PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 83 (2003) 199–209

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Basis of progesterone protection in spinal cord neurodegeneration☆

Maria Claudia Gonzalez Deniselle[a], Juan José Lopez Costa[b], Susana L. Gonzalez[a], Florencia Labombarda[a], Laura Garay[a], Rachida Guennoun[c], Michael Schumacher[c], Alejandro F. De Nicola[a,*]

[a] *Laboratory of Neuroendocrine Biochemistry, Department of Human Biochemistry, Faculty of Medicine, Instituto de Biologia y Medicina Experimental, University of Buenos Aires, Obligado 2490, 1428 Buenos Aires, Argentina*

[b] *Faculty of Medicine, Instituto de Biologia Celular y Neurociencias "Prof. E. De Robertis", University of Buenos Aires, Buenos Aires, Argentina*

[c] *INSERM U488, Kremlin-Bicêtre, France*

Abstract

Progesterone neuroprotection has been reported in experimental brain, peripheral nerve and spinal cord injury. To investigate for a similar role in neurodegeneration, we studied progesterone effects in the Wobbler mouse, a mutant presenting severe motoneuron degeneration and astrogliosis of the spinal cord. Implant of a single progesterone pellet (20 mg) during 15 days produced substantial changes in Wobbler mice spinal cord. Morphologically, motoneurons of untreated Wobbler mice showed severe vacuolation of intracellular organelles including mitochondria. In contrast, neuropathology was less pronounced in Wobbler mice receiving progesterone, together with a reduction of vacuolated cells and preservation of mitochondrial ultrastructure. Determination of mRNAs for the α3 and β1 subunits of neuronal Na, K-ATPase, showed that mRNA levels in untreated mice were significantly reduced, whereas progesterone therapy re-established the expression of both subunits. Additionally, progesterone treatment of Wobbler mice attenuated the aberrant expression of the growth-associated protein (GAP-43) mRNA which otherwise occurred in motoneurons of untreated animals. The hormone, however, was without effect on astrocytosis of Wobbler mice, determined by glial fibrillary acidic protein (GFAP)-immunostaining. Lastly, progesterone treatment of Wobbler mice enhanced grip strength and prolonged survival at the end of the 15-day observation period. Recovery of morphology and molecular motoneuron parameters of Wobbler mice receiving progesterone, suggest a new and important role for this hormone in the prevention of spinal cord neurodegenerative disorders.

Keywords: Progesterone; Neurodegeneration; Wobbler mouse; Spinal cord

1. Introduction: basis of steroid neuroprotection

Functions of female steroid hormones in the central nervous system (CNS) were classically associated to reproductive processes and sexual behavior. However, alternative roles for these hormones were increasingly discovered, in particular effects contributing to neuroprotection by modulation of gene expression and intracellular signaling pathways in areas of the CNS other than those related to endocrine functions [1–4]. In the case of estrogens, protective and neurotrophic effects are well documented using in vitro preparations, whole animal experiments and in patients with Alzheimer's disease [2,5–7]. Estradiol combined with progesterone also showed neuroprotective effects in an animal model of neurodegeneration [8].

In addition to estrogens, single progesterone treatment produced protective and regenerative effects in models of injury [3]. For instance, progesterone stimulated myelinogenesis of injured peripheral nerves [9,10] and prevented neuronal loss following brain contusion, ischemia and edema [11–13]. In the spinal cord, treatment of rats with progesterone increased motoneuron survival after axotomy or injury [14,15], protected cultured neurons against glutamate toxicity [16] and normalized defective functional parameters of injured motoneurons [17]. The presence of a progesterone receptor in peripheral nerves and central nervous system, suggested that neuroprotection may be due to transcriptional events following receptor interaction with hormone-responsive elements in DNA. However, non-genomic actions should not be disregarded. In this sense, progesterone binding to neurotransmitter receptors, to the membrane protein 25-Dx and inhibition of membrane lipid peroxidation may play a crucial role in steroid effects

☆ Proceedings of the 15th International Symposium of the Journal of Steroid Biochemistry and Molecular biology, "Recent Advances in Steroid Biochemistry and Molecular Biology", Munich, Germany, 17–20 May 2002.

* Corresponding author. Tel.: +54-11-4783-2869; fax: +54-11-4786-2564.

E-mail address: denicola@dna.uba.ar (A.F. De Nicola).

0960-0760/03/$ – see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-0760(02)00262-5

[12,18]. However, while the bulk of progesterone neuroprotection was reported in central or peripheral nervous system trauma, demonstrations in neurodegeneration models are generally scarce [8].

2. The Wobbler mouse, an animal model of spinal cord neurodegeneration

A prototype neurodegenerative disease in humans is amyotrophic lateral sclerosis (ALS), a devastating illness affecting motoneurons that innervate voluntary muscles. Ninety five % of ALS patients do not report a family history of the disease, for which reason they belong to the "sporadic form", while the remaining 5% present the "familial form", transmitted by a dominant gene. In both forms, however, surviving time is limited to about 5 years [19]. The drama imposed by ALS is due to its fatal outcome and the minor effectiveness of available treatments, making animal models useful tools to explore therapeutic alternatives for human motoneuron diseases.

The Wobbler mouse is a genetic model of spinal cord neurodegeneration. In homozygous Wobbler mice, a mutation of autosomic recessive expression (*wr*) produces motor neuron loss and astrocyte reactivity in the spinal cord and brain stem [20]. Although the *wr* gene has not been identified, it maps to chromosome 11 close to the glutamine synthetase gene [21]. Wobblers become useful models to study amyotrophic lateral sclerosis (ALS) and infantile spinal muscular atrophy (Werdenig–Hoffman disease) [19,22].

Former morphological studies of anterior horn cells of Wobbler mice spinal cord found a dramatic perikaryal vacuolar degeneration, in addition to swelling of α and γ motor neurons, interneurons and Renshaw cells [23–25]. This change was accompanied by pronounced astrocytosis [26,27] and increased density of activated microglia [28]. Whereas motoneurons in 3–4-week-old Wobbler mice are TUNEL negative [29,30] a transient massive DNA fragmentation of neurons and glial cells was reported by Blondet et al. [31] before the manifestation of clinical symptoms. Therefore, while apoptosis may cause cell death in the early stages, motoneuron pathology of symptomatic mice resembled the type II or cytoplasmic form of cell death described by Clarke [32] and attributed to increased oxidative stress. In Wobbler mice, participation of oxidative stress is supported by abnormalities of mitochondrial function [33] and the clinical, biochemical and morphological improvement caused by treatment with common antioxidants, antioxidant steroids or nitric oxide (NO) inhibitors [27,34–36]. Thus, both oxidative stress and apoptosis acting in a different time sequence may cause cell death in the Wobbler mouse and other motor neuron degeneration models including the superoxide dismutase transgenic mouse [31,37].

Therefore, the Wobbler mouse spinal cord seemed particulary vulnerable to oxidative attack. In this respect, progesterone treatment was considered a valuable therapeutic tool for several reasons: first, progesterone can diffuse and associate with phospholipids by intercalation between polyunsaturated fatty acid chains [12,38]. This mechanism caused membrane stabilization and attenuated injury-induced lipid peroxidation [12]. Second, progesterone itself can behave as a free-radical scavenger [39]. Although important, inhibition of lipid peroxidation may not be an exclusive mechanism of progesterone action in the spinal cord, since detection of an intracellular progesterone receptor capable of transcriptional regulation was reported in this tissue [40,41]. Therefore, functional multiplicity and not a single form of steroid action may account for progesterone effects in the Wobbler mouse.

In our experiments, we used 3-month-old homozygous Wobbler mice (*wr*/*wr*) (The Animal Center of the National Institutes of Health, Bethesda, MD, USA), representing stages 3–4 of the disease according to the criteria of Yung et al. [42]. All affected animals showed reduced body weight; and clinically presented tremor, ambulatory difficulty, flexion of proximal limbs, distal limb extension, positive clasp knife reflex response and diminished muscle strength. Routine histology showed motor neuron loss and astrogliosis of the spinal cord [27]. A group of Wobbler mice remained untreated, whereas another group received under the skin of the neck a 20 mg pellet of progesterone under light ether anesthesia. Animals were used 15 days after steroid pellet implantation. The efficiency of this treatment was checked by measuring progesterone levels by gas chromatography/mass-spectrometry [43], which demonstrated that progesterone pellet implantation resulted in 10-fold higher hormone levels in the cervical spinal cord compared to untreated Wobbler mice.

3. Parameters measured in motoneurons and astrocytes

The reported success of progesterone treatment in nervous system trauma, decided us to investigate for similar effects in Wobbler mouse motor neuron degeneration. On these premises, several objectives were pursued: first, to establish in 3 months old, clinically affected Wobbler mice if progesterone treatment modified the existing neuropathology, assessed by light and electron microscopy. Second, to explore if progesterone modulated some parameters important for motoneuron function, including the mRNAs for the α3 and β1 subunits of the Na, K-ATPase and the growth-associated protein (GAP-43). The Na, K-ATPase is an essential enzyme, playing a pivotal role in neurotransmission, nutrient uptake and maintenance of membrane potential [44,45]. In addition, it showed sensitivity to progesterone treatment after spinal cord injury [17]. In contrast to the high expression of the Na, K-ATPase in mature neurons, GAP-43 is expressed only by embryonic cells [46]. Considering that GAP-43 protein and mRNA are both aberrantly expressed in Wobbler mice, its regulation might

have important implications for the fate of degenerating motoneurons [46]. Third, to analyze whether progesterone treatment regulated the pre-existing spinal cord astrocytosis of Wobbler mice. This may be an important issue, in view of the fact that astrocytes have a protective role for motoneuron function and disease outcome [47–49]. The last aim was based on previous demonstrations of increased expression of the astrocyte glial fibrillary acidic protein (GFAP) following treatment of Wobbler mice with the neuroprotectant and antioxidant steroid U-74389F [50]. Fourth, we studied the effects of progesterone on muscle strength and survival time of Wobbler mice in order to extrapolate to the in vivo situation findings obtained at the morphological and biochemical level. As will be shown below, our data suggest that progesterone retarded neurodegeneration of Wobbler mice spinal cord and in this context, it may be potentially valuable for treatment of human neurodegenerative diseases.

4. Effects of progesterone treatment on Wobbler mice neuropathology: an electron microscopic study

Light microscopy demonstrated an increased frequency of vacuolated motor neurons in the ventral horn of Wobbler mice, in agreement with the finding of others [23–25]. The effect of progesterone was quantitatively evaluated using computerized image analysis, which demonstrated that the total number of vacuolated neurons per unit area found in untreated Wobblers ($(18 \pm 2.2) \times 10^{-5}\,\mu m^{-2}$) was significantly reduced in the group of Wobbler mice receiving progesterone ($(11 \pm 0.8) \times 10^{-5}\,\mu m^{-2}$, $P < 0.05$). Although the origin of these vacuoles was uncertain, electron microscopy suggested that vacuolization derived from degenerating mitochondria, cysternae of smooth and rough endoplasmic reticulum and Golgi apparatus. In Wobbler mice receiving progesterone, neuronal degeneration was moderate. In the perinuclear area, few vacuolae remained and rough endoplasmic reticulum was largely preserved. The shape of the nuclear membrane was partly restored and some perinuclear mitochondria regained a normal appearance.

Considering that abnormal mitochondria may be a source of free radicals which further impaired motoneuron function, we compared mitochondrial ultrastructure in untreated and progesterone-treated Wobbler mice. Fig. 1 (left-hand photomicrograph) shows a massive vacuolation suffered by a mitochondria in a Wobbler mouse motoneuron. The ultrastructure of the organelle was highly disorganized, with rupture of the outer and inner membranes. Progesterone treatment of Wobbler mice reverted in part these abnormalities, in that some mitochondria reassumed a normal ultrastructure. As shown in the right-hand photomicrograph of Fig. 1, the outer and inner membranes, cristae and matrix were better preserved in the motoneuron of the progesterone-treated Wobbler mice These observations suggested that cytoplasmic and mitochondrial vacuolation, a predominant form of cell death in Wobbler mice motoneuron disease, may be overturned by progesterone. In very few cases, such as the motoneuron of an untreated Wobbler mouse shown in Fig. 2, we detected nuclear chromatin fragmentation suggestive of apoptosis [31]. However, in contrast to the high incidence of motoneuron vacuolation of Wobbler mice, the number of cell nuclei showing chromatin cumpling was very low and similar in both treated and untreated mutant mice. The absence of apoptotic changes was

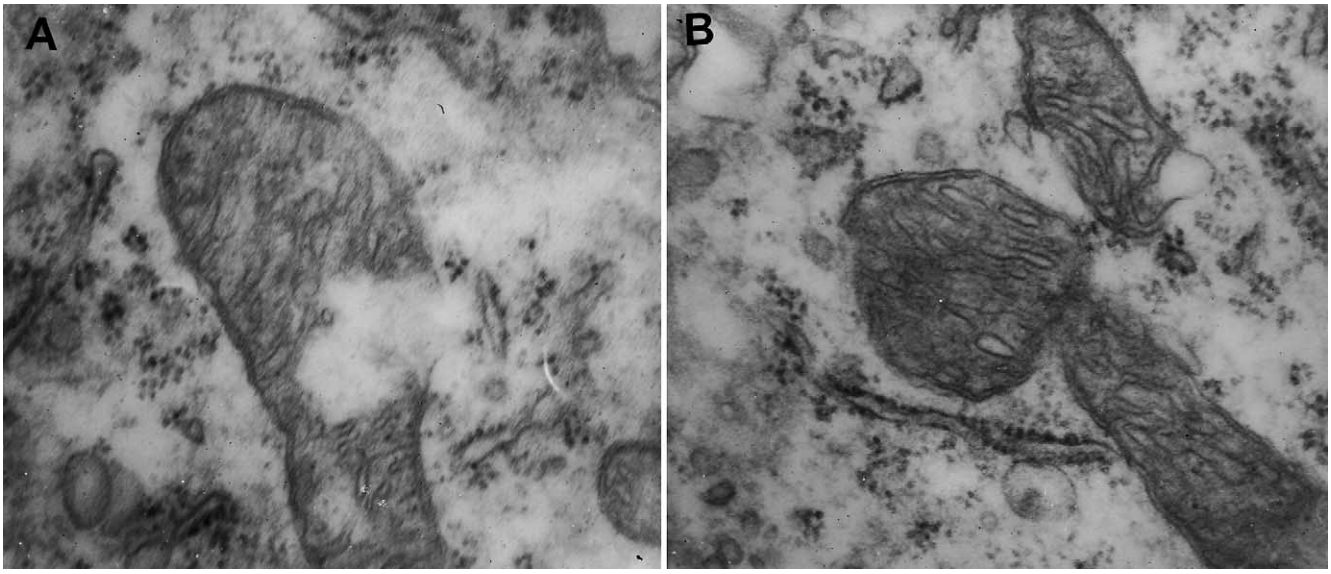

Fig. 1. Electron microscopy of Wobbler mice ventral horn motoneurons. The left-hand photomicrograph (A) shows a degenerating motoneuron from an untreated Wobbler mouse with massive vacuolation disrupting the outer, inner mitochondrial membranes and cristae. The right-hand photomicrograph (B) corresponds to a Wobbler mouse receiving progesterone, showing that mitochondrial membrane system, including the cristae, are better conserved. Magnification: 50,000×.

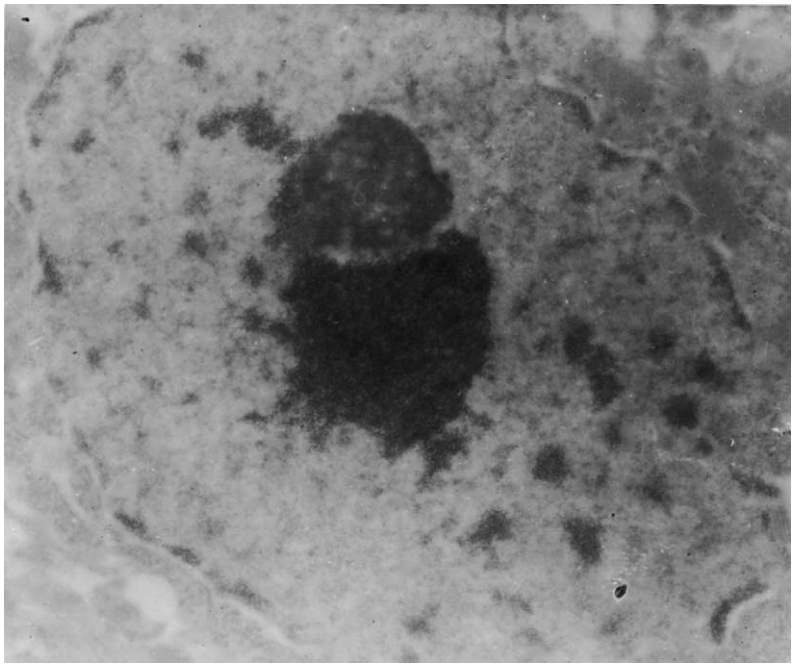

Fig. 2. Apoptoptic features in a motoneuron. Nuclear chromatin fragmentation, as exemplified in this photomicrograph, is observed with very low frequency in 3-month-old symptomatic Wobbler mice. In these mice, cytoplasmic and mitochondrial vacuolation of the type exemplified in Fig. 1 constituted the main ultrastructural abnormalities. Magnification: 12,000×.

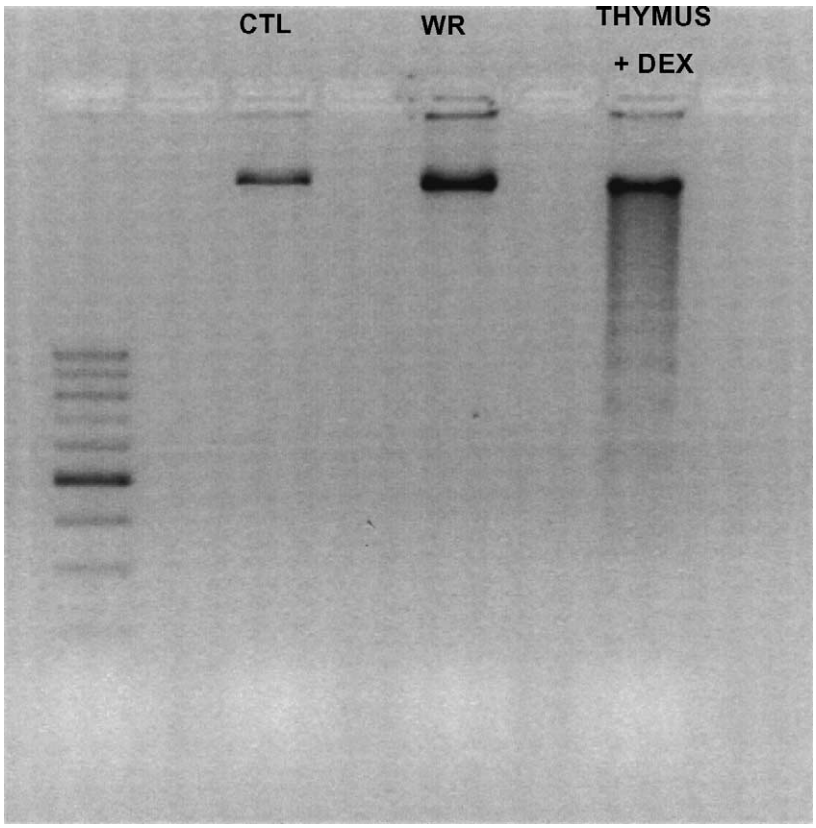

Fig. 3. DNA analysis by agarose gel electrophoresis. Genomic DNA was extracted from the spinal cord of control mice (CTL), spinal cord of Wobbler mice (WR) and thymus of normal mice receiving 0.5 mg dexamethasone 2 and 16 h previous to killing (thymus + DEX). The bands on the left correspond to DNA markers. DNA laddering was absent from control and Wobbler mice spinal cords, but multiple bands were detected in the gel corresponding to the thymus of glucocorticoid-exposed mice run as a positive control.

also evident using agarose gel electrophoresis. In this case, the DNA laddering profile indicative of apoptotic DNA fragmentation, was negative in both control and Wobbler mice spinal cords (Fig. 3, CTL and WR). As a positive control we used the thymus from normal mice receiving two s.c. injections of 0.5 mg dexamethasone 2 and 16 h before killing. In this case, a DNA ladder typical of apoptosis was obtained on the gels (Fig. 3, thymus + DEX).

5. Effects of progesterone on neuronal Na, K-ATPase subunit mRNA

The effects of progesterone treatment on the α3 and β1 subunit mRNAs for the neuronal NA, K-ATPase was studied in control littermates and Wobbler mice. In these experiments, ^{35}S-labeled oligonucleotide probes with sequences coding for the α3 subunit and the β1 subunit mRNA were used in conjunction with in situ hybridization procedures [51,52]. The chosen oligonucleotides produced strong hybridization signals in ventral horn motoneurons without labeling of glial cells [51,52].

The hormone's effect was determined in Wobbler mice subjected to 15-day steroid exposure as well as in untreated Wobbler mice and age-matched controls. In this experiment, neurons measuring >300 μm^2 localized in Lamina IX were considered to be α motoneurons based on anatomical location and size. Control motoneurons showed a substantial grain density representing oligonucleotide probe hybridized to neuronal α3 or β1 subunits of Na, K-ATPase mRNA. This pattern contrasted with the grain depletion in motor neurons from Wobbler mice. Following 15 days of progesterone exposure, the hybridization signal in Wobbler mice was higher than in untreated Wobblers. The lower part of Fig. 4 shows the expression of the β1 subunit by motor neurons of control mice (A, left-hand graph), the grain depletion of untreated Wobbler mice (B, center graph) and the recovered mRNA levels in a Wobbler mouse treated with progesterone (C, right-hand photomicrograph). Quantitative analysis of ISH data (Fig. 4) further confirmed in untreated Wobbler mice a reduction in the expression of the β1 subunit mRNA for the Na, K-ATPase (41 ± 2.7 grains/100 μm^2) when compared to control animals (52 ± 2.8, $P < 0.05$). In Wobbler mice treated with progesterone, grain density was higher than in untreated Wobblers (55 ± 3.5, $P < 0.05$), whereas there were non-significantly differences with controls. In addition to its effect on the β1 subunit, progesterone treatment also restored the reduction of α3 Na, K-ATPase mRNA expression shown by Wobbler mice in ventral horn motor neurons (results not shown).

6. Attenuation of GAP-43 mRNA expression by progesterone treatment of Wobbler mice

A protein with abnormal expression in Wobbler spinal cord (as well as in patients with ALS) is the growth-associated protein or GAP-43. In the mRNA studies, a

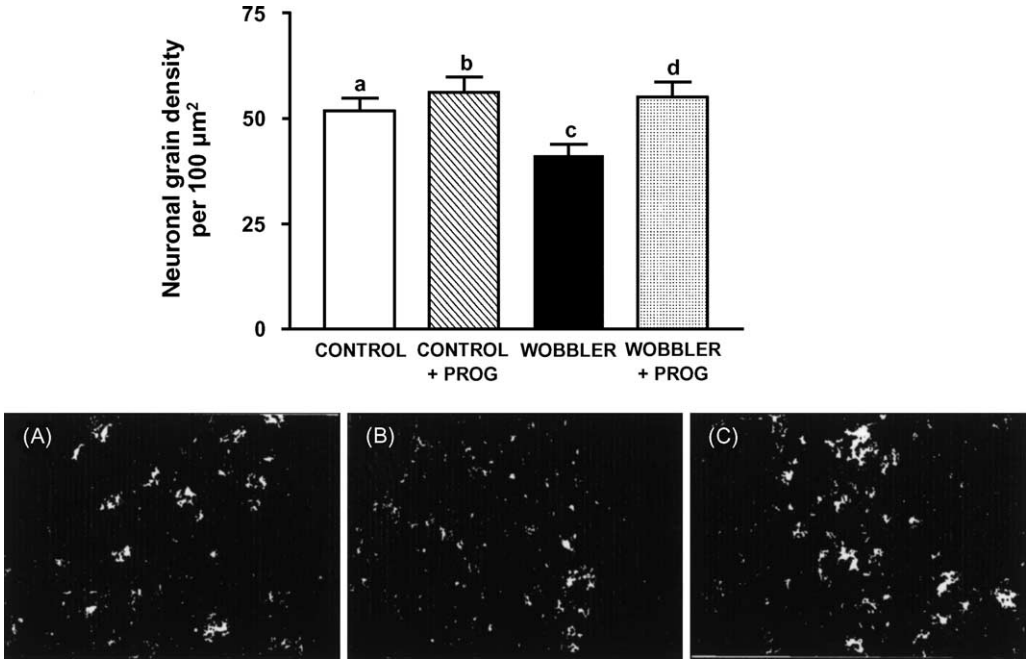

Fig. 4. In situ hybridization for the β1 subunit mRNA of the Na, K-ATPase in the spinal cord. Upper graph: quantitative analysis of grain density in control mice (a: open column), controls receiving progesterone (b: cross-hatched column), untreated Wobbler mice (c: filled column) and steroid-treated Wobbler mice (d: dotted column). Significance—a vs. c: $P < 0.05$; c vs. d: $P < 0.05$) (ANOVA and Newman–Keuls's test). Lower graph: dark field photomicrographs of a control mouse (A, left-hand graph), Wobbler mouse (B, center graph) and a Wobbler mouse treated with progesterone (C, right-hand graph). Magnification: 100×.

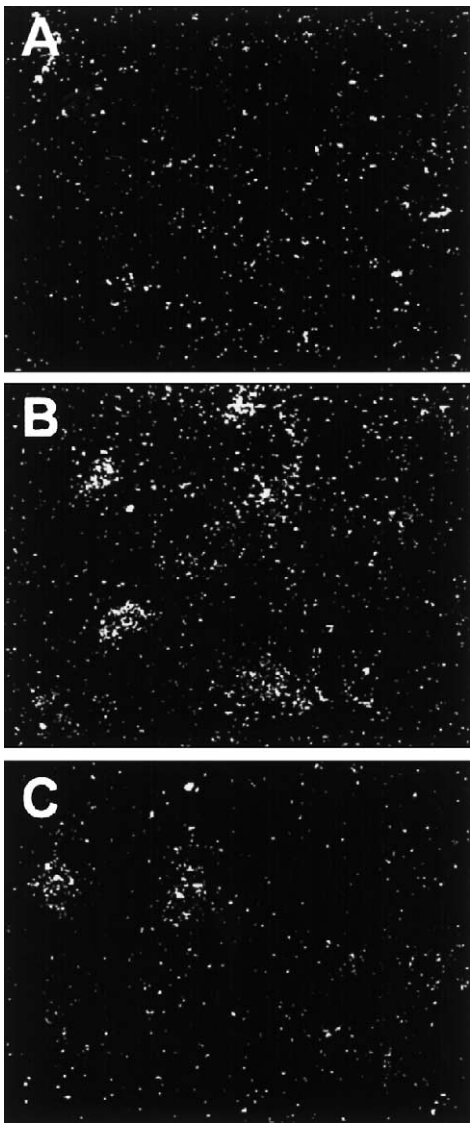

Fig. 5. Dark field photomicrographs representing the low expression of GAP-43 mRNA in motoneurons of a normal mouse (A, upper graph), the high expression of this mRNA in an untreated Wobbler mouse (B, middle graph) and the attenuation of GAP-43 mRNA in a Wobbler mouse receiving progesterone (C, lower graph). Magnification 200×.

synthetic ^{35}S-labeled oligonucleotide complementary to mouse GAP-43 mRNA [53] was hybridized to spinal cord sections. Computer-assisted image analysis was used to quantitate the number of grains per cell, a measurement proportional to the amount of cytoplasmic mRNA according to Mitsumoto and Gambetti [54]. GAP-43 mRNA was almost undetectable in control mice motoneurons, in contrast with the intense expression in Wobbler mice spinal cord, as shown in the bright field and dark field photomicrographs of Fig. 5. As also observed in Fig. 5, progesterone treatment depressed the aberrant expression of Wobbler mouse GAP-43 mRNA.

Quantitative analysis confirmed the very low levels of GAP-43 mRNA in control mice spinal cord, which was unaffected by progesterone treatment. In Wobbler mice not receiving steroid therapy, the average number of grains per cell (85 ± 2.6) was significantly higher than controls levels (8 ± 1.4, $P < 0.001$). Fifteen days after progesterone pellet implant, although GAP-43 mRNA in Wobbler mice (63 ± 2.8) was still higher than in controls, it was significantly reduced respect of untreated Wobbler mice ($P < 0.01$).

7. Astrocytosis of Wobbler mice spinal cord was unaffected by progesterone

Wobbler mice present in common with ALS patients astrogliosis in gray and white matters of the spinal cord, primary motor cortex and subpial regions [26]. The reactive astrocytes found in Wobbler and ALS spinal cord expressed high levels of GFAP [55]. Some authors consider this event a secondary response to neuronal illness, in which case transforming growth factor α could act as the neuronal inducer of astrogliosis [56]. Previously, we found that treatment of Wobbler mice with the antioxidant aminosteroid U-74389F enhanced the existing astrocytosis [50]. This finding encouraged us to search for progesterone effects on GFAP immunostaining in astrocytes of control and Wobbler mice.

Four groups of animals were studied: controls, controls receiving a single progesterone pellet during 15 days, untreated Wobbler mice and Wobbler mice implanted with progesterone. GFAP expression was carried out in spinal cord slices using a rabbit anti-GFAP polyclonal antibody and routine immunohistochemical procedures [50]. Fig. 6 shows that the number of GFAP-immunoreactive astrocytes/mm^2 in the ventral horn of the spinal cord was eight-fold higher in Wobbler compared to control mice (a versus c: $P < 0.001$). However, progesterone was unable to change the high number of GFAP positive astrocytes in Wobbler mice or modified the low number of cells found in control mice (Fig. 6—a versus b: NS; c versus d: NS).

8. Test of muscle strength and survival of Wobbler mice receiving progesterone

As already mentioned, Wobbler mice develop forelimb paralysis and denervation muscle atrophy. These changes are secondary to degeneration and loss of spinal cord motoneurons. In order to study whether progesterone slowed the deterioration of muscle function at the time that prevented motoneuron degeneration, a grip strength test was used [57]. Grip strength was measured by placing mice on a vertical grid and measuring the time in seconds they remained in the grid without falling down. The results showed that untreated Wobbler mice remained in the grid for 5 ± 1.5 s only, whereas Wobbler mice receiving progesterone for 15 days tripled the time spent in the grid (16.4 ± 3.2 s, $P < 0.05$).

Since Wobbler mice die prematurely, we also studied if progesterone treatment prolonged survival in the mutant

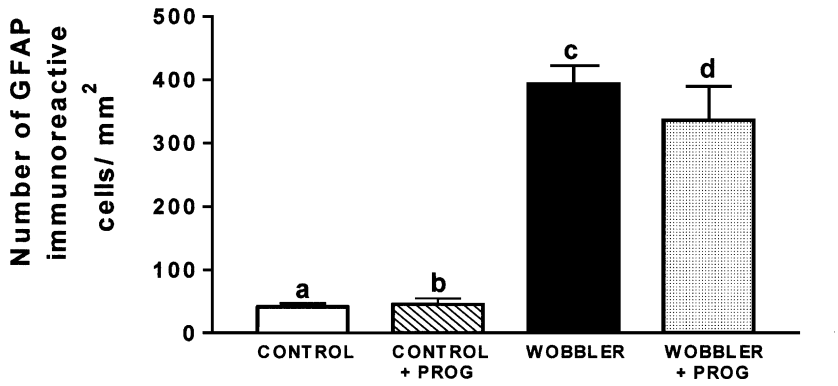

Fig. 6. Number of GFAP-immunoreactive astrocytes/mm^2 in the spinal cord of control mice (a: open columns), controls receiving progesterone (b: cross-hatched columns), Wobbler mice (c: filled column), and Wobblers receiving progesterone (d: dotted column). Results represent the mean ± S.E. of $n = 5$–6 animals per group. Statistical significance—a vs. b: NS; a vs. c: $P < 0.001$; c vs. d: NS (ANOVA and post hoc Newman–Keuls's test).

mice. In this study, a group of Wobbler mice received a single progesterone pellet, while an age-matched group was left untreated. Observations were prolonged for 15 days after progesterone implantation. Percent survival time in both groups was identical for the first 7 days, after which progesterone-treated Wobbler mice deviated from the untreated group. At the end of the study period (15 days), a significant difference was measured due to increased survival of Wobbler mice receiving progesterone compared to the untreated mice ($P < 0.03$).

9. Discussion

The present experiments investigated progesterone effects in a neurodegeneration model, based on previous evidences for progesterone beneficial effects in ischemia and trauma of the central and peripheral nervous system. The resulting data demonstrated that progesterone treatment of symptomatic Wobbler mice: (1) reverted motoneuron neuropathology according to light and electron microscopic observations, (2) up-regulated the mRNAs of the α3 and β1 subunits of neuronal Na, K-ATPase, (3) reduced the aberrant hyperexpression of GAP-43 mRNA, (4) did not affect the number of GFAP-immunostained astrocytes in the ventral horn, and (5) enhanced muscle strength and prolonged survival of clinically ill Wobbler mice.

The prevalent neuropathology found in symptomatic Wobbler mice consisted in motoneurons with cytoplasmic vacuolation and damaged mitochondria but with relative preservation of nuclear ultrastructure. Previous observations using Wobbler mice at stages 3–4 of the disease described similar changes [24,25,29,42,57]. At this stage of the disease, signs of overt apoptosis such as pyknotic nuclei, nuclear membrane fracture, apoptotic bodies and plasma membrane blebbing were unfrequent. In 3-month-old Wobbler mice chromatin clumping was very scarce, although it was found in some animals. In contrast, massive DNA fragmentation reportedly occurred in presymptomatic Wobbler mice [31], although DNA laddering typical of apoptosis was absent in our clinically affected Wobbler mice.

When morphological features of different types of cell death, i.e. apoptosis, necrosis and cytoplasmic cell death were confronted to the present findings, we concluded that neurodegeneration in symptomatic Wobbler mouse resembled the last form, also known as type II or the autolytic type [32,58]. In this process, dilatation of smooth endoplasmic reticulum cysternae and Golgi system lead to their autolytic breakdown, as already shown in clinically affected Wobbler mice [29]. Another important ultrastructural observation in untreated Wobbler mice was the presence of vacuolized mitochondria, with cristolisis, edema and lack of outer membrane integrity. At the functional level, a mitochondrial respiratory chain dysfunction was observed in Wobbler mice by Guang-Ping et al. [33]. This group reported that superoxide production, a leading cause of lipid peroxidation may cause motor neuron death. Besides, lipid peroxidation was proposed as a leading causative factor for the cytoplasmic and mitochondrial changes in neurodegeneration [59]. A cytoplasmic form of cell death may also play a role in the mutant superoxide dismutase type 1 (SOD1) transgenic model (G93A and G37R mutations), in which a toxic gain of function of the mutated enzyme led to oxygen radical-induced lipid peroxidation of intracellular membranes and mitochondrial abnormalities [60–62]. Interestingly, spinal cord neuropathology of Wobbler mice bear striking similarities to that of SOD1 transgenics, which also showed extensive vacuolation of mitochondria, Golgi apparatus and endoplasmic reticulum [61,63,64]. Similarly, while apoptotic changes and chromatin clumping were reported by some workers in SOD1 transgenic and Wobbler mice [31,65,66], others sustained that motor neuron death occurs primarily by massive vacuolation in the absence of apoptosis [29,67]. Thus, it is possible that at least two mechanisms—apoptosis and cytoplasmic vacuolization—caused motor neuron degeneration in the genetic and

transgenic models of ALS, although with a different time course.

Cytoplasmic vacuolization suggests that free radicals produced in neurons or glial cells originated and/or exacerbated neurodegeneration of untreated Wobbler mice. Blockage of free radical damage to motor neurons with the antioxidants OPC-14117, *n*-acetylcysteine, lecythinized superoxide dismutase or T-588 corrected the motor dysfunction of Wobbler mice [35,36,68,69]. Excessive production of nitric oxide is another important risk factor for neurodegeneration, considering the Wobbler mice showed increased activity of nitric oxide synthase (NOS) in ventral horn motoneurons [49]. Also, the NOS inhibitor nitroindazol produced beneficial effects [34]. We previously reported that Wobbler mouse spinal cord showed increased number of neurons giving strong histochemical staining for NOS–NADPH–diaphorase [49], whereas treatment with the antioxidant 21-aminosteroid U-74389F reduced this activity and, presumably, nitric oxide (NO) production. Therefore, NO toxicity may contribute to neurodegeneration, considering that after coupling with superoxide anion, NO forms peroxinitrites which damages lipids, proteins and DNA. Whether progesterone treatment interrupted NO generation is unknown but experiments are under way to study this possibility.

Another important finding of the present experiments using symptomatic Wobbler mice was the pronounced reduction of the α3 catalytic subunit and β1 non-catalytic subunit mRNAs of the Na, K-ATPase in motoneurons. Increased lipid peroxidation was reported to dramatically reduce the enzyme activity [70]; whether it also affects mRNA levels is unknown. Conceivably, this reduction could curtail pivotal functions of the enzyme such as Na/K exchange, maintenance of membrane potential, nutrient uptake and neurotransmission [44,45]. In connection with the last property, a reduction of Na, K-ATPase would also impair acetylcholine release at the neuromuscular plate. This might cause further ambulatory difficulty and weakness. Continuous inhibition of this enzyme, as occurred in epilepsy, ischemia, hypoglycemia and aging first caused malfunction and eventually neuronal death [41,71]. Therefore, reduction of the Na, K-ATPase may exacerbate neuropathology, because the enzyme plays an important role for motor neuron function in the spinal cord.

In this scenario, it was highly rewarding that a relatively short course of progesterone treatment partially restored motoneuron morphology and also mRNAs for the Na, K-ATPase α3 and β1 subunits in clinically affected Wobbler mice. Although our experiments did not elucidate the mechanism involved in this effect, direct modulation of the α3 or β1 subunit Na, K-ATPase genes by the progesterone receptor was not previously reported. Nevertheless, a glucocorticoid receptor (GR) consensus sequence responsive element was found in both the Na, K-ATPase α and β genes [72,73]. Reportedly, this DNA sequence is common to both GR and the progesterone receptor [74,75], opening the possibility that the Na, K-ATPase gene may be a direct target of progesterone in the degenerating tissue. Using this or an indirect mechanism, increased expression of the mRNA for the Na, K-ATPase subunits could have rescued motor neurons from the devastating consequences of degeneration. Therefore, several neuronal functions would return after reinstating the mRNA for the Na, K-ATPase subunits [44,45], including the reestablishment of cholinergic neurotransmission. Indeed, synaptic transmission at the neuromuscular junction is under progesterone regulation [76].

A protein with abnormal expression in Wobbler spinal cord (as well as in patients with ALS) is the growth-associated protein or GAP-43. Physiologically, this phosphoprotein is found in high concentrations in embryonic life, during synaptogenesis and in axons regenerating after injury [77]. Usually, the high expression of GAP-43 subsides when growing axons reach their target or myelinize [78]. In the normal adult spinal cord, moderate levels of GAP-43 are found in axon terminals of the dorsal horn, in the corticospinal tract and in the area surrounding the central canal Lamina X but is absent in motoneurons [79]. However, GAP-43 is up-regulated in motoneurons after experimental spinal cord trauma [80]. In humans, ventral horn motoneurons are normally devoid of GAP-43 but high mRNA levels appeared in ALS patients [81].

It is not unreasonable to hypothesize that down-regulation of GAP-43 mRNA and protein may be closely related to progesterone beneficial effects in Wobbler mice. A priori, increased accumulation of GAP-43 protein may be caused by slow transportation from perikaryon to the axonal growth cone [77]. However, the severe muscle atrophy typical of Wobbler mice suggests, as an alternative mechanism, that muscle denervation could originate the GAP-43 hyperexpression of motoneurons. Degenerating motoneurons may react to denervation and synaptic loss by compensatory but useless strengthening of the remaining synapsis. Hyperexpression of GAP-43 may be of little help, considering that abnormal synaptic contacts including collateral synaptogenesis and hypomyelination usually accompany motoneuron degeneration in Wobbler mice and ALS patients [77,81,82]. Actually, in these cases as well as in other neurophatologies (Alzheimer' s disease and schizophrenia) GAP-43 may be a marker for neurodegeneration.

In agreement with previous reports [25–27], we found an increased number of GFAP-immunoreactive astrocytes in Wobbler mice spinal cord. Although the biological significance of this finding and its relation to neuronal sickness of Wobbler mice is still unclear, we interpret it in terms of the known beneficial role of astrocytes on neuronal function. Thus, neuronal damage caused by ischemia, toxins, excitotoxicity, injury and neurodegeneration was first followed by astrocyte hypertrophy and then hyperplasia with GFAP hyperexpression [83]. In this way, stimulated astrocytes would be endowed to provide neuroprotection. For a number of years, it has been realized that among other properties, astrocytes take up excess glutamate and potassium released

during neurotransmission, provide neurons with trophic factors, glucose and lactate used for energy purposes and even play a role in myelination of axons [47,48]. Thus, stimulation of a preexisting astrocytosis would be advantageous, in contrast to traditional concepts which associated astrocytosis with increased pathology and inhibition of neuronal regeneration [84]. Recently, Ikeda et al. [35] treated Wobbler mice with T-588, a non-steroidal antioxidant. By the time T-588 enhanced motor function, muscle parameters and survival time of the affected animals, it also stimulated astrocytosis. These data, in conjunction with previous work using the 21-aminosteroid U-74389F [49] supported that some antioxidant neuroprotectants also up-regulate astrocyte number and/or function. However, we did not find an effect of progesterone on GFAP immunostaining of astrocytes, suggesting that in contrast to the effects of U-74389F and T-588, progesterone protection may have occurred primarily on motoneurons. However, it is still possible that progesterone can affect in astrocytes molecules other than GFAP but not considered in our investigation.

10. Conclusions

We would like to suggest that restoration of motoneuron morphology, up-regulation of Na, K-ATPase subunit mRNAs, and down-regulation of aberrantly expressed GAP-43 mRNA may be linked to progesterone neuroprotection of symptomatic Wobbler mice. This assumption is supported by the increased muscle strength and prolonged survival of mutant mice receiving progesterone. The present work expanded to a genetic degeneration model multiple evidences of progesterone beneficial effects in experimental brain, peripheral nerve and spinal cord injury [3,9–17]. As already discussed, some of these effects may be due to progesterone binding to a transcriptionally-active progesterone intracellular receptor. However, membrane-based non-genomic mechanisms, including inhibition of free-radical attack of intracellular membranes, may also provide clues towards the molecular basis of steroid neuroprotection. Future studies and clinical trials will unravel the importance of progesterone to evade neuronal loss and accelerate reparative responses in human degenerative diseases.

Acknowledgements

This work was supported by grants from the University of Buenos Aires (M048), National Research Council of Argentina (PIP No. 02007), Beca Carrillo Oñativia from the Minister of Health, FONCYT (BID 802 OC AR PICT 2000 05-08663) and a Cooperative Program between the Governments of France and Argentina (ECOS/SECYT # A98SO1). The laboratory assistance of Paulina Roig, Analia Lima and Esther I. Nievas and EM assistance of Emerita Jorge Vilela and Mariana López Ravasio is gratefully acknowledged.

References

[1] I. Azcoitia, C. Fernández-Galaz, A. Sierra, L.M. Garcia-Segura, Gonadal hormones affect neuronal vulnerability to excitotoxin-induced degeneration, J. Neurocytol. 28 (2001) 699–710.
[2] B.S. Mc Ewen, The molecular and neuroanatomical basis for estrogen effects in the central nervous system, J. Clin. Endocrinol. Metab. 84 (1999) 1790–1797.
[3] M. Schumacher, I. Akwa, R. Guennoun, F. Robert, F. Labombarda, F. Desarnaud, P. Robel, A.F. De Nicola, E.E. Baulieu, Steroid synthesis and metabolism in the nervous system: trophic and protective effects, J. Neurocytol. 29 (2000) 307–326.
[4] A.F. De Nicola, Steroid hormones and neuronal regeneration, in: F.J. Seil (Ed.), Advances in Neurology, vol. 52, Raven Press, New York, 1993, pp. 199–206.
[5] R.D. Brinton, Cellular & molecular mechanisms of estrogen regulation of memory function and neuroprotection against Alzheimer's disease: recent insight and remaining challenges, Learning & Memory 8 (2001) 121–133.
[6] V.W. Henderson, The epidemiology of estrogen replacement therapy and Alzheimer's disease, Neurology 48 (7) (1997) S27–S28.
[7] C.D. Toran-Allerand, Novel sites and mechanisms of oestrogen action in the brain, Novartis Found. Symp. 230 (2000) 56–69.
[8] J.M. Vongher, C.A. Frye, Progesterone in conjunction with estradiol has neuroprotective effects in an animal model of neurodegeneration, Pharmacol. Biochem. Behav. 64 (1999) 777–785.
[9] H.L. Koenig, M. Schumacher, B. Ferzaz, A.N. Do Thi, A. Ressouches, R. Guennoun, I. Jung-Testas, P. Robel, Y. Akwa, E.E. Baulieu, Progesterone synthesis and myelin formation by Schwann cells, Science 268 (1995) 1500–1502.
[10] F. Desarnaud, A.N. Do Thi, A.M. Brown, G. Lemke, U. Suter, E.E. Baulieu, M. Schumacher, Progesterone stimulates the activity of the promoters of peripheral myelin protein-22 and protein zero genes in Schwann cells, J. Neurochem. 71 (1998) 1765–1768.
[11] J. Chen, M. Chopp, Y. Li, Neuroprotective effects of progesterone after transient middle cerebral artery occlusion in rat, J. Neurol. Sci. 171 (1999) 24–30.
[12] R. Roof, E. Hall, Gender differences in acute CNS trauma and stroke: neuroprotective effects and progesterone, J. Neurotrauma 17 (2000) 367–388.
[13] D.G. Stein, Z.L. Fulop, Progesterone and recovery after traumatic brain injury: an overview, Neuroscientist 4 (1998) 435–442.
[14] W.H. Yu, Survival of motoneurons following axotomy is enhanced by lactation or by progesterone treatment, Brain Res. 49 (2001) 379–382.
[15] A. Thomas, R. Nockels, H. Pan, C. Shaffrey, M. Chopp, Progesterone is neuroprotective after acute experimental spinal cord trauma in rats, Spine 24 (1999) 2134–2138.
[16] T. Ogata, Y. Nakamura, K. Tsuji, T. Shibata, T. Kataoka, Steroid hormones protect spinal cord neurons from glutamate toxicity, Neuroscience 55 (1993) 445–449.
[17] F. Labombarda, S. Gonzalez, M.C. Gonzalez Deniselle, R. Guennoun, M. Schumacher, A.F. De Nicola, Cellular basis for progesterone neuroprotection in the injured spinal cord, J. Neurotrauma 19 (2002) 343–355.
[18] C.J. Krebs, E.D. Jarvis, J. Chan, J.P. Lydon, S. Ogawa, D.W. Pfaff, D. W, A membrane-associated progesterone-binding protein, 25-Dx, is regulated by progesterone in brain regions involved in female reproductive behaviors, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 12816–12821.
[19] M.E. Cudkowicz, R.H. Brown, Amyotrophic lateral sclerosis and related motor neuron diseases, in: J.L. Jameson (Ed.), Principles of Molecular Medicine, Humana Press, NJ, 1988, pp. 907–911.
[20] L.W. Duchen, S.J. Strick, An hereditary motor neurone disease with progressive denervation of muscle in the mouse: the mutant Wobbler, J. Neurol. Neurosurg. Psychiatr. 31 (1968) 535–542.

[21] K. Kaupmann, D. Simon-Chazottes, J.L. Guener, H. Jokusch, H. Wobbler, a mutation affecting motoneuron survival and gonadal functions in the mouse, maps to proximal chromosome 11s, Genomics 13 (1992) 39–43.
[22] D.L. Price, D.W. Cleveland, V.E. Koliatsis, Motor neuron disease and animal models, Neurobiol. Dis. 1 (1994) 3–11.
[23] M.P. Junier, M. Coulpier, N. Le Forestier, J. Cadusseau, F. Suzuki, M. Peschanski, P.A. Dreyfus, Transforming growth factor alpha (TGF alpha) expression in degenerating motoneurons of the murine mutant Wobbler: a neuronal signal for astrogliosis? J. Neurosci. 14 (1994) 4206–4216.
[24] P. Dockery, Y. Tang, M. Morais, L.L. Vacca-Galloway, Neuron volume in the ventral horn in Wobbler mouse motoneuron disease: a light microscope stereological study, J. Anat. 191 (1997) 89–98.
[25] H. Mitsumoto, W.G. Bradley, Murine motor neuron disease (the Wobbler mouse): degeneration and regeneration of the lower motor neuron, Brain Res. 105 (1982) 811–834.
[26] S. Muyarama, K. Inoue, H. Kawakami, T.W. Bouidin, K. Suzuki, A unique pattern of astrocytosis in the primary motor area in amyotrophic lateral sclerosis, Acta Neuropathol. Berlin 82 (1991) 456–461.
[27] M.C. González Deniselle, S. González, A.F. De Nicola, Cellular basis of steroid neuroprotection in the Wobbler mouse, a model for motoneuron disease, Cell. Mol. Neurobiol. 21 (2001) 237–254.
[28] S. Boillee, L. Viala, M. Peschanski, P.A. Dreyfus, Differential microglial response to progressive neurodegeneration in the murine mutant Wobbler, Glia 33 (2001) 277–287.
[29] P. Popper, D.B. Farber, P.E. Micevych, K. Minoofar, J.M. Bronstein, RTPM-2 expression and tunel staining in neurodegenerative diseases: studies in Wobbler and rd mice, Exp. Neurol. 143 (1997) 246–254.
[30] R. Pernas-Alonso, C. Perrone-Capano, F. Volpicelli, U. di Porzio, Regionalized neurofilament accumulation and motoneuron degeneration are linked phenotypes in Wobbler neuromuscular disease, Neurobiol. Dis. 8 (2001) 581–589.
[31] B. Blondet, A. Ait-Ikhlef, M. Murawsky, F. Rieger, Transient massine DNA fragmentation in nervous system during the early course of a murine neurodegenerative disease, Neurosci. Lett. 305 (2001) 202–206.
[32] P.G.H. Clarke, Developmental cell death: morphological diversity and multiple mechanisms, Anat. Embryol. 181 (1990) 195–213.
[33] X. Guang-Ping, K.R. Dave, C.T. Moraes, R. Busto, T.J. Sick, W.G. Bradley, M.A. Perez-Pinzón, Dysfunctional mitochondrial respiration in the Wobbler mouse brain, Neurosci. Lett. 300 (2001) 141–144.
[34] K. Ikeda, Y. Iwasaki, M. Kinoshita, Neuronal nitric oxide synthase inhibitor 7-nitroindazole delays motor dysfunction and spinal motoneuron degeneration in the Wobbler mouse, J. Neurol. Sci. 160 (1998) 9–15.
[35] K. Ikeda, Y. Iwasaki, M. Kinoshita, S. Marubuchi, S. Ono, T-588, a novel neuroprotective agent, delays progression of neuromuscular dysfunction in Wobbler mouse motoneuron disease, Brain Res. 858 (2000) 84–91.
[36] J.T. Henderson, M. Javaheri, S. Kopko, J.C. Roder, Reduction of lower motor neuron degeneration in Wobbler mice by *n*-acetyl-L-cysteine, J. Neurosci. 16 (1996) 7574–7582.
[37] M. Li, M.V.O. Ona, C. Guegan, M. Chen, V. Jackson-Lewis, L.J. Andrews, A.J. Olsewski, P.E. Stieg, J.P. Lee, S. Przedborski, R.M. Friedlander, Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model, Science 288 (2000) 335–339.
[38] J.C. Carlson, M.Y. Gruber, J.E. Thompson, A study of the interaction between progesterone and membrane lipids, Endocrinology 113 (1983) 190–194.
[39] A.L. Betz, H.C. Coester, Effects of steroids on edema and sodium uptake of the brain during focal ischemia in rats, Stroke 21 (1990) 199–204.
[40] F. Labombarda, R. Guennoun, S. Gonzalez, P. Roig, A. Lima, M. Schumacher, A.F. De Nicola, Immunocytochemical evidence for a progesterone receptor in neurons and glial cells of the rat spinal cord, Neurosci. Lett. 288 (2000) 29–32.
[41] D.A. Monks, G. Arciszewska, N.V. Watson, Estrogen-inducible progesterone receptors in the rat lumbar spinal cord: regulation by ovarian steroids and fluctuation across the estrous cycle, Horm. Behav. 40 (2001) 490–496.
[42] K.K.L. Yung, F. Tang, L.L. Vacca-Galloway, Changes of neuropeptides in spinal cord and brain stem of Wobbler mouse at different stages of motoneuron disease, Neuroscience 50 (1982) 209–222.
[43] P. Liere, Y. Akwa, S. Weill-Engerer, B. Eychenne, A. Pianos, P. Robel, J. Sjovall, M. Schumacher, E.E. Baulieu, Validation of an analytical procedure to measure trace amounts of neurosteroids in brain tissue by gas chromatography–mass spectrometry, J. Chromatogr. Biomed. Sci. Appl. 10 (2000) 301–312.
[44] G. Lees, Inhibition of sodium-potassium-ATPase: a potencial ubiquitous mechanism contributing to central nervous system neurophatology, Brain Res. Rev. 16 (1991) 283–300.
[45] W. Stahl, The Na, K-ATPase of nervous tissue, Neurochemistry 8 (1986) 449–476.
[46] M.C. González Deniselle, C. Grillo, S. González, P. Roig, A.F. De Nicola, Evidence for down-regulation of GAP-43 mRNA in Wobbler mouse spinal motoneurons by corticosterone and a 21-aminosteroid, Brain Res. 841 (1999) 78–84.
[47] P. Magistretti, L. Pellerin, Astrocytes couple synaptic activity to glucose utilization in the brain, New Physiol. Sci. 14 (1999) 177–182.
[48] W. Liedtke, W. Edelman, P.L. Bieri, F.C. Chiu, N.J. Cowan, R. Kucherlapati, S. Raine, GFAP is necessary for the integrity of CNS white matter architecture and long-term maintenance of myelination, Neuron 17 (1996) 607–615.
[49] M.C. Gonzalez Deniselle, S. Gonzalez, A. Lima, G. Wilkin, A.F. De Nicola, The 21-aminosteroid U-74389F attenuates hyperexpression of GAP-43 and NADPH-diaphorase in the spinal cord of Wobbler mouse, a model for amyotrophic lateral sclerosis, Neurochem. Res. 24 (1999) 1–8.
[50] M.C. Gonzalez Deniselle, S. Gonzalez, G.G. Piroli, A. Lima, A.F. De Nicola, The 21-aminosteroid U-74389 F increases the number of glial fibrillary acidic protein-expressing astrocytes in the spinal cord of control and Wobbler mice, Cell. Mol. Neurobiol. 16 (1996) 61–72.
[51] S. González, C. Grillo, M.C. González Deniselle, A. Lima, B.S. McEwen, A.F. De Nicola, Dexamethasone up-regulates mRNA for Na, K-ATPase in some spinal cord neurons after cord transection, NeuroReport 7 (1996) 1041–1044.
[52] C. Grillo, G. Piroli, A. Lima, B.S. McEwen, A.F. De Nicola, Aldosterone up-regulates mRNA for the α3 and β1 isoforms of (Na, K)-ATPase in several brain regions from adrenalectomized rats, Brain Res. 767 (1997) 120–127.
[53] E.J. Elliott, D.A. Parks, P.S. Fishman, Effect of proximal axotomy on GAP-43 expression in cortical neurons in the mouse, Brain Res. 755 (1997) 221–228.
[54] H. Mitsumoto, P. Gambetti, Impaired slow axonal transport in Wobbler mouse motor neuron disease, Ann. Neurol. 19 (1986) 36–43.
[55] S. Laage, G. Zoberl, H. Jockusch, Astrocyte overgrowth in the brain stem and spinal cord of mice affected by spinal atrophy, Wobbler, Dev. Neurosci. 10 (1998) 190–198.
[56] M.P. Junier, M. Coulpier, N. Le Forestier, J. Cadusseau, F. Suzuki, M. Peschanski, P.A. Dreyfus, Transforming growth factor alpha (TGF alpha) expression in degerenating motoneurons of the murine mutant Wobbler: a neuronal signal for astrogliosis? J. Neurosci. 14 (1994) 4206–4216.
[57] M. Coulpier, M.-P. Junier, M. Peschanski, P.A. Dreyfus, Bcl-2 sensitivity differentiates two pathways for motoneuronal death in the Wobbler mutant mouse, J. Neurosci. 16 (1996) 5897–5904.
[58] I.-W. Chu-Wang, R.W. Oppenheimer, Cell death of motoneurons in the chick embryo spinal cord, J. Comp. Neurol. 177 (1978) 33–58.
[59] M.P. Mattson, Modification of ion homeostasis by lipid peroxidation: roles in neuronal degeneration and adaptive plasticity, Trends Neurosci. 20 (1998) 53–57.
[60] E.D. Hall, P.K. Andrus, J.A. Oostveen, T.J. Fleck, M.E. Gurney, Relationship of oxygen radical-induced lipid peroxidative damage to

disease onset and progression in a transgenic model of familial ALS, J. Neurosci. Res. 53 (1998) 66–77.
[61] J. Kong, Z. Zu, Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1, J. Neurosci. 18 (1998) 3241–3250.
[62] Z. Mourelatos, N.K. Gonatas, A. Stieber, M.E. Gurney, M.C. Dal Canto, The Golgi apparatus of spinal cord motor neurons in transgenic mice expressing mutant Cu, Zn superoxide dismutase becomes fragmented in early, preclinical stages of the disease, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 5472–5477.
[63] M.E. Gurney, What transgenic mice tell us about neurodegenerative disease, Bio. Essays 22 (2000) 297–304.
[64] P.C. Wong, C.A. Pardo, D.R. Borchelt, M.K. Lee, N.G. Copeland, N.A. Jenkins, S.S. Sisodia, D.W. Cleveland, D.L. Price, An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria, Science 271 (1995) 515–518.
[65] W.P.J.M. Spooren, B. Hengerer, DNA laddering and caspase 3-like activity in the spinal cord of a mouse model of amyotrophic lateral sclerosis, Cell. Mol. Biol. 46 (2000) 63–69.
[66] B.M. Morrison, W.G. Janssen, J.W. Gordon, J.H. Morrison, Time course of neuropathology in the spinal cord of G86R superoxide dismutase transgenic mice, J. Comp. Neurol. 391 (1998) 64–77.
[67] A. Migheli, C. Atzori, R. Piva, M. Tortarolo, M. Girelli, D. Schiffer, C. Bendotti, Lack of apoptosis in mice with ALS, Nat. Med. 5 (1999) 966–967.
[68] K. Abe, S. Morita, T. Kikuchi, Y. Itoyama, Protective effect of a novel free-radical scavenger, OPC-14117, on Wobbler mouse motor neuron disease, J. Neurosci. Res. 48 (1997) 63–70.
[69] K. Ikeda, M. Kinoshita, Y. Iwasaki, N. Tagaya, T. Shiojima, Lecithinized superoxide dismutase retards Wobbler mouse motor neuron disease, J. Neurosci. Res. 48 (1995) 63–70.
[70] E.D. Hall, J.M. Braughler, Effects of intravenous methylprednisolone on spinal cord lipid peroxidation and (Na, K)-ATPase activity. Dose response analysis during 1st hour after contusion injury in the cat, J. Neurosurg. 49 (1982) 563–568.
[71] N.B. Chauhan, G.J. Siegel, In situ analysis of Na, K-ATPase α1 and α3-isoform mRNAs in aging rat hippocampus, J. Neurochem. 66 (1996) 1742–1751.
[72] A. Derfoul, N. Robertson, J. Lingrel, J. Hall, R. Litwack, Regulation of the human Na/K-ATPase β1 gene promoter by mineralocorticiod and glucocorticoid receptors, J. Biol. Chem. 273 (1998) 20702–20711.
[73] N. Farman, J. Bonvalet, J. Seckl, Aldosterone selectively increases Na/K-ATPase α3-subunit mRNA expression in rat hippocampus, Am. J. Physiol. 266 (1994) C423–C428.
[74] W. Vedekis, Nuclear receptors, transcriptional regulation, and oncogenesis, Proc. Soc. Exp. Biol. Med. 199 (1992) 1–12.
[75] J. Zilliacus, A. Wright, J. Carlstedt-Duke, J. Ake-Gustafsson, Structural determinants of DNA-binding specificity by steroid receptor, Mol. Endocrinol. 4 (1995) 389–400.
[76] H. Meiri, Is synaptic transmission modulated by progesterone? Brain Res. 385 (1986) 193–196.
[77] L.I. Benowitz, A. Routtenberg, GAP-43: an intrinsic determinant of neuronal development and plasticity, TINS 20 (1997) 84–91.
[78] A.B. Oestreicher, P.N.E. De Graan, W.H. Guispen, J. Verhaagen, L.H. Schrame, B-50, the growth associated protein-43: modulation of cell morphology and communication in the nervous system, Prog. Neurobiol. 53 (1997) 627–686.
[79] R. Curtis, S. Averill, J.V. Priestley, G.P. Wilkin, The distribution of GAP-43 in the normal spinal cord, J. Neurocytol. 22 (1993) 9–50.
[80] R. Curtis, D. Green, R.M. Lindsay, G.P. Wilkin, Up-regulation of GAP-43 and growth of axons in rat spinal cord after compression, J. Neurocytol. 22 (1993) 51–64.
[81] I.M. Parhad, R. Oishi, A.W. Clarck, GAP-43 gene expression is increased in anterior horn cells of amyotrophic lateral sclerosis, Ann. Neurol. 31 (1992) 593–597.
[82] J.P. Kapfhammer, M.E. Schwab, Inverse patterns of myelination and GAP-43 expression in the adult CNS: neurite growth inhibitors as regulators of neuronal plasticity, J. Comp. Neurol. 340 (1994) 194–206.
[83] M.D. Norenberg, Astrocytes responses to CNS injury, J. Neuropathol. Exp. Neurol. 53 (1994) 213–220.
[84] R.J. McKeon, R.C. Schreiber, J.S. Rudge, J. Silver, Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes, J. Neurosci. 11 (1991) 3398–3411.