly, a possible field of study may deal with the analysis of mPR activation in Schwann-like cells, such as Schwann cell-like differentiated human mesenchymal stem cells, well-suited for clinical approaches in tissue engineering and regenerative medicine. Indeed, as previously mentioned, these cells recapitulate the pro-regenerative effects of Schwann cells *in vitro* and *in vivo*, while presenting some advantages like better *in vitro* proliferation and easier availability (Faroni et al., 2015).

A deep comprehension of the mechanisms involved in mPR activity in Schwann cells may be also exploited for neuronal cell regeneration in very high-impact conditions such as the spinal cord injury. Therefore, the identification of novel mPR-based therapeutic strategies might be a promising tool in central/peripheral neuroregeneration.

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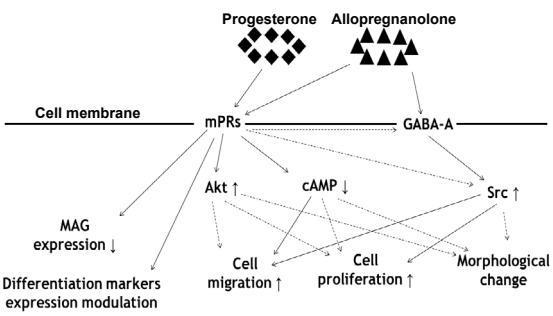

Figure 1 | **Graphical illustration of progestogen actions in Schwann cells.**
Effects reported in literature are indicated by solid arrows, hypothesized effects by broken arrows. cAMP: Cyclic AMP: GABA-A: Gamma-aminobutyric acid type A; MAG: myelin associated glycoprotein; mPRs: membrane progesterone receptors.

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