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# PROGESTERONE INCREASES OLIGODENDROGLIAL CELL PROLIFERATION IN RAT CEREBELLAR SLICE CULTURES

**A. M. GHOUMARI,* E. E. BAULIEU AND M. SCHUMACHER**

*INSERM U488, Batiment Gregory Pincus, 80 rue du Général Leclerc, 94276 Bicêtre, France*

**Abstract—We have previously demonstrated that progesterone significantly increases the rate of myelination in organotypic slice cultures of 7-day-old rat and mouse cerebellum. Here, we show that progesterone (20 μM) stimulates the proliferation of oligodendrocyte precursors in cultured cerebellar slices of 7-day-old rats. The steroid increased the number of pre-oligodendrocytes ($NG2^+$, $O4^+$) and to some extent of oligodendrocyte precursors, corresponding to an earlier developmental stage (nestin$^+$, PDGFαR$^+$, $NG2^+$, $O4^-$). Progesterone stimulated the proliferation of both $NG2^+$ and $O4^+$ cells as shown by increased double-immunolabeling with the cell proliferation marker Ki67. The mitogenic effect of progesterone was inhibited by the progesterone receptor antagonist mifepristone (10 μM) and could not be mimicked by its GABA-active metabolite 3α,5α-tetrahydroprogesterone (allopregnanolone), even at the high concentration of 50 μM. Results indicate that progesterone first strongly and transiently stimulates the proliferation of oligodendrocyte precursors, and that it may thereafter accelerate their maturation into myelinating oligodendrocytes. Although oligodendrocyte precursors may be a direct target for the actions of progesterone, their number may also be indirectly influenced by the effects of the steroid on neurons and microglial cells, since treatment of the cerebellar slices with progesterone enhanced staining of the neuronal cytoskeleton marker microtubule-associated protein-2 and increased the number of $OX\text{-}42^+$ microglia. A small percentage (about 0.1%) of the $NG2^+$ cells transiently became $OX\text{-}42^+$ in response to progesterone. These results point to novel mechanisms by which progesterone may promote myelination in the CNS, specifically by stimulating the proliferation and maturation of oligodendrocyte precursors into myelinating oligodendrocytes.** 

**Key words: progesterone, oligodendrocytes, microglial cells, cerebellum.**

There is a growing recognition that the physiological functions of gonadal steroid hormones need to be extended beyond their role in reproduction. Thus, progesterone (PROG) has been shown to play an important role in the viability of neurons and in the formation of myelin sheaths (Schumacher et al., 2002, 2004). Moreover, PROG present in nervous tissues originates either from the steroidogenic endocrine glands as this steroid easily crosses the blood–brain barrier, or from local synthesis. Steroids which can be synthesized within the nervous system qualify as neurosteroids (Baulieu, 1997).

An important role for PROG in myelination has first been demonstrated in the regenerating mouse sciatic nerve and in co-cultures of sensory neurons and Schwann cells (Koenig et al., 1995). More recently, it has been shown that the administration of PROG allows to reduce aging-associated morphological abnormalities of myelin in the rat sciatic nerve and that the progesterone receptor (PR) of myelin-forming Schwann cells is a pharmacological target for the treatment of peripheral neuropathies (Azcoitia et al., 2003; Sereda et al., 2003). Recent work has shown that the increase in peripheral myelin protein synthesis by progestins involves the activation of both intracellular PR and membrane $GABA_A$ receptors located on Schwann cells (Magnaghi et al., 2001). The PROG metabolite 3α,5α-THP (3α,5α-tetrahydroprogesterone, allopregnanolone), is a potent positive modulator of $GABA_A$ receptors (Lambert et al., 2003).

There is now increasing evidence that PROG also promotes myelination by oligodendrocytes in the CNS. Already several years ago, PROG has been shown to increase the number of myelin basic protein (MBP)-positive oligodendrocytes in cultures of glial cells prepared from neonatal rat brain (Jung-Testas et al., 1989). More recently, the prolonged administration of PROG was found to improve the slow and inefficient remyelination that occurs in the brain of old male rats (Ibanez et al., 2003, 2004). In organotypic slice cultures of 7-day-old (P7) rat and mouse cerebellum, PROG significantly increased the rate of myelination, evaluated by immunofluorescence analysis of MBP (Ghoumari et al., 2003). As in peripheral nerves, the promyelinating effects of PROG in cerebellar slices involved both the intracellular PR and $GABA_A$ receptors, although activation of the former was required. In fact, PROG had no effect on myelination in PR knockout mice (Ghoumari et al., 2003).

PROG may accelerate CNS myelination by stimulating the proliferation or maturation of oligodendrocyte precursors. Such a mechanism was suggested by the observation that PROG also increased the immunostaining of two

*Corresponding author. Tel: +33-1-49-59-18-97; fax: +33-1-45-21-19-40.
E-mail address: ghoumari@kb.inserm.fr (A. M. Ghoumari).

*Abbreviations:* DIV, days *in vitro*; GalC, galactocerebroside; GFAP, glial fibrillary acidic protein; MAP-2, microtubule-associated protein-2; MBP, myelin basic protein; NeuN, neuronal nuclei; OPC, oligodendrocyte progenitor cell; PBS, phosphate-buffered saline; PBSGTA, phosphate-buffered saline 0.12 M (pH 7.4) containing 0.25% Triton X-100, 0.2% gelatin, 0.1% sodium azide; PDGFαR, platelet-derived growth factor α receptor; PR, progesterone receptor; PROG, progesterone; P7, 7-day-old, postnatal day 7; RU486, mifepristone; 3α,5α-THP, 3α,5α-tetrahydroprogesterone, allopregnanolone; 5α-DHP, 5α-dihydroprogesterone.

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other markers of the oligodendroglial lineage in cerebellar slices, namely, galactocerebroside (GalC) and O4 antigen (Ghoumari et al., 2003). Thus, PROG could be part of the growth factors involved in the regulation of oligodendrocyte precursor proliferation and maturation (Miller, 2002).

As summarized in Fig. 1, the differentiation of oligodendrocytes progresses through a series of stages with distinct functional, morphological and antigenic characteristics (Hardy and Renolds, 1991; Levine et al., 2001; Gago et al., 2003). The oligodendrocyte precursor cells express platelet-derived growth factor $\alpha$ receptors (PDGF$\alpha$R) (Pringle and Richardson, 1993; Grinspan et al., 1995), the intermediate filament protein nestin and the chondroitin-sulfate proteoglycan NG2 (Gallo and Armstrong, 1995; Nishiyama et al., 1996a; Dawson et al., 2000) (Fig. 1). Both PDGF$\alpha$R and NG2 are highly co-localized on oligodendrocyte precursor cells, and it has been proposed that their interaction may be necessary for the cells to effectively respond to the mitogenic stimulus of PDGF (Nishiyama et al., 1996b). The oligodendrocyte precursor cells arise in germinal zones and then migrate throughout the CNS (Hardy and Reynolds, 1991; Pringle and Richardson, 1993; Lee et al., 2000). In the cerebellum, $NG2^+$ cells already appear during the last embryonic days and then proliferate over the first 10 days of postnatal life (Levine et al., 1993).

Co-expression of NG2 and O4 antigen defines a distinct stage of oligodendroglial maturation, referred to as pre-oligodendrocytes (Reynolds and Hardy, 1997). As $O4^+$ cells continue to differentiate, they no longer express NG2 and begin to produce GalC, an early marker of differentiated oligodendroglia (Compston et al., 1997). Fully mature myelinating oligodendrocytes continue to be labeled by the O4 antibody, as it also recognizes components of the myelin sheaths such as sulfatides (Bansal et al., 1992; Reynolds and Hardy, 1997) (Fig. 1).

Organotypic slice cultures provide a powerful tool for studying maturational processes such as myelination (Berger and Frotscher, 1994; Gähwiler et al., 1997). In the present study, we used organotypic slice cultures of P7 rat cerebellum to examine the effects of PROG on oligodendrocyte precursor proliferation and differentiation. At this developmental stage, a large number of Purkinje cells survive and axons start to be myelinated (Dusart et al., 1997; Ghoumari et al., 2000; Notterpek et al., 1993).

Our results show that PROG induces a strong and transient increase in the proliferation of $NG2^+$ and $O4^+$ pre-oligodendrocytes within the cerebellar slices. The mitogenic effect of PROG on oligodendrocyte precursors was mediated by the intracellular PR and could not be mimicked by the GABA-active metabolite 3$\alpha$,5$\alpha$-THP. PROG also significantly increased the proliferation of oligodendroglial cells at an earlier $NG2^+$ developmental stage, when cells still express nestin. In addition, PROG may favor the differentiation of pre-oligodendrocytes into premyelinating $O4^+$ and $GalC^+$ oligodendrocytes, in agreement with our previous observation that PROG increases O4- and GalC-immunoreactivities in cerebellar slices. Concerning other cell types, PROG did not influence the number of neurons, but increased the density of the neuronal cytoskeleton,

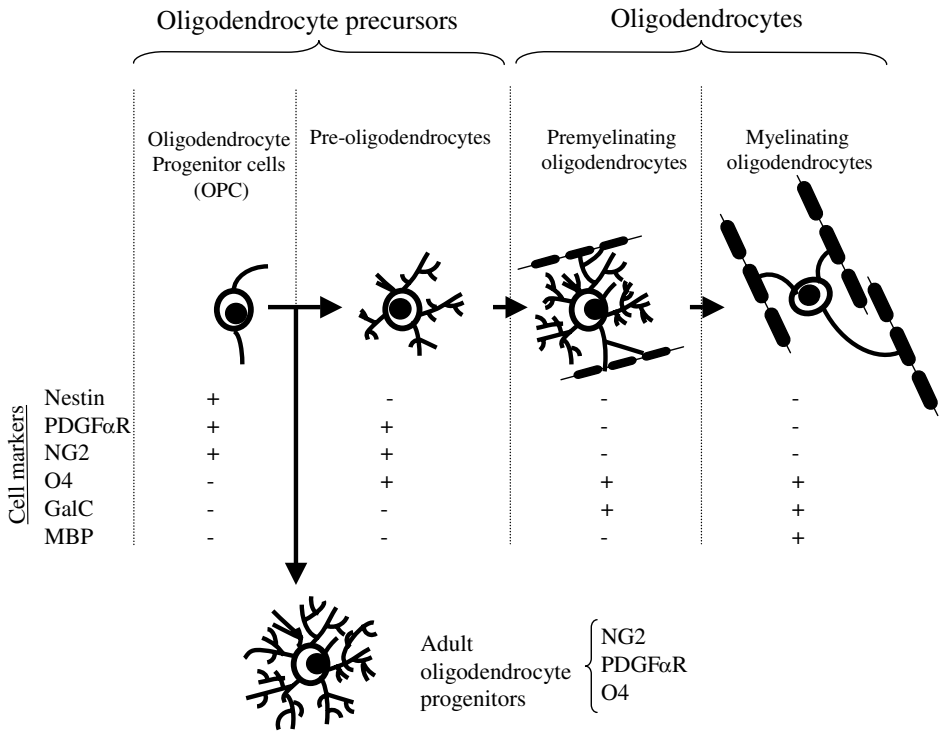


**Fig. 1.** Schema illustrating the different stages of development of the oligodendrocyte lineage. Different cell markers were used to identify the developmental status of the glial cells (NG2, a membrane chondroitin sulfate proteoglycan; O4 antigen) (adapted from Levine et al., 2001; Gago et al., 2003).