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Review

Progesterone neuroprotection in traumatic CNS injury and motoneuron degeneration

Alejandro F. De Nicola [a,b,*], Florencia Labombarda [a,b], Maria Claudia Gonzalez Deniselle [a,b], Susana L. Gonzalez [a,b], Laura Garay [a,b], Maria Meyer [a], Gisella Gargiulo [a], Rachida Guennoun [c], Michael Schumacher [c]

[a] Laboratory of Neuroendocrine Biochemistry, Instituto de Biologia y Medicina Experimental-CONICET, Obligado 2490, 1428 Buenos Aires, Argentina
[b] Dept. of Human Biochemistry, Faculty of Medicine, University of Buenos Aires, Buenos Aires, Argentina
[c] UMR788 Inserm and University Paris-Sud 11, Kremlin-Bicêtre, France

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ABSTRACT

Studies on the neuroprotective and promyelinating effects of progesterone in the nervous system are of great interest due to their potential clinical connotations. In peripheral neuropathies, progesterone and reduced derivatives promote remyelination, axonal regeneration and the recovery of function. In traumatic brain injury (TBI), progesterone has the ability to reduce edema and inflammatory cytokines, prevent neuronal loss and improve functional outcomes. Clinical trials have shown that short-and long-term progesterone treatment induces a significant improvement in the level of disability among patients with brain injury. In experimental spinal cord injury (SCI), molecular markers of functional motoneurons become impaired, including brain-derived neurotrophic factor (BDNF) mRNA, Na,K-ATPase mRNA, microtubule-associated protein 2 and choline acetyltransferase (ChAT). SCI also produces motoneuron chromatolysis. Progesterone treatment restores the expression of these molecules while chromatolysis subsided. SCI also causes oligodendrocyte loss and demyelination. In this case, a short progesterone treatment enhances proliferation and differentiation of oligodendrocyte progenitors into mature myelin-producing cells, whereas prolonged treatment increases a transcription factor (Olig1) needed to repair injury-induced demyelination. Progesterone neuroprotection has also been shown in motoneuron neurodegeneration. In Wobbler mice spinal cord, progesterone reverses the impaired expression of BDNF, ChAT and Na,K-ATPase, prevents vacuolar motoneuron degeneration and the development of mitochondrial abnormalities, while functionally increases muscle strength and the survival of Wobbler mice. Multiple mechanisms contribute to these progesterone effects, and the role played by classical nuclear receptors, extra nuclear receptors, membrane receptors, and the reduced metabolites of progesterone in neuroprotection and myelin formation remain an exciting field worth of exploration.

Introduction

The array of biological effects described for steroid hormones has been modified throughout the years. The original concept of progesterone as a reproduction-restricted hormone has now been modified to incorporate previously unforeseen neuroprotective, myelinating and anti-inflammatory effects in the nervous system [16,29,30,151,152,161,162]. The isolation of progesterone from the corpus luteum [17], its high concentration in ovary and placenta and the biological activity exerted in reproductive tissues, made progesterone a prototypical reproductive hormone. In line with this concept, a majority of reports have been devoted to the responses produced by progesterone actions in genital and reproductive tissues, including the secretory transformation of the uterine mucosa, promotion of the glandular growth of the mammary gland, implantation of the fertilized ovum, maintenance of pregnancy, and control of the function of the hypothalamic–pituitary–gonadal axis, reviewed in [18]. Biological effects on the reproductive system have been supported by the preferential localization of an estrogen-inducible progesterone receptor (PR) in the uterus, placenta, breast, anterior pituitary and reproduction-related areas of the brain such as the preoptic area and nuclei of the mediobasal hypothalamus [18,91]. Further research has shown that PR derives from a single gene, although alternative gene splicing produces a family of related molecules, with two main isoforms expressed in several tissues: PR-A and PR-B. In the periphery, PR-B is a more potent transactivator than PR-A, which in turn represses

* Corresponding author. Address: Laboratory of Neuroendocrine Biochemistry, Instituto de Biología y Medicina Experimental-CONICET, Obligado 2490, 1428 Buenos Aires, Argentina. Fax: +54 11 4786 2564.
E-mail address: denicola@dna.uba.ar (A.F. De Nicola).

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PR-B in many systems [106,110]. Both PR-A and PR-B are expressed in the central nervous system [16,82,114,182].

As additional functions of progesterone came into light, the regulation, localization and function of PR in the nervous system also underwent modifications. Originally, brain PR has been considered the mediator of hormonal effects on sex behavior, on the synthesis and release of the hypothalamic gonadotrophin-releasing hormone and pituitary gonadotrophins [91]. In 1980, MacLusky and McEwen [112] localized progesterone-binding sites in areas of the brain akin to reproduction as well as in others unrelated to this function, such as the cerebral cortex, cerebellum and midbrain. Similarly, the PR is found in the spinal cord and in brainstem nuclei involved in pain and autonomic modulation [90,98,100]. Recently, immunoelectron microscopy has shown PR in the synaptic compartment of the CA1, CA3 areas and the dentate gyrus of the hippocampus [173], which may be linked to control of neuronal excitability and synaptic plasticity. In certain extrahypothalamic areas, PR are expressed by neurons and glial cells of the astrocyte, oligodendrocyte and Schwann cell lineage, which are likely involved in neuronal protection, regulation of astrocyte reactivity, cell proliferation, myelination, response to injury and immunomodulation, i.e. functions not linked to known reproductive effects [22,49,87,89,98,107,173].

Similarly to the receptor story, the relationship between progesterone and nervous system function underwent substantial changes throughout the years. In a fundamental discovery, Selye [153] was the first to show that progesterone could induce anesthesia in rats within minutes after its administration, pointing out to membrane effects of the hormone. In 1981, Baulieu and coworkers have demonstrated that progesterone (as well as other steroids) are synthesized *de novo* from cholesterol in the central nervous system (CNS), for which he coined the term "neurosteroids" [5]. Clearly, the physiology of neurosteroids is different from the functions of the endocrine progesterone, a fact supported by variations of the neurosteroid content following brain aging, Alzheimer's disease and spinal cord trauma [37,102,151]. The molecular basis for non-classical effects of progesterone has been provided several years ago by Majewska et al. [113], who described the binding of the progesterone-reduced derivative 3α,5α-tetrahydroprogesterone (allopregnanolone,THP) to the GABA-A receptor. This molecular interaction produced barbiturate-like effects, explaining the anxiolitic, hypnotic and anesthetic effects caused by progesterone administration. Modulation by endogenous progesterone and THP of GABA-A receptors could be relevant to mood and behavioral changes observed in humans suffering from the premenstrual syndrome, the premenstrual dysphoric syndrome and several psychiatric disorders including depression and schizophrenia [70,125,142].

Further reports uncover additional non-reproductive effects of progesterone in the CNS. For instance, progesterone exerts a negative regulation of astrogliosis after lesions of the hippocampus and cerebral cortex, increases neuronal survival under pathological conditions, supports a more efficient respiratory activity and attenuates oxidative damage of mitochondria. Progesterone also prevents neuronal loss, edema formation and functional abnormalities after brain contusion injury, and it shows a promyelinating effect on the lesioned, degenerating and aged peripheral nervous system (PNS) and CNS [48,83,104,135]. Additionally, it exerts anti-inflammatory effects in brain trauma and in rodent models of multiple sclerosis [47,162]. These investigations have stimulated further research to uncover the effectiveness of progesterone for traumatic, neurodegenerative and autoimmune diseases.

Neuroprotective effects of progesterone effects in the PNS

As first shown in the sciatic nerve of the PNS, progesterone promotes the formation of new myelin sheaths by regenerating nerves of male mice following a cryolesion [97], whereas the parent hormone and its derivatives dihydroprogesterone (DHP) and THP reduce the abnormalities of myelinated fibers of aging animals [4,121]. In Schwann cells, the myelinating glia of the PNS, progesterone activates genes encoding the myelin proteins Po and PMP22, induces the expression of Krox 20, a transcription factor related to myelinogenesis, and enhances the initiation and rate of myelin formation [19,34,67]. Recent work has provided substantial evidences that progesterone and DHP counteracts several abnormalities following nerve crush injury and in diabetes-induced peripheral nerve degeneration. After lesion of the sciatic nerve, a considerable reduction of neurosteroid levels appeared, but treatment with progesterone and DHP exert neuroprotection at the molecular, morphological and functional levels [144]. In diabetic rats, progesterone, DHP and THP normalize the deficient expression of Po and PMP22, increase conduction velocity and sodium pump activity and promote skin innervation [107]. Therefore, progesterone and derivatives seem to promote remyelination, stimulate axonal regeneration and the recovery of function in peripheral neuropathies.

Neuroprotective effects of progesterone after traumatic brain injury

The pathophysiology of traumatic brain injury (TBI) is a multifactorial process involving both primary and secondary insults. Pathophysiological changes include increased extracellular glutamate concentrations, increased intracellular Ca^{2+}, free radical overproduction, alteration in the blood–brain barrier, and exacerbated inflammatory response. Edema formation and glial swelling is also a common early consequence of TBI [14,15,64]. The ideal drugs should be able to block the multiple cellular events that lead to brain damage after TBI. Different experimental studies mostly conducted by Stein's group have shown that progesterone is an attractive potential neuroprotective candidate after TBI [160–162]. A systematic meta-analysis of experimental studies has confirmed that progesterone treatment is efficient in reducing the lesion volume following TBI [52] Recognition of the neuroprotective potential of progesterone has recently led to the completion of two clinical trials [175–176].

Experimental studies

The neuroprotective effects of progesterone have been demonstrated after experimental TBI. A much studied model of TBI uses a bilateral contusion lesion of the rat medial prefrontal cortex [160]. It results in edema, secondary excitotoxic neuronal death in the vicinity of the lesion, and subsequent retrograde neuronal degeneration in both the mediodorsal thalamic nucleus (MDN) and the nucleus basalis magnocellularis (NBM) [76].

Gender influences

Evaluation of estrus cycle stage and gender on behavioral outcome after experimental TBI, suggests that the presence of endogenous circulating hormones, may confer early neuroprotection in females [171]. Normally cycling females exhibited significantly less edema than males following TBI and pseudopregnant females, the group with the highest levels of progesterone, developed almost no post-injury cerebral edema [146,147]. Subsequent studies using ovariectomized female rats, indicated that the reduction of cerebral edema was associated primarily with the presence of circulating progesterone [146]. In brain, steroids arise from the peripheral endocrine glands and local synthesis. Since in TBI, the endogenous circulating hormones at the time of injury are impor-

tant for neuroprotection, we have investigated the effect of pseudopregnancy and TBI on steroid levels in plasma and in brain after prefrontal cortex injury. Results have shown different steroid profiles in brain and plasma of male and pseudopregnant female rats and specific profile changes after TBI. In sham-operated pseudopregnant females, much higher levels of progesterone, 5α-dihydroprogesterone (DHP), THP, and 3β,5α-tetrahydroprogesterone have been measured in both brain and plasma, compared with sham-operated males. Six hours after TBI, the levels of pregnenolone, progesterone, and DHP are increased, and those of testosterone decreased in male brain, whereas levels of DHP and 3β,5α-tetrahydroprogesterone are increased in brain of pseudopregnant female rats. Plasma levels of DHP have not changed after TBI, suggesting a local activation of the 5α-reduction pathway of progesterone in both male and pseudopregnant female brain. The significant increase in neurosteroid levels in the male brain after TBI is consistent with their role in neuroprotection [120].

Pharmacological treatment with progesterone

It is known that progesterone regulates different cellular events after TBI. Experimental evidence has shown that post-injury treatment with progesterone decreases brain edema [148], attenuates free radical damage, protects against lipid peroxidation and reduces neuronal loss and cognitive deficits after TBI [35,36,147,149]. Progesterone also reduces the inflammatory response [76,136,137]. Reduction of cerebral edema by progesterone may be due to the stabilization of the blood–brain barrier and to the modulation of the expression of aquaporin 4 [72]. Progesterone reduces inflammation through induction of CD55, a potent inhibitor of the complement convertases which are activators of the inflammatory cascade and by reducing inflammatory immune cytokines [137]. Progesterone has also been shown to decrease the expression of NF kappa B implicated in both inflammation and apoptosis after TBI [26–28,136].

Dose, duration, window of effectiveness

A progesterone's dose-response study has demonstrated that low (8 mg) and moderate (16 mg) doses of progesterone are optimal for improving recovery, while high dose (32 mg) are disruptive to some animals [63]. Progesterone is effective in reducing edema and in protecting neurons after TBI if treatment is delayed as much as 24 h after injury [148]. Pretreating ovariectomized female rats with low physiological concentrations of progesterone reduce hippocampal neuron loss in response to TBI [143]. With respect to the duration of the progesterone treatment and its mode of administration, available experimental data show that both prolonged and continuous administration of the hormone leads to more complete behavioral recovery after TBI [155]. The benefits of continuous progesterone release via subcutaneous silastic capsule implants have been compared to daily subcutaneous injections. Both slow-release and injected progesterone treatments enhance acute recovery after TBI. However, a continuous mode of administration is more beneficial than bolus injection [28]. Tapered progesterone withdrawal promotes long-term recovery following TBI comparatively to acute progesterone withdrawal which causes an increase in anxiety behavior [26–28].

Progesterone is also effective in other models of TBI. These important findings have been now extended to other models of brain injury. In a model of diffuse TBI, post-injury administration of progesterone reduces blood–brain barrier permeability and edema formation and improved the motor and cognitive performance [132,133]. In lateral fluid percussion brain injury of moderate severity, progesterone increases the expression of the anti-apoptotic gene Bcl2 and down-regulates the expression of the pro-apoptotic genes Bax and Bad in the cerebral cortex [179].

Progesterone metabolites and synthetic progestins have also been tested in TBI. For example, THP decreases cell death and cognitive deficits after TBI [34,35]. However, it has been shown recently that progesterone and THP differentially regulate hemostatic proteins after TBI. Progesterone maintains procoagulant whereas THP increases anticoagulant protein expression [169]. These results suggest that in TBI, where blood loss may exacerbate injury, it may be preferable to treat patients with progesterone. The enantiomer of progesterone acts as a neuroprotectant after TBI. Indeed, this compound decreases cerebral edema, cell death mediators, inflammatory cytokines and reactive gliosis, and increases antioxidant activity [168]. Medroxyprogesterone acetate (MPA) is widely used in clinical practice. In some conditions MPA can exhibit pharmacological actions that are different from those of natural progesterone. In a study to assess the efficacy of MPA, it has been shown that MPA produce a dose-related reduction of cerebral edema at 48 h post-TBI but it does not enhance behavioral recovery [174].

Human studies

Human studies have also provided interesting data that support progesterone neuroprotection [167]. Predicted outcome at the time of discharge from an in-patient rehabilitation program was evaluated according to work capacity in 72 females and 262 males. Female TBI patients have a better predicted outcome [66]. A more recent study has shown that lipid peroxidation after severe TBI is more prominent in males than females [7]. This observation shows that gender is an important consideration in clinical trial design.

Two clinical trials have assessed the protective effect of progesterone after TBI. In the first phase II trial named ProTECT, patients have received state-of-the-art emergency treatment within 11 h of TBI plus 3 days of intravenous progesterone or vehicle. At 30 days post-injury, progesterone have reduced mortality in severely injured patients and has improved the functional outcome in patients with moderate TBI [175]. The aim of the second clinical study has been to analyze the longer term efficacy of progesterone on neurologic outcome in TBI patients. Within 8 h of injury, they have received intramuscular injections of progesterone or placebo for five consecutive days. Patients in the progesterone group have shown improved neurological scores, and a significantly lower mortality rate at 6-month follow-up [176]. The same group has shown in patients treated with progesterone a decreased level of 15-F(2t)-isoprostane, and TNFα, indicating that successive early treatment with progesterone will benefit the patients with acute head injury by improving the recovery and reducing the disability, which may be related to its alleviating inflammatory and lipid peroxidation response [176]. The results of the two trials have supported progesterone to be safe and potentially efficacious in the treatment of TBI. Larger phase III trails will be necessary to verify results prior to clinical implementation. A summary of progesterone effects after TBI is shown in Table 1.

Progesterone neuroprotection after spinal cord injury

Due to the fact that spinal cord injury often results in complete loss of motor and sensory function, a search for novel pharmacological therapies is constantly under way [139,163]. After spinal cord injury, ventral horn motoneurons show early degeneration and chromatolysis, with death occurring by necrosis or apoptosis depending on the severity and/or type of the lesion [8,39,117]. Several strategies have been developed to preserve neuronal function and repair damage, including transplant of peripheral nerves,

Table 1
Factors regulated by progesterone after traumatic brain injury.

Factors	Function	Progesterone effect	Refs
AQP4	Water channel involved in edema formation and resorption	↗ Near the lesion site ↘ Far from the lesion site	[72]
NFκB	Regulation of transcription of apoptotic and inflammatory factors	↘	[27,137]
IκB	Inhibition of nuclear transport of NFκB	↗	[27]
IL1β	Pro-inflammatory cytokine	↘	[76]
TNFα	Inflammation factor that initiates different signaling pathways leading to apoptosis, astrogliosis and demyelination	↘	[27,76,136]
cFos	Transcription factor involved in apoptosis and inflammation	↘	[27,137]
C 3	Complement involved in inflammation	↘	[27,137]
Thrombin, Fibrinogen, Coagulant factor XIII	Procoagulant factors	↗	[169]
Caspase 3	Protease involved in apoptosis	↘	[27,36]
Bax, Bad	Pro-apoptotic gene	↘	[179]
Bcl2	Anti-apoptotic gene	↗	[179]
		↘	[36]

olfactory ensheating cells, stem cells or Schwann cells and enhancement of axonal growth using fibronectin conduits [82]. Pharmacological approaches have also been employed, such as delivery of neurotrophic factors, antioxidant compounds, antiglutamatergic drugs and steroids [29,73,74,139].

Steroid hormones offer promising therapeutic perspectives during the acute phase of spinal cord injury. Glucocorticoids, in this respect, have been the standard treatment for patients with acute spinal cord injury [73,74]. Early reports have shown that progesterone preserves neurons after section of the hypoglossal and facial motor nuclei [86], whereas in the spinal cord, treatment of rats with progesterone increases motoneuron survival after axotomy or injury, protects cultured neurons against glutamate toxicity and normalizes defective functional parameters of injured neurons [99,135,165]. In spite of these evidences, there is no general consensus that progesterone confers protection to the injured spinal cord [43].

Molecular parameters regulated by progesterone in spinal cord–injured rats

Our laboratory has shown in rats with spinal cord injury due to complete transection, that progesterone brings a strong neuroprotection, measured by the response of different neuronal parameters, including the sodium pump, the cholinergic marker choline acetyltransferase (ChAT), the growth-associated protein GAP-43, the myelin basic protein (MBP) and brain-derived neurotrophic factor (BDNF). Injury-induced deafferentiation reduces the number of ChAT immunopositive motoneurons and the expression of mRNAs of the alpha3 and β1 subunits of the Na,K-ATPase, while moderately up-regulates the mRNA of the growth-associated protein GAP-43 in ventral horn motoneurons [31,99]. In injured animals, *in vivo* progesterone treatment for 72 h restores to normal the reduced levels of the sodium pump mRNA and ChAT, whereas levels of GAP-43 mRNA are further enhanced. These are important effects, because ChAT catalyzes acetylcholine synthesis, the release of which at the neuromuscular junction starts muscle contraction. In turn, the Na,K-ATPase maintains the membrane potential, neuronal excitability and entry of metabolites and ions into the soma, whereas GAP-43, due to its location at the growth cone, is involved in axonal regeneration. Therefore, the responses of these markers to progesterone in injured rats are interpreted as protective and regenerative for the damaged tissue.

Additional data regarding progesterone neuroprotection has been obtained from studies involving BDNF. Interest in BDNF derived from the fact that the neurotrophic factor mimics some of the progesterone effects on the spinal cord [60]. For example, application of BDNF prevents the axotomy-induced decrease of ChAT in motoneurons, stimulates sprouting of cholinergic fibers and increases the expression of the regeneration-associated gene GAP-43 after spinal cord injury [1,95,178]. Additionally, BDNF administration promotes the recovery of MBP after compression-induced spinal cord injury [81]. Neurotrophic factors and their receptors are present not only in the developing but also in adult spinal cord neurons, indicating they may play an important role for neuronal survival and axonal regeneration [164].

To study progesterone effects on BDNF, we have treated rats with complete spinal cord transection at thoracic level T10 with four injections of 4 mg/kg progesterone at times 1 h (ip), and again at 24, 48 and 72 h (s.c.) post-lesion. An identical protocol has been used to study progesterone effects on the above-mentioned neuronal markers [99]. *In situ* hybridization techniques using a ^{35}S-oligonucleotide probe coding for bp 562–609 of rat BDNF and immunocytochemistry to localize BDNF protein, has shown strong expression of BDNF mRNA and protein in large ventral horn neurons (>500 μm^2) of Rexed Lamina IX in sham-operated rats, but a marked depletion of both BDNF mRNA and protein by 3 days following spinal cord injury [60]. This period of time coincided with intense chromatolytic changes (see below) and, as shown before, with depletion of ChAT and the α3 subunit mRNA of Na,K-ATPase [99]. Thus, failure to sustain BDNF expression may cause impairment of cell function, induce neuronal degeneration and inhibit axonal regeneration, as previously suggested by Nakamura and Bregman [128]. In spinal cord-injured rats, progesterone treatment enhances by 200% BDNF mRNA and substantially increases neuronal BDNF protein expression and immunopositive fiber density compared to untreated animals. Again, this time period of progesterone effects coincided with repletion of ChAT, increased levels of mRNA for the Na,K-ATPase and GAP-43 and with preservation of Nissl bodies, suggesting inhibition of chromatolysis (see below).

The finding that progesterone enhances the BDNF-immunopositive fiber network has raised the possibility that the steroid may be also modulating BDNF availability to the injured spinal cord from peripheral sources, in addition to the enhancement of BDNF mRNA and protein expression in motoneurons.

Morphological aspects of progesterone neuroprotection

We have recently complemented the neurochemical studies using a morphological and ultrastructural approach. Employing the same injury model and progesterone treatment protocol, we have observed that progesterone partly normalizes the ultrastructural abnormalities and preserve the microtubule-associated protein 2 (MAP2) immunostaining of deafferented motoneurons. Electron microscopy observations have further shown that after complete spinal transection, changes developed in the nucleus, karyoplasm and nucleolus. The nucleus of lesioned motoneurons displays reduced electron density, scattered chromatin and adopts an eccentric position, accompanied by chromatolytic changes in the cytoplasm, such as fragmented RER, dissolution of Nissl bodies and vacuolation [62]. These abnormalities clearly differentiate the chromatolytic process – a reversible process – from apoptosis. Typical apoptosis is characterized by plasma and nuclear membrane blebbing, presence of apoptotic bodies and pyknotic nuclei, ultimately leading to cell death. Instead, injured motoneuron morphology fits better with a form of cell damage known as paraptosis [92,105,158]. Ultrastructural changes also occurred in the nucleolus, the site of synthesis of the ribosomes, which are the basophilic component of Nissl substance. Electron microscopy observations in the injured rats have shown that the nucleolus presented small light areas, possibly related to its high synthetic activity [138]. In this respect, the centrifugal disappearance of Nissl bodies seems to stimulate the nucleolus to elaborate extra material for replacement of the depleted ribonucleoproteins. Interestingly, in progesterone-treated rats, nuclear morphology is intermediate between the normal pattern and the pattern found in injured animals, suggesting a partial recovery. Second, the nucleolus of both hormone naïve and hormone-treated animals has shown morphological changes in the fibrilar portion, which in the case of progesterone-receiving rats accompanies cytoplasmic replenishment of Nissl bodies. This finding suggests a more efficient synthesis of ribonucleoproteins in hormone-repleted animals. Third, progesterone also modifies the nuclear eccentricity showed by injured motoneurons. In this case, a large percentage of the motoneurons present a centrally located nucleus resembling normal motoneurons, in contrast to the injured group in which at least half of the motoneurons shows nuclear eccentricity and translocation of Nissl bodies from the karioplasm to the cell membrane (Fig. 1). A suggested mechanism for the development of nuclear eccentricity includes the interference with axonal transport and accumulation of axonal components in the injured cell body [111].

Several studies have emphasized that axonal lesions deprive cells of neurotrophic factors, trigger the chromatolytic reaction and lead to an eccentric localization of the nucleus [54]. In addition to trans-neuronal signals [39,126] depletion of endogenous neurotrophic factors after injury, in particular BDNF may lay behind the structural abnormalities of motoneurons leading to cell dysfunction and neurodegeneration. The involvement of neurotrophic factors is of outmost importance to understand the present findings. As mentioned above, it is relevant that progesterone enhances BDNF expression after injury [61]. The same effect occurs in neurodegeneration of the spinal cord and in cortical explants in culture [59,92].

As previously demonstrated by other authors [159,180,183] spinal cord injury also brought changes of MAP2 staining. Loss of MAP2 immunoreactivity also occurs in a variety of pathological conditions exposed to excess calcium influx and calpain activation, NMDA-activation, oxidative stress and dephosphorylation [41,93,180]. We have observed that progesterone treatment of rats with spinal injury up-regulates MAP2 staining in dendrites and perikaryon, suggesting effects on the cytoskeleton [38]. Besides spinal injury, progesterone also enhances MAP2 protein expression in other circumstances [141]. Of further interest is the notion that MAP2 immunoreactivity is associated with polyribosomes and that

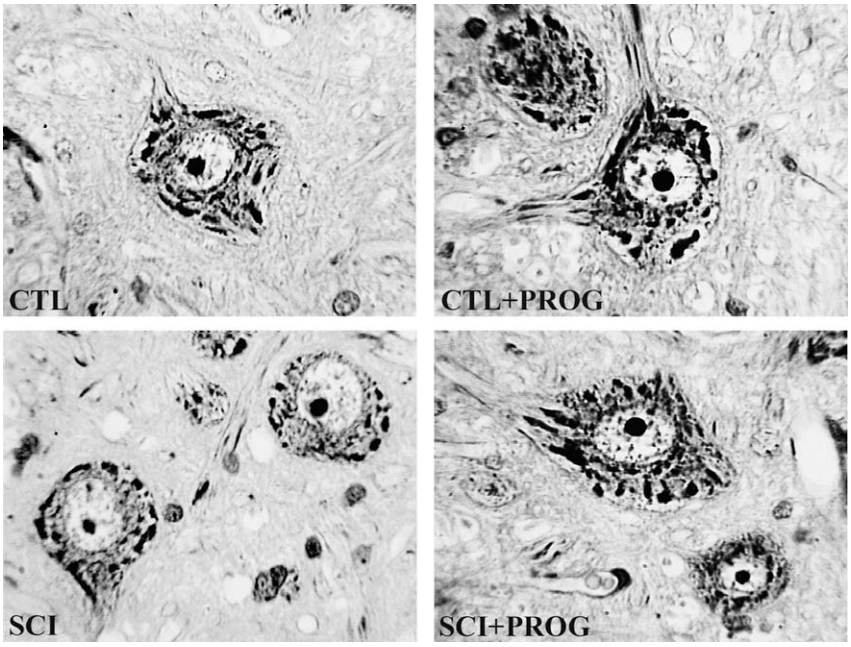

Fig. 1. Motoneuron morphology in the spinal cord of a control rat (CTL), a control receiving progesterone (CTL + PROG), rat with spinal cord injury (SCI) and spinal cord injury receiving progesterone (SCI + PROG). In comparison to the normal phenotype of motoneurons of the CTL and CTL + PROG groups, chromatolysis developes in motoneurons of spinal cord-injured rats (SCI), characterized by nuclear eccentricity and translocation of the Nissl bodies to the cell membrane. The chromatolytic pattern is reversed in injured rats receiving progesterone during 3 days (SCI + PROG), which resemble the normal motoneuron phenotype. Cresyl-violet staining. Magnification: 1000×.

MAP2 may play a role in RER membrane positioning [41]. Our study has shown that the injury-induced loss of MAP2 and the disruption of RER membranes are modulated by steroid administration. Furthermore, cytoskeletal proteins are downstream targets of neurotrophins, which recover the cytoskeleton and protect its proteins from proteolytic degradation. BDNF positively modulates the expression and phosphorylation state of MAP2 [45,115], suggesting that BDNF may be one of the factors mediating progesterone effects on the cytoskeleton and on attenuation of the chromatolytic reaction.

In conclusion, ultrastructural observations proceeds in parallel with neurochemical evidences of survival of damaged motoneurons, favoring the view that progesterone supports spinal cord function by a direct or indirect action on motoneurons. The already mentioned similarities in the regulation of molecular parameters and some cellular events attributed to progesterone and shown for BNDF, suggests that BDNF and progesterone actions may share common intracellular pathways, and sustain that BDNF may be one of the intermediates in progesterone action. Progesterone-induced BDNF may act in a paracrine or autocrine fashion to positively regulate the function of neurons and perhaps other cell types, such as oligodendrocytes. This last aspect will be discussed in the next subheading.

Promyelinating effects of progesterone after spinal cord injury

Oligodendrocyte lineage and the response to injury

Oligodendrocytes, the myelinating cells of the CNS, are extremely labile to the effects of injury. Injury provokes oligodendrocyte death by apoptosis with secondary axonal demyelination [65,108,145]. A number of factors contribute to injury-induced oligodendrocyte death, including glutamatergic toxicity with increased oxidative stress, release of pro-inflammatory cytokines and increased extracellular ATP levels, as reviewed by McTigue and Tripathi [118]. Mature oligodendrocytes are the source of myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) [6]. Spinal cord contusion or transection induces loss of these myelin proteins [65,154,182]. However, mature oligodendrocytes cannot repair myelin after injury-induced demyelination, and remyelination derives from recruitment of an endogenous population of oligodendrocyte precursor cells (OPC) that express the NG2 surface marker [78,79,115,118,182]. The oligodendrocyte lineage traverses several steps, from OPC expressing the proteoglycan NG2, to mature oligodendrocytes that express the MBP, PLP and MOG genes and their product proteins. Regulation of the oligodendrocyte pathway is multifactorial, and the transcription factors Olig1, Olig2 and Nkx2.2, among others, play specific roles during OPC differentiation and remyelination [40,109,111,140]. Therefore, analysis of agents influencing remyelination, requires the study of their effects on OPC proliferation, expression of myelin genes and transcription factors that control oligodendrogenesis [129].

Progesterone effects on OPC and myelin transcription factors

In this section, we discuss results pertaining to the promyelinating effects of progesterone after spinal cord injury, whereas the myelin-producing effects of progesterone on the peripheral neuropathies have been mentioned previously in this review paper [34,67,97,121]. Existing proofs for the myelinating effects of progesterone in the CNS include the increased expression of MBP and the mature oligodendrocyte marker cyclic nucleotide 3′-phosphodieterase (CNPase) in cultures of mixed glial cells and organotypic slice cultures of cerebellum [50,88,89], remyelination after toxin-induced lesions of the cerebellar peduncle of aging rats [80] and stimulation by endogenous progesterone and their reduced derivatives of the proliferation and differentiation of OPC *in vitro* [46,51].

Employing the spinal cord injury model used to study neuroprotection [100,101] we have shown that OPC bearing the NG2 marker, albeit absent in controls, appeared moderately in the gray and white matter of the spinal cord after injury. In these conditions, a reduction of the mRNA and protein expression of MBP indicates the presence of injury-induced demyelination. Progesterone treatment enhances the expression of MBP protein and mRNA in the corticospinal tract and dorsal ascending tract and highly stimulated $NG2^+$ cell density over untreated spinal cord-lesioned rats. In a second study, we have used two different times of progesterone treatment (3 and 21 days) and increased the progesterone dose to 16 mg/kg per day. In both the 3 and 21 day progesterone-treated groups, we have determined the number of OPC and the number of

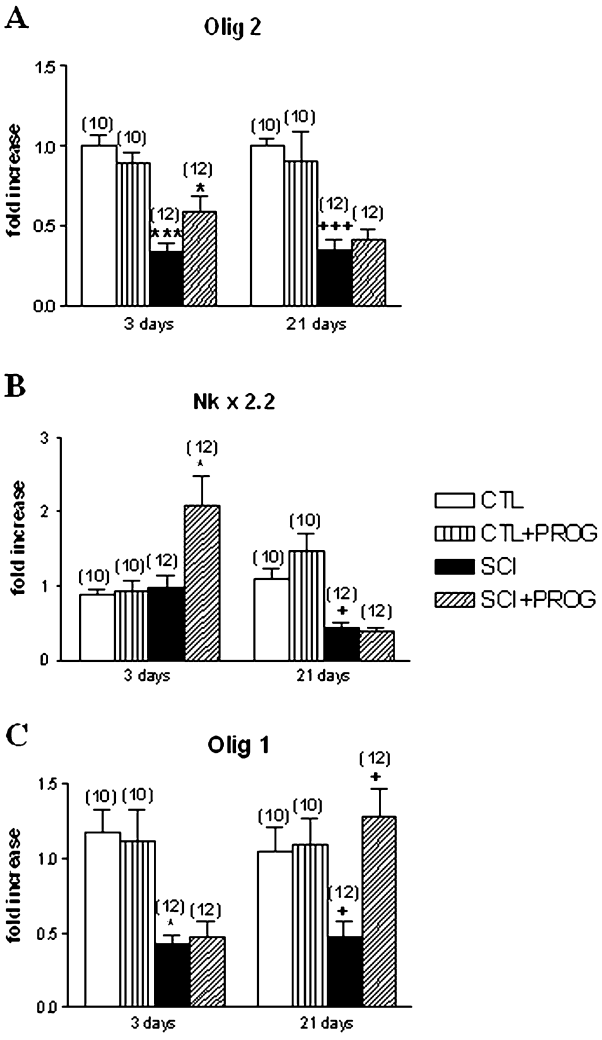

Fig. 2. Progesterone effects on myelin transcription factors. mRNAs for the oligodendrocyte transcription factors Olig2, Nkx2.2 and Olig1, determined by real-time PCR show pronounced changes after progesterone treatment during 3 or 21 days to spinal injured rats.. Spinal cord injury alone induces a down regulation of mRNA for Olig2 ($^{***}p < 0.001$ vs CTL 3 days and $^{+++}p < 0.001$ vs CTL 21 days), for Nkx2.2 ($^{*}p < 0.05$ vs CTL 21 days) and for Olig1 ($^{*}p < 0.05$ vs CTL 3 days and $^{+}p < 0.05$ vs CTL 21 days). Progesterone treatment for 3 days increases Olig 2 ($^{*}p < 0.05$ vs SCI 3 days) and Nkx 2.2 mRNAs ($^{*}p < 0.05$ vs SCI 3 days), whereas 21 days of progesterone treatment up-regulates Olig1 mRNA levels ($^{*}p < 0.05$ vs SCI 21 days) without effect on Olig2 or Nkx2.2 mRNAs. Abbreviations as specified in the legend to Fig. 1. The number of rats used is indicated between parentheses (Reproduced with permission from Ref. [104]).

mature oligodendrocytes, the myelin proteins MBP, PLP and MOG at the mRNA and protein levels, and the expression of transcription factors that are involved in differentiation of the oligodendrocyte lineage (Olig2 and Nkx2.2) and in remyelination (Olig1).

In agreement with literature reports and our own data [78,79,101,130] we have observed that spinal lesion stimulates the recruitment of OPC in dorsal, lateral and ventral tracts of the white matter at both 3 and 21 days periods post-injury. However, this recruitment does not preclude demyelination, because immunostaining for the myelin proteins MBP and PLP, their mRNAs as well as the number of mature oligodendrocytes (i.e. CC1 + cells) suffer a pronounced depletion at 3 and 21 days. A depletion of transcription factors involved in myelination and oligodendrocyte maturation accompanies the myelination failure. According to Franklin and Kotter [44], impaired remyelination is caused by defective differentiation and maturation of OPC, rather than a defective recruitment of OPC. Interestingly, we have observed that progesterone treatment changes this scenario into one leading to remyelination. Thus, after 3 days of progesterone treatment, recruitment of OPC showing a reactive morphology is further increased, as well as the levels of MBP mRNA and protein and of the mRNA for the transcription factors Olig2 and Nkx2.2 (Fig. 2). Nkx2.2 has consensus binding sites on the PLP and MBP promoters, allowing for a direct control of the synthesis of the major myelin proteins. Progesterone stimulation of Olig2 and Nkx.2.2 in OPC should induce their differentiation into mature forms [109,172], and at the same time, acting on surviving oligodendrocytes, progesterone may enhance synthesis of MBP mRNA and protein (Fig. 3).

Additionally, the changing pattern of oligodendrogenesis obtained after 21 days of progesterone treatment again suggests a myelination drive. At this time period, the early rise of OPC promoted by early progesterone treatment has subsided, with a concomitant increase of mature oligodendrocytes, suggesting differentiation of the OPC. In order to ascertain this possibility, we have studied the appearance of the proliferation marker bromodeoxyouridine (BrdU) into maturing cells. Our experiment has shown that an increased number of oligodendrocytes bearing the CC1 marker also become BrdU positive, indicating that a progesterone early influence on OPC ends into their differentiation and maturation. These changes are accompanied by increased expression of PLP mRNA and protein and to increased mRNA of the transcription factor Olig1 (Fig. 2). Olig1 is required for oligodendrogenesis and to repair demyelinated lesions during demyelination caused by cuprizone or lysolecithin application [2], and the same reasoning may be applied to our data in spinal cord-injured rats. In conclusion, these experiments strongly support the hypothesis that progesterone imposes a myelination drive favouring differentiation of proliferating progenitors into a mature, myelinating oligodendrocyte phenotype. Thus, current and past experimental evidences underline that progesterone behaves as a promyelinating factor for the injured tissue.

Progesterone protection in spinal cord neurodegeneration

The Wobbler mouse mutant

Progesterone neuroprotection is well documented for the injured brain, spinal cord and peripheral nerves, but experimental evidences indicate that it may exert a similar role in degenerative diseases. To investigate the role of progesterone in neurodegeneration, we have employed the Wobbler mouse, a murine model that suffers a mutation in the gene coding for VPS54 (vacuolar vesicular protein sorting) present in chromosome 11. Wobblers are useful models of neurodegenerative diseases in humans, namely amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy [11,12,55,123]. Recent data by Dennis and Citron [32] greatly support the value of Wobblers for ALS research. These authors have demonstrated that Wobbler motoneurons suffer a relocation of TDP-43 (nuclear transactive DNA-binding protein) from the nuclear to the cytoplasmic compartment and changes of ubiquitination commonly found in motoneurons from patients with sporadic forms of ALS. Interestingly, these abnormalities are absent in the SOD1 transgenic mice, a model for the familial form of the disease (FALS) [32]. Epidemiological studies have shown that sporadic ALS

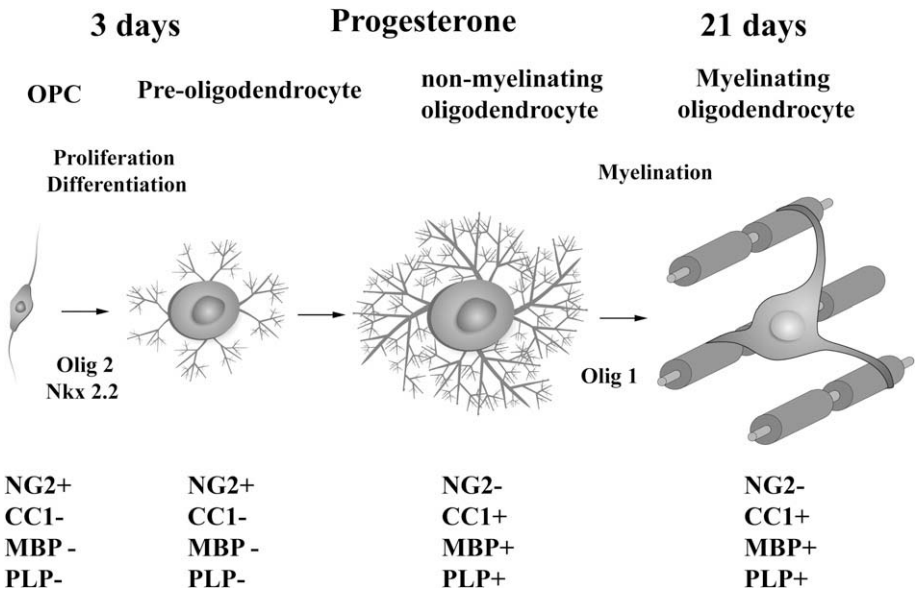

Fig. 3. A summary of progesterone effects on the oligodendrocyte cell lineage following spinal cord injury. Oligodendrocyte precursor cells (OPC) and pre-oligodendrocytes are immunopositive for NG2 but show negative staining for markers of mature oligodendrocytes (CC1) and for myelin proteins (MBP, PLP). Non-myelinating and myelinating oligondendrocytes are NG2 negative but become immunopositive for CC1, MBP and PLP. We hypothesized that 3 days of progesterone treatment enhances the density of OPC and induces their differentiation by increasing the expression of the transcription factors Olig2 and Nkx2.2. Twenty one days after injury, progesterone favors remyelination by increasing Olig1 (involved in repair of demyelinated lesions) and enhancement of oligodendrocyte maturation. These effects on the oligodendrocyte lineage run in parallel with progesterone effects on myelin proteins, and may constitute fundamental steps for repairing damage induced by traumatic spinal cord injury.

accounts for about 85–90% of all affected patients and 10–15% are inherited as an autosomal dominant trait. About 20% of FALS cases bear the SOD1 mutation [25].

Morphologically, anterior horn motoneurons of Wobbler mice undergo a dramatic perikaryal vacuolar degeneration that are accompanied by astrocytosis and microglial activation [12,55,123]. There are no signs of apoptosis, and neuropathology resembles the type II or cytoplasmic form of cell death [23,105]. Participation of oxidative stress in this mechanism is supported by abnormalities of mitochondrial function in Wobbler mice [150] and by the clinical, biochemical and morphological improvement caused by treatment with antioxidants, antiglutamatergic drugs, steroids and nitric oxide inhibitors [55,77,81]. BDNF also shows a protective effect in Wobbler mice, which could be mediated in part by the reduction of reactive oxygen species [166].

In addition to vacuolation, motoneuron abnormalities of Wobbler mice include increased activity of neuronal nitric oxide synthase (NOS), decreased levels of the mRNAs for the Na,K-ATpase and BNDF, decreased immunoreactivity and activity for ChAT and deficits of retrograde axonal transport [24,55–57,59,177]. Morphologically, Wobbler mitochondria shows massive vacuolation disrupting the outer and inner mitochondrial membranes and cristae, coupled to decreased oxygen consumption and respiratory complex I activity [55,56,150]. Increases of NOS activity could generate toxic levels of NO, which becomes a noxious factor for mitochondrial respiratory chain complexes [150]. Survival of Wobbler mice is also compromised, and afflicted animals show muscle atrophy and reduced muscle strength [181].

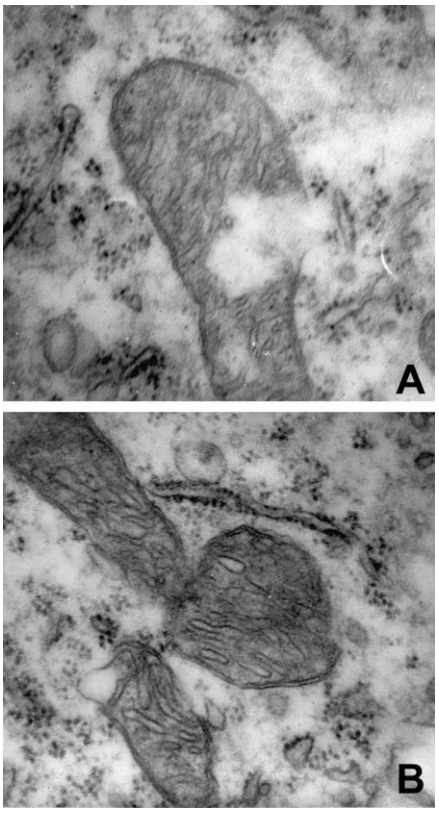

Fig. 4. High-power electron microscopy (20.000×) of mitochondria from motoneurons of an untreated Wobbler mouse and a Wobbler mouse receiving progesterone for 18 days. (A) This photomicrograph shows grossly abnormal mitochondria with cristolysis and rupture of mitochondrial membranes in an untreated Wobbler mouse. (B) Photomicrograph of a progesterone-treated Wobbler mouse in which the mitochondrial ultrastructure is better conserved, including the cristae and outer membrane. (reproduced with permission from Ref. [56]).

Progesterone effects in Wobbler mice

To study progesterone effects, we have initially employed 2 month-old symptomatic Wobbler mice showing ambulatory difficulties, muscle atrophy and forelimb flexion [181]. These mice have remained untreated or received a single progesterone pellet (20 mg) under the skin of the neck. After 18 days of treatment, vacuolated motoneurons in the spinal cord of 2 month-old Wobblers are reduced 6-fold by progesterone treatment, with a concomitant reduction of NOS active neurons. This effect could relieve motoneurons from increased oxidative stress [57]. Electron microscopic observations have demonstrated a considerable reduction of disrupted mitochondrial membranes in progesterone-treated Wobblers (Fig. 4). Although the expression levels of PR have not been studied in Wobbler motoneurons, in other CNS areas PR are found closely apposed to mitochondria [173]. After traumatic brain injury, progesterone reverses the early post-injury alterations in mitochondrial respiration [143]. It must be clarified in future studies if the PR plays a significant role in prevention of mitochondrial and motoneuron abnormalities of the Wobbler mouse, or if PR-independent mechanisms are also engaged.

Progesterone and BDNF regulation in Wobbler mice

A more prolonged exposure of Wobbler mice to progesterone for 60 days also increases neuronal BDNF mRNA, changes the subcellular distribution of BDNF protein and restores the number of neurons expressing ChAT-IR in the spinal cord, as well as the activity of this enzyme in nerve terminals [59]. With respect to trophic effects, progesterone attenuates the ongoing atrophy of Wobbler mice forelimb biceps bracchii, implying that the whole neuromuscular function is probably enhanced by treatment with the exogenous neuroactive steroid.

Restoration of BDNF expression following long-term exposure to progesterone may be essential for the ailing motoneurons, considering the beneficial roles assigned to neurotrophins in pathological situations. It has been shown that in mouse neurons, the pro-BDNF precursor is rapidly converted intracellularly to mature BDNF, the latter being stored and released by excitatory input [116]. We have observed that progesterone increases BDNF mRNA levels in large α-motoneurons which innervate the forelimbs muscles, smaller neurons such as γ-motoneurons that control muscle spindle organs, Renshaw inhibitory cells and interneurons [59]. These responses point to an intrinsically complex regulation of BDNF expression in the neuronal network of the ventral horn. *Prima facie*, up-regulation of endogenous BDNF may be helpful to Wobbler mice, because treatment of these animals with exogenous BDNF slows motoneuron degeneration, diminishes axon loss and enhances behavioral parameters related to locomotor activity [81,82,85,124].

It is accepted that working in an autocrine manner, BDNF binds to neuronal tyrosine-kinase type B receptors (TrkB) and activates gene transcription employing different signaling cascades [127]. However, preliminary data has shown that neuronal TrkB receptors are not modulated by progesterone, although steroid treatment down-regulates the expression of $p75^{ntr}$, a pan neurotrophin receptor type that mediates death signals after binding neurotrophins (Gonzalez Deniselle et al. unpublished results). Thus, progesterone could play a dual role, in part by stimulation of endogenous BDNF, and in part by decreasing its binding to $p75^{ntr}$. If BDNF is released from motoneurons after progesterone stimulation, it may bind to the normally-expressed TrkB receptors in nearby neurons in a paracrine manner [71]. A paracrine role for BDNF has been proposed following axotomy, degenerative diseases and ischemia [9,21,53]. Oligodendrocytes are also targets of secreted BDNF,

which increases survival and myelin synthesis by these cells [96]. As described before in rats with spinal cord injury, progesterone enhances myelinogenesis and BDNF expression [60,101,102]. Therefore, it would be important to investigate if progesterone-stimulated BDNF is connected to oligodendrocyte function in the Wobbler mouse. In addition to neuronal-derived BDNF, progesterone may also enhance the transport of target (muscle)-derived BDNF. In connection with this new role of progesterone, we have shown that the steroid normalizes the retrograde axonal transport of Wobbler mice, which may include BDNF among other molecules [58] (Fig. 5).

Progesterone treatment of Wobbler mice has also resulted in changes in ChAT, the enzyme responsible for acetylcholine synthesis. Untreated Wobbler mice present a significant reduction in ChAT-immunoreactive motoneurons, indicating they are dysfunctional neurons that lost the ChAT phenotype [59]. Results from other laboratories have shown that ChAT-immunoreactivity and enzyme activity in the ventral horn are significantly lower in clinically symptomatic Wobbler mice [10,124,181] and also in ALS patients [91,134]. We have found that in the forelimb muscles of Wobbler mice, ChAT activity is reduced by 55.3% respect of control animals, which also agrees with similar findings in other laboratories [10,11,81]. Depletion of ChAT-IR in neurons and enzyme activity in nerve terminals is reversible, because progesterone induces a slight but significant increment in ChAT-positive neuronal density in the spinal cord and increases enzyme activity in the muscle. These findings reflect that progesterone recovers the ChAT phenotype of motoneurons, most likely through stimulation of BDNF, which colocalizes with ChAT according to the results of dual-labeling immunofluorescence histochemistry. These effects have correlated with a significant increment of muscle weight, and enhancement of muscle strength

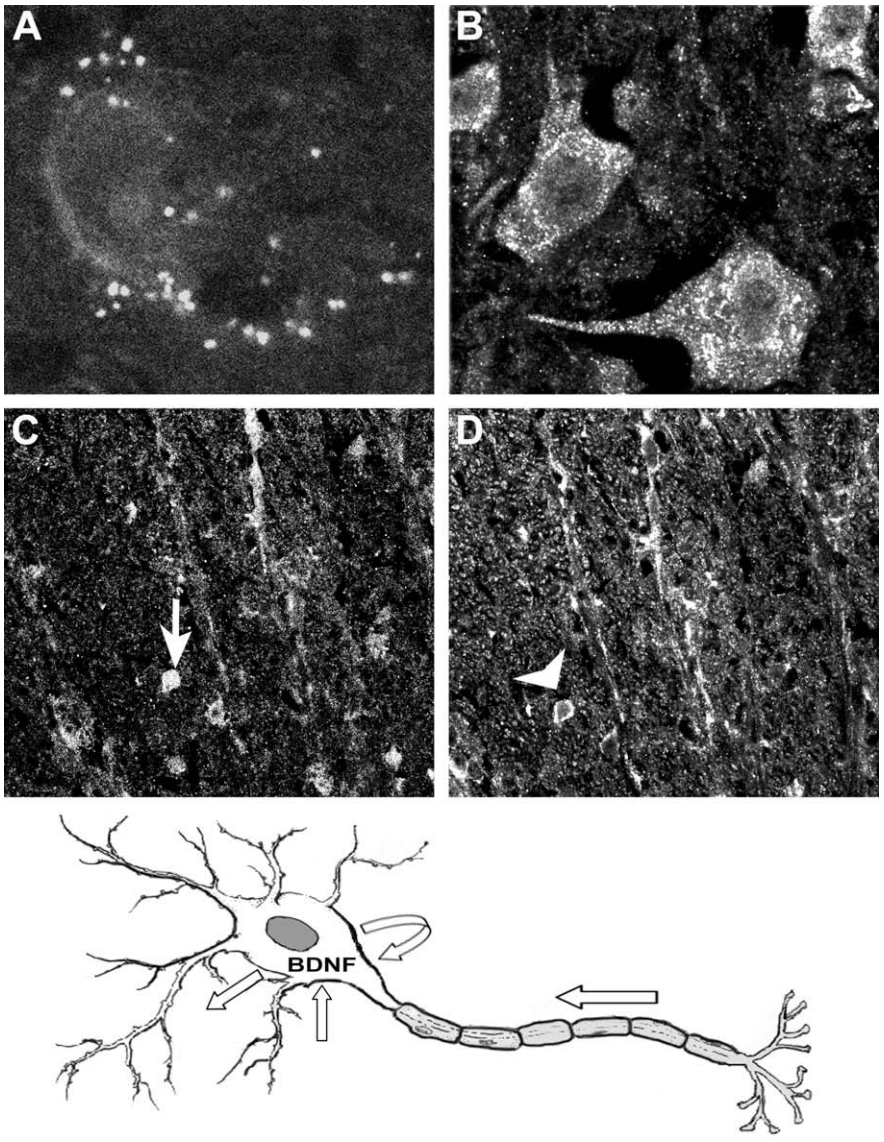

Fig. 5. Effects of progesterone on brain-derived neurotrophic factor (BDNF) expression in Wobbler mouse motoneurons and glial cells. (A) In motoneurons from progesterone-treated Wobbler mice, BDNF protein is detected as cytoplasmic aggregates suggesting a secretory pathway. (C) BDNF protein is also present in glial cells of the white matter (white arrow). Motoneurons (B) and glial cells (D, arrowhead) also express the tyrosine kinase receptor (TrkB) for BDNF detected by fluorescence immunohistochemistry. Lower cartoon: A working hypothesis proposing that in progesterone-treated Wobblers, BDNF derives from neuronal synthesis, although a peripheral source cannot be discarded (arrow in axon). BDNF contributes to neuroprotection and myelination using autocrine or paracrine mechanisms, following binding to TrkB receptors in neurons and glial cells.

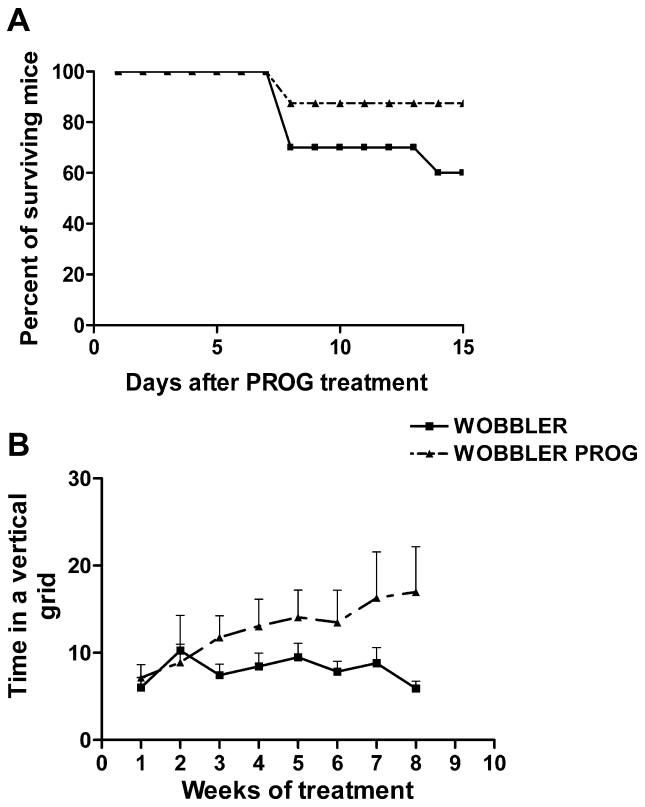

Fig. 6. Effects of progesterone treatment on muscle strength and survival time of Wobbler mice. (A) Survival time of Wobbler mice measured from day 0 (progesterone pellet implantation) to day 15 (time of sacrifice). On day 15th, significantly more steroid-treated Wobbler mice survives compared to untreated mice ($p < 0.03$). (B) Plot of time spent on a vertical grid vs. time (weeks) of progesterone treatment of Wobbler mice. The time spent on a vertical grid is increased by progesterone treatment of Wobbler mice, in comparison to untreated mice ($^{*}p < 0.05$). (reproduced with permission from Ref. [55]).

in Wobbler mice receiving progesterone [55] (Fig. 6). Interestingly, BDNF treatment of Wobbler mice attenuates motoneuron degeneration, neuronal atrophy and axonal loss, and slows down disease progression according to measures of grip strength, rotarod test and muscle potentials [81,84,85,124]. These observations contribute to sustain that BDNF could mediate some late effects of progesterone in the Wobbler mouse.

At this time, it seems important to impose the following question: does the Wobbler mouse respond to progesterone treatment at all time periods of the disease? To elucidate this point, we have employed genotypified Wobbler mice at the initial (1–3 months old), symptomatic (5–8 months) and late (12–13 months) periods. According to evidences provided by counting the vacuolated motoneurons, the density of ChAT+ motoneurons, and markers of glial cells including GFAP (astrocytes) and glutamine synthase (astrocytes and oligodendrocytes), Wobblers always remain sensitive to progesterone. Thus, Wobbler mice receiving a progesterone pellet 18 days before sacrifice, have shown decreased number of vacuolated motoneurons, increased ChAT immunostaining, decreased GFAP + astrogliosis and increased expression of glutamine synthase, the enzyme that prevents glutamatergic excitotoxicity. Therefore, it seems likely that neurodegeneration is not irreversible, and may be subject to restoration by progesterone. In summary, it seems now clear that progesterone plays a beneficial role in Wobbler mouse motoneuron degeneration, suggesting that the steroid protective effects could be extended to patients suffering from neurodegenerative diseases.

Mechanism(s) of action of progesterone in trauma and neurodegeneration

Progesterone receptors

Progesterone effects are mediated by an array of progesterone receptors (PR) that include the classic nuclear PR-A and PR-B receptors, the newly cloned membrane receptors mPRs and the membrane-associated protein PGRMC1 [16,68,151]. PGRMC1 is a membrane-bound progesterone-binding site that shows several potential signaling mechanisms, including the Jak/STAT and Src pathways and the activation of protein kinase G. However, transducing signals for PGRMC1 in the nervous system remain unknown [68]. Much remains to be discovered regarding the role of these receptors in the neuroprotective effects of progesterone in TBI. However, our observation that PGRMC1 is expressed in structures involved in cerebrospinal fluid production and osmoregulation, and is up-regulated in neurons and induced in astrocytes after TBI, point to a potentially important role of this progesterone-binding protein in the maintenance of water homeostasis after TBI [68,119].

The PR is also expressed in the spinal cord. Use of a specific antibody (KC146) recognizing the B isoform of PR, allow us to localize PR in neurons and glial cells. PR-B is a more potent transactivator of gene expression than PR-A [3]. Immunocytochemistry has demonstrated that neurons localized in ventral horn and Lamina IX, in addition to glial cells of the gray and white matter and ependymal cells are PR-B positive [98,100]. Evidence for estrogen-inducibility of PR in ovariectomized rats or gender differences in neuronal PR immunostaining intensity is not obtained for the spinal cord. In both neurons and glial cells of this tissue, PR-B localizes in cytoplasm and nucleus and in some cell processes, suggesting alternative mechanisms of hormone action [98,100]. The presence of extra nuclear PR has been also reported in the pre and postsynaptic areas of hippocampus [173].

In further investigations we have employed RT-PCR and immunocytochemistry to measure the levels and cellular localization of PR-B and PGRMC1 (25-Dx) in the spinal cord examined under different experimental conditions [100]. In male rats with spinal cord injury, levels of PR mRNA significantly decrease, while those of PGRMC1 are unchanged respect of control animals. When spinal cord-injured animals receive progesterone treatment during 72 h, PR mRNA levels remain similar to non-treated animals, while PGRMC1 mRNA levels are significantly increased. Immunostaining for PR-B shows intracellular localization in neurons and glial cells of the spinal cord, whereas PGRNC1 immunoreactivity localizes mainly to plasma membrane of dorsal horn and central canal neurons. Since the two binding systems for progesterone differ in their response to a lesion, hormone treatment and regional localization, their function may also differ under normal and pathological conditions. The response of PGRMC1 to injury suggests its involvement in the neuroprotective effects of progesterone in the spinal cord and brain [68]. However, other receptors besides the classical PR-B and PGRMC1 may also account for progesterone effects. Recently, three isoforms of a membrane receptor for progesterone (mPR) have been cloned from fish and the brain of mammals [185]. These mPRs contain seven integral transmembrane domains, mediate signaling via an inhibitory G-protein coupled pathway and increase the MAPK pathway [184]. In the mouse spinal cord, RT-PCR analysis and sequencing of the amplified products demonstrates the expression of mPRα, mPRβ and mPRγ mRNA, suggesting that mPR receptors may be implicated in some of the biological effects of progesterone in the spinal cord [103].

Progesterone and the control of gene expression

At this point, it seems relevant to discuss how progesterone controls gene expression in spinal cord neuropathology. As discussed before, genes up-regulated by progesterone comprise BDNF, GAP-43, ChAT, Na,K-ATPase, MAP2, MBP, PLP, Olig1, Olig2 and Nkx2. While a PR/glucocorticoid receptor (GR) hormone-response-element is present in a few gene promoters, it is absent from others [157]. In the case of BDNF, estrogen response elements – but not progesterone response elements – are present in the promoter [156]. In contrast, GR interact with proteins of the AP-1 complex to regulate BDNF gene transcription [75] but the role played by proteins of the AP-1 complex in progesterone regulation of BDNF remains to be established. In the case of the MBP and α Na,K-ATPase gene promoters, a GR consensus element [33,42,94], sharing the same bases in DNA with PR exists in several target genes [131,170,186]. However, the absence of a GR/PR consensus sequence from the PLP and GAP-43 genes [20,184], as well as for the oligodendrocyte transcription factors Olg1, Olg2 and Nkx2.2 genes, suggests that alternative mechanisms may operate for progesterone effects on these molecules.

In this regard, the identification of a specific motif in the PR that interacts with the SH3 domain of the Src tyrosine kinase has been shown to mediate rapid progesterone effects in target tissues. In this mechanism, the interaction of a cytoplasmic form of PR-B with Src activates the MAPK and ERK signaling pathway in TD47 breast cancer cells [13,122] and as shown recently, it also takes place in brain [69]. Thus, changes of gene expression could be triggered after interaction of Src with PR-B, bypassing the direct transcriptional activity of nuclear PR. The role of the MAPK/ERK pathway in progesterone effects in the spinal cord remains to be elucidated.

Neuroprotective effects of progesterone metabolites

Further possibilities for non-nuclear mediated actions of progesterone derive from the conversion into the metabolites DHP and THP, which are endowed with neuroactive properties. THP exerts neuroprotective activity after brain damage, promyelinating activity in the PNS and CNS and enhances OPC proliferation *in vitro* [4,22,35,36,46,50,169]. After spinal cord transection, levels of pregnenolone, progesterone, DHP and THP measured by a highly sensitive GC/MS method, are increased in tissue but not in plasma, suggesting an important function for the locally biosynthesized neurosteroids. Systemic progesterone administration to injured rats produces large increases in progesterone, DHT and THP. Because the ratio of reduced derivatives/progesterone is 65-times higher in spinal cord than in plasma, an important responsibility (neuroprotection, myelination) for the locally biosynthesized reduced derivatives may be envisaged [102]. Similarly, DHP constitutes a main compound extracted from the spinal cord of progesterone-implanted Wobbler mouse (unpublished data). To decipher the prevailing hormonal mechanisms in rats with spinal cord injury and the Wobbler mouse – as well as in similar animal models – has prospective applications to patients with trauma and degeneration of the central nervous system.

Concluding remarks

Trauma to the CNS is an extremely important issue for its devastating consequences and for the limited capacity for regeneration exhibited by the adult CNS. Therefore, identifying potential factors that may help restoring the missing functions becomes a crucial objective of neuropharmacological research. In this sense, progesterone has shown encouraging protective effects for brain trauma, as documented by data produced in experimental animals and in two human trials. For spinal cord injury, human studies are still lacking, although reports in laboratory models have shown that progesterone's protective effects apply during the acute and more prolonged periods following spinal cord transection. Therefore, it seems that progesterone is neuroprotective in trauma events independently where it occurs. This is an important point, and progesterone is also neuroprotective for different types of cell death. When comparing progesterone effects in brain and spinal cord, some similarities come to light suggesting common mechanisms of neuroprotection. Both the brain and spinal cord express classical intracellular PR and various forms of membrane progesterone receptors (mPRs, PGRMC1), supporting direct and multiple mechanisms of action in these tissues. Second, progesterone becomes a promyelinating factor, by stimulating the proliferation and differentiation of oligodendrocyte progenitors, increasing myelin protein synthesis and by inducing remyelination of the injured brain and spinal cord. Third, progesterone protects damaged neurons undergoing apoptosis in TBI, chromatolysis in the lesioned spinal cord and vacuolar degeneration in a motoneuron degeneration model. In brain and spinal cord trauma and neurodegeneration, progesterone induces the synthesis of neurotrophic growth factors and induces or stimulates specific enzymes involved in neurotransmission and metabolism. Additional data confirm progesterone's anti-inflammatory effect following TBI, but similar data are still needed for the spinal cord. Future studies should consider the examined properties of progesterone, progesterone metabolites, neuroactive steroids and neurosteroids as novel therapeutic agents for trauma and neurodegenerative diseases.

Acknowledgments

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