New Aspects of Progesterone Interactions With the Actin Cytoskeleton and the Neurosteroidogenesis in the Cerebellum and the Neuronal Growth Cone

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Abstract

The impact of progesterone on neuronal tissues in the central (CNS) and peripheral (PNS) nervous system is of significant scientific and therapeutic interest. Glial and neuronal cells of vertebrates express steroidogenic enzymes, and are able to synthesize progesterone de novo from cholesterol. Progesterone is described to have neuroprotective, neuroreparative, antidegenerative, and antiapoptotic effects in the CNS and the PNS. Thus, first clinical studies promise new therapeutic options using progesterone in the treatment of patients with traumatic brain injury. Additionally, experimental data from different animal models suggest further positive effects of progesterone on neurological diseases like cerebral ischemia, peripheral nerve injury and amyothropic lateral sclerosis. In regard to this future clinical use of progesterone, we discuss in this review the underlying physiological principles of progesterone effects in neuronal tissues. Mechanisms leading to morphological reorganizations of neurons in the CNS and PNS affected by progesterone are addressed with special focus on the actin cytoskeleton. Furthermore, new aspects of a progesterone-dependent regulation of neurosteroidogenesis mediated by the recently described progesterone binding protein PGRMC1 in the nervous system are discussed.

Key words: Purkinje cell, Dorsal root ganglia, growth cone, progesterone receptor, PGRMC1, neurosteroidogenesis, actin cytoskeleton, life cell imaging, confocal laser scanning microscopy, microinjection
Introduction

Progesterone was first isolated from the ovaries of female rats in 1935. Since then, the scientific interest in this hormone has been constantly high. Progesterone is a C21-steroid hormone, which plays an elementary role in the female menstruation cycle and is essential for the establishment and maintenance of pregnancy. Nevertheless, gestagenes like progesterone are also produced in male organisms. Since the beginning of the 1990’s, the already heightened interest in progesterone continued to increase when Baulieu and Robel (Baulieu and Robel 1990) first described that steroidogenic enzymes are also expressed in the central nervous system (CNS). In the following years different laboratories were able to show that both neuronal and glial cells in central and peripheral (PNS) nervous system are able to synthesize progesterone from cholesterol (Mellon et al. 1993; Compagnone et al. 1995; Guennoun et al. 1997, Furukawa et al. 1998; Tsutsui et al. 2000). From that time, the neuronally-derived steroids were termed neurosteroids and continue to be of high scientific and therapeutic interest.

Proceeding research gives evidence of the high significant physiological function of progesterone for the nervous system. Simultaneously, it can be deduced that progesterone might also be an effective option in the therapy of different severe neurological diseases (Deutsch et al. 2013). Indeed, different preclinical and early clinical studies revealed the protective effects of progesterone in patients with traumatic brain injuries (TBI) (Roof et al. 1994; Djebaili et al. 2004, 2005; Wright et al. 2007; Xiao et al. 2008). As TBI is a common and severe disorder and the up-to-date therapy of TBI patients cannot satisfyingly reduce morbidity and mortality, progesterone represents a new promising approach in TBI therapy. Additionally, experimental data from different animal models suggest further benefits of progesterone on other neurological disorders. Thus, progesterone might be a potential therapeutic device of apoplexies, as a post ischemic application of progesterone leads to a better outcome in different rat models (Sayeed et al. 2007; Ishrat et al. 2009; Wang et al. 2010). Furthermore anti-inflammatory protective effects in animal models of autoimmune encephalomyelitis were observed, which might suggest a future option in the therapy of patients with

Thus, progesterone seems to be a promising option in the therapy of a variety of neurological disorders. With regards to the molecular mechanisms that underlie the physiological and pathological effects and functions of progesterone in the nervous system, research is still in its early stages. This review provides an overview of current scientific knowledge about progesterone effects and underlying molecular mechanisms in cerebellar Purkinje cells (CNS) and dorsal root ganglia neurons (PNS), with a special focus on its influence on neurosteroidogenesis and the modulation of the actin cytoskeleton.

**Progesterone effects in immature Purkinje cells are mediated through classical progesterone receptors**

Purkinje cells, named after Jan Evangelista Purkyně, are large GABAergic neurons, located in the cortex of the cerebellum (Fig. 1a), which is divided into three layers: the outer high-fibre molecular layer, the Purkinje cell layer and the inner granule layer. The Purkinje cell bodies are 50-70µm in size and characteristically reveal a line-shaped arrangement in the Purkinje cell layer (Fig. 1b). The stem dendrites arise from the Purkinje cell body and usually divide into two or three main dendrites, which branch into numerous smaller secondary and tertiary dendrites (Fig. 1). The emerged dendritic arbour is totally covered with dendritic spines, which represent the postsynaptic component of excitatory and inhibitory synapses (Fig. 1c, Fig. 3a) and are therefore crucial for a proper development and function of both, immature and mature Purkinje cells.

Purkinje cells are subjected to a complex process of maturation, which starts prenatally and proceeds into the late postnatal period. Simultaneously, these cells were shown to express the steroidogenic enzymes P450scc as well as 3ß-HSD (Do Rego et al. 2009) and are able to synthesize progesterone de novo from cholesterol (Ukena et al. 1998, 1999) (Fig. 3c). As the synthesis of progesterone in the cerebellum seems to be restricted to the neonatal period of Purkinje cells, it is well justified to consider that progesterone is probably involved in the development of a precise cerebellar circuit (Ukena et al. 1998, 1999). Indeed, application of progesterone to neo-
natal Purkinje cells was shown to induce an increase in spinogenesis, synaptogenesis and dendritogenesis in vitro and in vivo (Sakamoto et al. 2001, 2002, Wessel et al. 2014). Furthermore, a recent study strongly confirmed these findings by directly visualizing, for the first time, a significant increase in dendritic length and dendritic area in the same Purkinje cells before and after stimulation with progesterone for 24 hours (Fig. 1d, e). In this study single Purkinje cells within the complex cerebellar tissue of rollertube slice cultures were specifically transfected with the aid of microinjection (pEYFP-actin) to allow a non-overlapping imaging of these cells (Fig. 1d, e) (Wessel et al. 2014).

In different experimental studies the mentioned effects of progesterone on spinogenesis and dendritogenesis in Purkinje cells were shown to be absent when progesterone application was combined with mifepristone (RU486) (Sakamoto et al. 2001, 2002; Wessel et al. 2014). Even though such anti-progesterone activities of mifepristone are a well-known fact and established parts of clinically therapies, mifepristone was shown to also act as an agonist on the classical progesterone receptors under specific cellular conditions (Cadepond et al. 1997; Liu et al. 2002), depending on the cellular availability of certain co-activators and co-repressors (Meyer et al. 1990; Katzenellenbogen and Katzenellenbogen 2002). For this reason, mifepristone is now more appropriately termed as a selective progesterone receptor modulator (SPRM) (Leonhardt et al. 2003). In addition to its interaction with progesterone receptors, mifepristone was shown to have a further anti-glucocorticosteroid activity (Bertagna et al. 1984; Gaillard et al. 1984; Cadepond et al. 1997). Indeed, both glucocorticoid (GR) and mineralocorticoid (MR) receptors are expressed in developing as well as in adult Purkinje cells (Ahima et al. 1992; Lawson et al. 1992). Nevertheless, in the cerebellum, the anti-progesterone functions of mifepristone compensate the inhibition of glucocorticosteroid receptors as in Purkinje cells (i) it was also demonstrated that mifepristone-induced inhibition of GR is dose-dependent, thus GR are probably not affected by the single dose used of 2 µM mifepristone (Cadepond et al. 1997) and (ii) PCR analyses demonstrated an increase in the amount of classical progesterone receptor-mRNA subsequent to progesterone treatment (Wessel et al. 2014). Therefore, it can be assumed that progesterone induces its effects on spinogenesis and dendritogenesis in immature Purkinje cells via the classical progesterone receptors.
Indeed PCR and western blot analyses confirm the expression of classical progesterone receptors in neonatal as well as in mature Purkinje cells (Sakamoto et al. 2003; Wessel et al. 2014). The so-called classical progesterone receptors were first described in the 1970’s (O’Malley et al. 1970). These nuclear receptors are defined as ligand-activated transcription factors and are localized in the cytoplasm, linked to chaperone molecules (Smith and Toft 1993). After binding of progesterone to the receptors, they dissociate from the chaperone molecules and dimerize. Following dimerization the complexes translocate into the nucleus and modulate the transcription through interaction with specific progesterone-response elements (Tsai and O’Malley 1994; Leonhardt et al. 2003; DeMarzo et al. 1991; Edwards et al. 1991) (Fig. 3c).

Through these genomic mechanisms classic progesterone receptors are likely to induce the observed progesterone effects on dendritogenesis and spinogenesis. Taken together the observed induction of cerebellar development induced by progesterone might implicate promising options for a clinical use of progesterone in patients with traumatic, degenerative or ischemic deficits in the cerebellum.

Unfortunately though, recent data indicate that progesterone effects attributed to the classic progesterone receptors seem to be restricted to the neonatal period of the cerebellum (Wessel et al. 2014). Thus, a recent investigation of changes in the spine density in neonatal (7 days in vitro (div)), juvenile (15 div) and matured (30 div) Purkinje cells after incubation with progesterone confirm, that progesterone indeed induces an increase in spine number of immature and young Purkinje cells. But interestingly these new data strongly indicate, that in mature Purkinje cells effects of progesterone were no longer detectable (Wessel et al. 2014). Furthermore, neither the incubation with mifepristone nor with mifepristone plus progesterone showed measurable effects on dendritic length or spine density in mature Purkinje cells (Wessel et al. 2014). Thus, progesterone seems to induce neuronal growth in immature Purkinje cells by interaction with classical progesterone receptors, while it does not affect mature Purkinje cells through this mechanism anymore. These results implicate that progesterone might be a therapeutic option for children with cerebellar disorders, while a simple application of progesterone might not be helpful in the therapy of cerebellar diseases in adults. Here the US-American PECARN-Study, which is currently in the planning phase, dealing with progesterone application to children with TBI, will probably bring new perceptions.
**PGRMC1 induces spinogenesis even in mature Purkinje cells**

Further results give evidence that progesterone effects are also mediated by another progesterone binding protein, and that progesterone effects in Purkinje cells attributed to this receptor may continue into adulthood. The expression of this different kind of progesterone receptor class was shown in the cerebellum during the last decade (Sakamoto et al. 2004, 2008). Progesterone receptor membrane component 1 (PGRMC1) is a progesterone binding protein, which is associated with the membranes of the endoplasmatic reticulum, the Golgi apparatus, as well as mitochondria (Selmin et al. 1996; Sakamoto et al. 2004; Xu et al. 2011) (Fig. 3c). Its exact mechanism of action is still unclear, but different studies previously indicated an interaction with steroidogenesis (Hughes et al. 2007; Brinton et al. 2008; Rohe et al. 2009). Indeed, current results strongly suggest that PGRMC1 directly interacts with the production of neurosteroids in Purkinje cells (Wessel et al. 2014). Further research in different neuronal tissues revealed that a raised activation of PRGMC1 induced by progesterone lead to an increase in the expression of this receptor. Thus, in the hypothalamus of progesterone receptors A and B double-knock-out mice, the PGRMC1 amount was significantly increased compared to mice without this knock-out (Krebs et al. 2000). Furthermore, progesterone induced up-regulation of PGRMC1-mRNA was observed after a spinal cord injury in the rat spinal cord (Labombarda et al. 2003). Also in the hippocampus an increase in PGRMC1 was measured, induced by cyclic progesterone exposure (Zhao et al. 2012). A similar progesterone dependent up-regulation was currently described in Purkinje cells (Wessel et al. 2014). Here, not only the PRGMC1 protein amount but also the spine density was up-regulated when classic progesterone receptors were antagonized by mifepristone for more than 72 hours (mifepristone long-time incubation, MLTI) (Wessel et al. 2014). Even though the accompanied increase of the PGRMC1-amount strongly indicates that the increase of the spine density after MLTI is mediated by endogenous progesterone via PGRMC1, it need to be mentioned that mifepristone itself was shown to have a protective effect on different neuronal cells in experimental studies (Behl et al. 1997; McCullers et al. 2002; Ghoumari et al. 2003; Rakotomamonjy et al. 2011). Thus, RU 486 significantly improves Purkinje cell survival in cerebellar roller tube cultures due to an influence on neuronal depolarizations, independent of the classical progester-
one receptors (Ghoumari et al. 2003, 2006; Rakotomamonjy et al. 2011). Nevertheless none of these studies demonstrated an influence of mifepristone on the Purkinje cell morphology and spine density, but an increase of the cell survival of primary Purkinje cells during the critical postnatal week (Ghoumari et al. 2003, 2006; Rakotomamonjy et al. 2011). In contrast, MLTI was shown to increase the spine density and area, without an influence on the Purkinje cell survival (Wessel et al. 2014). Furthermore slice cultures used in the previous study were kept from 10 day’s old pups to avoid the well-known process of apoptosis in cerebellar slice cultures during the first postnatal days. As (i) mifepristone incubation started after the critical period of cell apoptosis, (ii) the PGRMC1 amount was shown to increase under MLTI, (iii) the Purkinje cell morphology was shown to be influenced and (iii) the effects were absent when PGRMC1 was blocked, it is well justified to consider that endogenous progesterone and the PGRMC1 are critical for the increase of spine density and area induced by MLTI.

Thus, classical progesterone receptors and PGRMC1 probably compete for progesterone. If the classical progesterone receptors are (i) absent (Krebs et al. 2000), (ii) blocked by mifepristone for a longer period (Wessel et al. 2014) or (iii) the PGRMC1 expression is increased through different mechanisms (Labombarda et al. 2003; Zhao et al. 2012), progesterone binding to PGRMC1 might be enhanced. This hypothesis is confirmed by the fact that the increase in spine density following MLTI in Purkinje cells was absent when MLTI incubation was combined with a blocker of the PGRMC1 receptor (AG 205). Thus, progesterone probably mediates its effects on the development, function and morphology of Purkinje cells not only through the classic progesterone receptors, but also through PGRMC1.

With regard to different studies, PGRMC1 seems to interact with the neurosteroidogenesis (Craven et al. 2007; Hughes et al. 2007; Rohe et al. 2009). Thus, Hughes et al. demonstrated that PGRMC1 is required for the P450 activity (Hughes et al. 2007) and Min et al. reported a specific increase in the hydroxylation of progesterone induced by an overexpression of the inner zone antigen (IZA) in the adrenal cortex, which is identical to PGRMC1 (Min et al. 2004). Indeed also in the cerebellum PGRMC1 seems to interact with the neurosteroidogenesis as we could previously prove that the increase in spinogenesis mediated through PGRMC1 is significantly
decreased when the 3ß-HSD was blocked with trilostane (Wessel et al. 2014). Thus, a progesterone-induced up-regulation of PGRMC1 in Purkinje cells seems to increase the activity of the cytochrome P450scс and 3ß-HSD, which in turn raises the internal progesterone concentration leading to the observed spinogenesis (Fig. 3c). Interestingly, this effect was not just observed in immature and juvenile Purkinje cells, but also in mature Purkinje cells (Wessel et al. 2014). This is especially promising for potential therapeutic use as PGRMC1 expression has already been shown to increase after TBI (Guennoun et al. 2008), which renders PGRMC1 a highly interesting target for further clinical research.

**Progesterone induces neuronal outgrowth in dorsal root ganglia (PNS)**

Also in the PNS, progesterone might be a prospective therapeutic option, as different studies suggest that progesterone improves at least the sensory function after peripheral nerve injury. Thus, injury induced allodynia was improved after progesterone treatment (Coronel et al. 2011; Dableh and Henry 2011). Even though positive effects for example on myelination (Koenig et al. 1995) and on the survival of motoneurons (Yu 1989) were already described, further information about molecular mechanisms beyond the effects of progesterone in the PNS are still missing. A recent in vitro study dealt with the basic effects of progesterone on primary cultures from chicken dorsal root ganglia (DRG). Interestingly, a highly significant increase in the outgrowth of neuronal DRG processes was detected 24 hours after treatment with progesterone compared to untreated controls (Fig. 2a,b) (Olbrich et al. 2013). Thus, progesterone also seems to induce neuronal outgrowth in developing neurons of the PNS. As it was shown that DRG neurons express the necessary enzymes for progesterone synthesis, P450scс as well as 3ß-HSD (Guennoun et al. 1997; Do Rego et al. 2009; Schaeffer et al. 2010), there is strong evidence to consider that DRG neurons themselves are able to synthesize progesterone. Taken together, progesterone probably has a crucial function for the physiological development of DRG neurons. Like in the adult cerebellum, progesterone effects were absent in DRG when progesterone treatment was combined with mifepristone (Olbrich et al. 2013). Regarding to this results classical progesterone receptors are expressed in DRG neurons (Chan et al. 2000). Interestingly, they were expressed not only in the soma but also over the entire surface of the neuronal growth cone (Olbrich et al. 2013). Such an extraneu-
ronal expression of classical progesterone receptors were previously also described by Waters et al., who revealed the expression in neurites and synapses in the rat hippocampus (Waters et al. 2008). Beside this, expression of classical progesterone receptors in DRG neurons was proven by RT-PCR-analysis (Olbrich et al. 2013). Thus, it is most reasonable that neuronal outgrowth following progesterone incubation is mediated by genomic mechanisms of the classical progesterone receptors. As progesterone seems to be involved in the formation of the peripheral neuronal circuits and axonal navigation plays a key role in the proper formation of neuronal connections during development, an influence of progesterone on axonal growth cones can be considered. The neuronal growth cone is crucial for axon navigation and acts as a sensory motile machine (Goldberg and Burmeister 1986; Dent and Gertler 2003; Lowery and Van Vactor 2009; Dent et al. 2011). Different guidance signals lead the axon to the final target and allow the formation of proper synaptic connections (Dickson 2002; Lowery and Van Vactor 2009; Marín et al. 2010). Indeed it could be shown, that progesterone induces an increase in the size and motility of neuronal growth cones in DRG neurons (Olbrich et al. 2013) (Fig. 2c,d).

In this study progesterone increases the appearance of lamellipodia and to a lesser extent of filopodia, which results in augmented growth cone circumference and growth cone area. No effects of progesterone on growth cone size were observed when progesterone treatment was combined with mifepristone (Fig. 2e), while mifepristone administration alone led to growth cone morphology comparable to controls (Olbrich et al. 2013). In consequence, exogenous stimulation with progesterone seems to provide functional benefits for peripheral neuronal tissues like the DRG. Therefore, further studies are needed to see whether and how the observed progesterone effects can be used therapeutically to generate positive effects on impaired peripheral neuronal tissues.

**Rapid interactions of Progesterone with the actin cytoskeleton**

Both endogenous and exogenous progesterone seem to induce morphological changes in neuronal tissues. As the morphology, and especially, the motility of cells are determined by the cytoskeleton (Bentley and Toroian-Raymond 1986; Chien et al. 1993; Lafont et al. 1993; Dent and Kalil 2001), it is well justified to consider that
progesterone induces such morphological changes by the modulation of the cytoskeleton.

The cytoskeleton of Purkinje cells reveals a characteristic distribution. Microtubules and neurofilaments (EYFP-Tub und GFP-NFM) build the scaffold of the cell body and the dendritic arbor of the Purkinje cells, while actin-filaments (pLife-Act Tag RFP) especially accumulate in the periphery of dendritic arbors, where the dendritic spines are located (Fig. 1d, Fig.3a). With the aid of time-series analyses in combination with Fluorescence Recovery After Photo bleaching (FRAP) and CLSM potential changes in this typical cytoskeletal distribution could be investigated. The fluorescence in a defined region of the dendritic arbor, the so-called region of interest (ROI), was bleached and afterwards, the time of fluorescence recovery in this area was measured. In untreated controls a local fluorescence recovery was measurable within 4 minutes (Wessel et al. 2014). As no transport of the fluorescence-labeled β-actin was detectable, but the fluorescence recovered especially within the dendritic spines of the ROI, it is most reasonable that there is a local protein biosynthesis in the dendritic arbor of Purkinje cells. This morphological evidence of local protein biosynthesis in dendrites is in line with results described by Bramham and Wells in 2007 (Bramham and Wells 2007). As local synthesis of proteins is much faster than transport of proteins from soma into the dendritic tree, it probably allows Purkinje cells to react very fast to internal and external stimulations. Thus, progesterone might induce morphological changes by the increase of actin biosynthesis within dendrites.

Indeed, Okabe and Hirokawa revealed that an anterograde flux of actin monomers induces the polymerization of actin-filaments in growth cones (Okabe and Hirokawa 1991). Thus, an increase of actin monomers might lead to an enhanced dendritogenesis and spinogenesis in Purkinje cells. Nevertheless, our recent study could not detect an increase in the time of the fluorescence recovery after photo bleaching in progesterone-treated Purkinje cells (Wessel et al. 2014). A possible explanation might be that progesterone has an impact on the amount of actin monomers within the dendritic arbor without changing the rate of the protein biosynthesis. This could be explained by the fact that FRAP is suitable to detect variations in the time of fluorescence recovery, for example based on transport mechanisms, whereas FRAP is not suitable to detect changes in the intensity of protein biosynthesis. Therefore, further
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studies are needed to clarify the role of the actin cytoskeleton for the genomic mechanisms of progesterone receptors in cerebellar Purkinje cells.

In DRG neurons, neurofilaments are prominently expressed in the neurites and build up the central region in the neuronal growth cone. Actin filaments instead are detectable in the peripheral zone of the neuronal growth cone forming lamellipodia and filopodia, even though some scattered neurofilaments also penetrate the periphery (Fig. 3b) (Marsh and Letourneau 1984; Schaefer et al. 2002; Dent and Gertler, 2003). Interestingly, treatment with progesterone leads to significant morphologic alterations of the growth cone’s shape and its characteristic distribution, by inducing a clear shift of cytoskeletal components. Thus, the amount of lamellipodia and filopodia, densely packed with actin-filaments, increases after progesterone treatment in comparison to controls. The shift of the actin-neurofilament-ratio was in favor of actin-filaments, leading to a considerably enlarged peripheral zone induced by progesterone treatment (Olbrich et al. 2013). This result suggests that interaction with the actin cytoskeleton is indeed an important mechanism of progesterone to effect neuronal tissues.

Further studies postulated a correlation between size and motility of neuronal growth cones (Argiro et al. 1984). Argiro et al. supposed that a reduced growth cone size leads to a slower neurite extension, probably mediated by either a decreased protein synthesis in the cell body or a reduced axonal transport of proteins towards the leading edge (Argiro et al. 1984). Thus, a stimulant like progesterone, which enlarges the growth cone size, might also enhance the growth cone’s motility. A recent study examined this hypothesis. Growth cones were transfected via microinjection and observed for a couple of hours with the aid of CLSM. In controls, active and motile neurons with apparently normal organelle movement could be monitored. Especially actin-filaments, but also neurofilaments, showed a constant motion and turnover. Actin-filaments of the peripheral zone formed new motile cell protrusions, which constantly changed their shape to explore the environment (Olbrich et al. 2013). It is remarkable that both filopodia and lamellipodia were newly built, but lamellipodia are not as dynamic as filopodia. Additionally an actin-turnover was observed within the growth cone that was increased prior to its outspread. The actin-turnover seemed to initiate new filopodia and lamellipodia, whereas neurofilaments just followed new actin branches to consolidate such new protrusions. While controls seemed to scan their
environment without moving forward, progesterone-treated growth cones showed increased cytoskeletal motility and changes in their morphology, induced by a rapid turnover of filopodia and lamellipodia within minutes. These progesterone-treated growth cones started growing out after approximately 30 minutes of incubation. All observed effects of progesterone were completely blocked after combined incubation with progesterone and mifepristone. Thus, progesterone does not just increase the size of DRG neuronal growth cones but also seems to raise the rate of motility and the outgrowth of the growth cone by interaction with the actin cytoskeleton.

As Argiro et al. concluded, slow neurite extension might be mediated by a decreased protein synthesis in the cell body or a reduced axonal transport of proteins towards the leading edge (Argiro et al. 1984). In turn, progesterone might induce outgrowth and motility of the growth cone by (i) increase of the neuronal protein biosynthesis, (ii) induction of the axonal transport of proteins or (iii) increase of the protein biosynthesis and the axonal mRNA-transport into the dendrite and following local protein biosynthesis in the dendrite. Indeed, Gu et al. described a direct axonal transport of β-actin mRNA from the soma to the neuronal growth cone, mediated by ZPB1 (Gu et al. 2002) (Fig. 3c). A progesterone-induced increase in the β-actin transcription, in line with an enhanced mRNA transport into the growth cone, followed by local protein biosynthesis of β-actin might be an explanation for the observed changes in the actin cytoskeleton after progesterone incubation (Fig. 3c, d). Interestingly, some of the effects were detected within a few minutes after progesterone administration, which indicate that there are different coexisting progesterone mechanisms of interaction with the actin cytoskeleton, mechanisms which are not all mediated genomically. Indeed progesterone receptors have been identified to mediate such rapid activation of kinase cascades within the cytoplasm (Simoncini et al. 2003; Fu et al. 2007). Such extra genomic progesterone-induced mechanisms probably include different molecular cascades, which submit fast actin rearrangements by direct interaction with actin binding proteins (Giretti and Simoncini 2008) (Fig. 3c).

Further studies are needed to get a closer idea of the exact mechanisms involved in the interaction between progesterone and the cytoskeleton. Nevertheless, it seems very likely that different mechanisms and receptors are involved in the mediation of progesterone effects.
Thus, progesterone is a multilateral neurosteroid with varying functions in diverging regions of the central and peripheral nervous system. It acts through a couple of specific and unspecific receptors, inducing genomic and non-genomic receptor mechanisms. This degree of versatility gives rise to a broad range of potential therapeutic options for different neurological disorders.
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Figure Legends

Figure 1. (a) Three layered cortex structure of the cerebellum with Calbindin- (red) labeled Purkinje cell bodies located in the Purkinje cell layer and their dendritic arbours, which extend to the outer molecular layer of the cerebellar cortex. The inner granule layer is characterized by incoming neuronal axons (green) and huge numbers of granule cell nuclei (blue). (b) Purkinje cell bodies are line-shaped arranged within the Purkinje cell layer. (c) Dendritic spines (green, pEYFP-actin transfected) of Purkinje cells are co-localized with PSD95-labeled functional synapses (red, anti-PSD95-AK, IHC). (d) Untreated Purkinje cell (15 div) 1 day after transfection with pEYFP-actin (yellow). (e) The same Purkinje cell after progesterone treatment for 1 day. Progesterone significantly increased the dendritic length and cell area of Purkinje cells within 1 day of cultivation. Scale bars: (a+b) 100µm, (c) 5µm, (d+e) 50µm.

Figure 2. Immunostaining of NF-H in chicken dorsal root ganglia using confocal LSM after cultivation for 3 days in (a) nutrient medium lacking any stimulating factor and (b) with addition of 10nM progesterone, in latter case followed by a significantly enhanced outgrowth and crosslinking. Scale bar: 500µm.

Changes in cytoskeletal properties within the neuronal growth cone show similar variances. (c) Controls showed typical distribution of neurofilaments (anti-NF-M-FITC) and actin (phalloidin-TRITC). Incubation with progesterone (d) led to a distinct enlargement of the peripheral zone, with additional lamellipodia and filopodia. (e) Morphological analysis confirm the descriptonal impression, measuring the area and circumference starting from the base of the neuronal growth cone defined as maximum distance perpendicular to the axonal axis measured 2 µm from the beginning of the axonal enlargement forming the growth cone. Scale bar: 5µm.

Figure 3. Signaling pathways within the neuronal growth cone of DRG neurons. Neurons were shown to undergo cytoskeletal rearrangements especially within the neuronal growth cones and (a) spine due to progesterone treatment. Spines are mainly composed of actin units, while monomer G-Actin is mostly located in the periphery polymeric F- actin can be found in the centre. In contrast the cytoskeleton of growth cones (b) is classically divided into three domains based on distribution of cy-
toskeletal proteins. The central domain is mainly characterized by neurofilaments and microtubules, reaching through the arc of actomyosin, representing the transition zone, into the peripheral zone build of actin filaments. (c) Underlying mechanism of cytoskeletal reorganization subsequent progesterone incubation include genomic, long-term effects after activation of the classic progesterone receptors A and B. These progesterone receptors undergo conformational changes subsequent progesterone binding. Thereupon the receptors dissociate from chaperone proteins, dimerize, translocate into the nucleus, and interact with specific progesterone response elements (PRE) to modulate the transcription of target genes involving dentritogenesis, spinogenesis, and synaptogenesis.

Contrary PGRMC1 is mainly known to be part of the regulation of neurosteroidogenesis, the cerebellar maturation process, and neuroprotective mechanisms. It is associated to membranes of different cell organelles including the Golgi-apparatus, endoplasmatic reticulum and mitochondria. (d) For short-term effects of progesterone various interactions of sex steroids with different, non-classical signalling cascades have been described, directly activating kinase cascades within the cytoplasm or at the cell membrane. Both signalling pathways might lead to changes in the cytoskeletal composition of the neuronal growth cones following progesterone incubation.
(a) Three layered cortex structure of the cerebellum with Calbindin- (red) labeled Purkinje cell bodies located in the Purkinje cell layer and their dendritic arbours, which extend to the outer molecular layer of the cerebellar cortex. The inner granule layer is characterized by incoming neuronal axons (green) and huge numbers of granule cell nuclei (blue). (b) Purkinje cell bodies are line-shaped arranged within the Purkinje cell layer. (c) Dendritic spines (green, pEYFP-actin transfected) of Purkinje cells are co-localized with PSD95-labeled functional synapses (red, anti-PSD95-AK, IHC). (d) Untreated Purkinje cell (15 div) 1 day after transfection with pEYFP-actin (yellow). (e) The same Purkinje cell after progesterone treatment for 1 day. Progesterone significantly increased the dendritic length and cell area of Purkinje cells within 1 day of cultivation. Scale bars: (a+b) 100µm, (c) 5µm, (d+e) 50 µm
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Changes in cytoskeletal properties within the neuronal growth cone show similar variances. (c) Controls showed typical distribution of neurofilaments (anti-NF-M-FITC) and actin (phalloidin-TRITC). Incubation with progesterone (d) led to a distinct enlargement of the peripheral zone, with additional lamellipodia and filopodia. (e) Morphological analysis confirm the descriptonal impression, measuring the area and circumference starting from the base of the neuronal growth cone defined as maximum distance perpendicular to the axonal axis measured 2 µm from the beginning of the axonal enlargement forming the growth cone. Scale bar: 5 µm

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Signaling pathways within the neuronal growth cone of DRG neurons. Neurons were shown to undergo cytoskeletal rearrangements especially within the neuronal growth cones and (a) spine due to progesterone treatment. Spines are mainly composed of actin units, while monomer G-Actin is mostly located in the periphery polymeric F-actin can be found in the centre. In contrast the cytoskeleton of growth cones (b) is classically divided into three domains based on distribution of cytoskeletal proteins. The central domain is mainly characterized by neurofilaments and microtubules, reaching through the arc of actomyosin, representing the transition zone, into the peripheral zone build of actin filaments. (c) Underlying mechanism of cytoskeletal reorganization subsequent progesterone incubation include genomic, long-term effects after activation of the classic progesterone receptors A and B. These progesterone receptors undergo conformational changes subsequent progesterone binding. Thereupon the receptors dissociate from chaperone proteins, dimerize, translocate into the nucleus, and interact with specific progesterone response elements (PRE) to modulate the transcription of target genes involving dentritogenesis, spinogenesis, and synaptogenesis. Contrary PGRMC1 is mainly known to be part of the regulation of neurosteroidogenesis, the cerebellar maturation process, and neuroprotective mechanisms. It is associated to membranes of different cell organelles including the Golgi-apparatus, endoplasmatic reticulum and mitochondria. (d) For short-term effects of progesterone various interactions of sex steroids with different, non-classical signalling cascades have been described, directly activating kinase cascades within the cytoplasm or at the cell membrane. Both signalling pathways might lead to changes in the cytoskeletal composition of the neuronal growth cones following progesterone incubation.


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