Sigma-1 Receptor in Motoneuron Disease

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Abstract

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease affecting spinal cord and brain motoneurons, leading to paralysis and early death. Multiple etiopathogenic mechanisms appear to contribute in the development of ALS, including glutamate excitotoxicity, oxidative stress, protein misfolding, mitochondrial defects, impaired axonal transport, inflammation and glial cell alterations. The Sigma-1 receptor is highly expressed in motoneurons of the spinal cord, particularly enriched in the endoplasmic reticulum (ER) at postsynaptic cisternae of cholinergic C-terminals. Several evidences point to participation of Sigma-1R alterations in motoneuron degeneration. Thus, mutations of the transmembrane domain of the Sigma-1R have been described in familial ALS cases. Interestingly, Sigma-1R KO mice display muscle weakness and motoneuron loss. On the other hand, Sigma-1R agonists promote neuroprotection and neurite elongation through activation of protein kinase C on motoneurons in vitro and in vivo after ventral root avulsion. Remarkably, treatment of SOD1 mice, the most usual animal model of ALS, with Sigma-1R agonists resulted in significantly enhanced motoneuron function and preservation, and increased animal survival. Sigma-1R activation also reduced microglial reactivity and increased the glial expression of neurotrophic factors. Two main interconnected mechanisms seem to underlie the effects of Sigma-1R manipulation on motoneurons: modulation of neuronal excitability and regulation of calcium homeostasis. In addition, Sigma-1R also contributes to regulating protein degradation, and reducing oxidative stress. Therefore, the multi-functional nature of the Sigma-1R represents an attractive target for treating aspects of ALS and other motoneuron diseases.

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16.1 Introduction: Motoneuron Diseases

Motoneuron diseases (MND) are progressive neurodegenerative disorders of wide etiology and clinical spectra, but with a common feature: the loss of lower and/or upper motoneurons. Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are the most frequent forms of MND and therefore the most studied. ALS was first described by Charcot in 1869 and is the most common type of MND in adults, with an incidence of 1–5 per 100.000. Concomitant degeneration of both upper (corticospinal/corticobulbar) and lower (spinal/bulbar) motoneurons distinguishes ALS from other forms of MND [1–3]. The main neuropathological features of ALS include extensive loss of motoneurons in the anterior horns of the spinal cord and motor nuclei of the brainstem, degeneration of the corticospinal tract, and degeneration and loss of large pyramidal neurons in the primary motor cortex, also accompanied by reactive gliosis around the areas of degeneration [3]. Cytoplasmic protein inclusions are common in the degenerating neurons, which predominantly comprise a nuclear RNA processing protein, TDP-43 (TAR-DNA binding protein 43) [4] It has been classically considered that despite most ALS cases are sporadic (sALS), 5–10 % are familiar (fALS), related with several genetic mutations [1, 5]. No matter if they are sporadic or familiar, patients develop progressive weakness and muscle atrophy, with spasticity and contractures. Progressive weakness may start distally or proximally in the upper or lower limbs and finally affect all muscles, including those related with breathing, speaking and swallowing. Patients die, mostly due to respiratory failure, by 2–5 years after diagnosis [2, 6].

No effective treatment is presently available for ALS [1]. Patient care focuses on symptomatic treatments and physical therapy. Assisted ventilation and nutrition can transiently overcome the loss of upper airway and respiratory muscular control [2]. A large number of therapeutic trials have been attempted, but it was not until the early 1990s that the first drug approved by the FDA for the treatment of patients with ALS reached the market: riluzole, an antiglutamatergic agent that blocks the presynaptic release of glutamate. However, the efficacy of riluzole is questionable, with minimal therapeutic benefits of about 3–4 months of survival increase [7]. One of the main concerns for developing new therapies is the lack of direct translation from promising preclinical findings to successful clinical results. Although the heterogeneous and complex nature of ALS has been studied extensively, the absence of early detection markers and proper biomarkers for the disease evaluation of patients does not allow identifying whether patients are at different stages or even developing the disease because of different underlying causes. These drawbacks often lead to a difficult interpretation of the results from clinical studies. In this sense, patients who develop the disease mainly because of defects in a particular pathway would display greatest benefit from the compounds that selectively target that pathway. Interestingly and supporting this idea, in most clinical trials, a subset of subjects showed improvement, but none of the compounds displayed an overarching effect on most patients. Therefore it seems that each clinical trial has been successful only in a select subset of individuals. Since ALS is a multifactorial disease, future strategies should be focused on multi-target drugs or on combinatorial treatments that might maximize the translational effects [1].

Frontotemporal lobal degeneration (FTLD or FTD) is the second most common type of dementia after Alzheimer's disease. It is caused by progressive neuronal atrophy and loss in the

frontotemporal cortex, and is characterized by personality and behavioral changes, as well as gradual impairment of language skills [8]. Traditionally, ALS and FTLD were considered as two distinct identities. However, novel evidence suggests that both pathologies belong to a clinical continuum, with pure forms linked by overlapping syndromes. The first link established between FTLD and ALS was the identification of TDP-43 positive ubiquitinated cytoplasmic inclusions in almost all ALS and more than a half FTLD patients [8, 9]. Although neuropsychological testing shows normal cognition in the majority of ALS patients, up to 50 % of them may present some degree of cognitive impairment, while 15–18 % meet the criteria for FTLD [10]. On the contrary, few patients with FLTD develop ALS [11]. Indeed, FTLD-only, ALS-only and coincident FTD-ALS cases were reported to occur inside the same family, supporting the hypothesis of a link between both pathologies. The recent finding of an hexanucleotid expansion in C9ORF72 constitutes a strong link between ALS and FTLD [12–15].

16.2 Pathophysiological Mechanisms Underlying Motoneuron Death

The exact molecular pathway causing motoneuron degeneration in ALS is unknown, but as with other neurodegenerative diseases, it is likely to be a complex interplay between multiple pathogenic mechanisms that may not be mutually exclusive and in which is still unknown the causative relation between them or whether they are the consequence of an upstream disturbance [1, 5, 16].

The identification of underlying genetic defects of familial cases of ALS has allowed the development of relevant animal models of the disease in mice, rats, zebra fish and drosophila [1, 4, 17–19], which have been essential for uncovering morphological and molecular pathogenic events in vivo that are not possible to investigate in humans. The most widely used ALS models are transgenic mice over-expressing human mutated forms of the SOD1 gene, which recapitulate the most relevant clinical and histopathological features of both familial and sporadic ALS.

Among the proposed pathophysiological mechanisms, excitotoxicity has been deeply explored. Neuronal injury caused by excitatory mediators may be due to failure in the neurotransmitter clearance from the synaptic cleft or increased postsynaptic sensitivity to glutamate. This enhanced excitatory input induces a massive calcium influx into the cytoplasm that damages the cells through the activation of calcium-dependent proteases, lipases and nucleases. A large body of evidence implicates excitotoxicity as a mechanism contributing to motoneuron death in ALS, such as threefold increased levels of glutamate in CSF from ALS patients [20, 21]. Furthermore, overactivation of NMDA receptors and increased calcium permeability of AMPA receptors have been described in ALS mouse models [1, 22–25]. Loss of the glial excitatory amino acid transporter 2 (EAAT2) was also reported in ALS mouse models [26, 27].

Oxidative stress results from the imbalance between the production of reactive oxygen species (ROS) and the biological capacity to remove ROS or repair ROS-induced damage. The analysis of CSF and serum from ALS patients showed increased concentration of ROS compared to healthy subjects [28–31]. Evidence of oxidative stress damage to proteins [32], lipids [30] and DNA [33] was also reported to occur in ALS patients. Oxidative stress has been also documented in ALS mouse models [34, 35].

Mitochondria are the cellular organelle in charge of ATP production, calcium homeostasis maintenance and intrinsic apoptosis regulation. An important core of evidences implicates mitochondria as key players in ALS physiopathology [36]. Reduced mitochondrial DNA content associated with increased mutations of mitochondrial DNA, and respiratory chain complexes dysfunction have been described in the spinal cord of ALS patients [37]. Mitochondrial function impairments affect also the skeletal muscle of ALS patients [38]. In vitro studies showed mitochondrial morphological and functional altera-

tions in NSC-34 cells expressing mutant SOD1 [39]. Experiments performed in mSOD1 mice also revealed early mitochondrial morphological abnormalities prior to onset of symptoms [40].

Neurons are polarized cells that require efficient mechanisms to direct axonal vs. dendritic transport. Since neurons transmit signals along long distances, proteins and organelles have to travel more than in other cell types (axons of human motoneurons can reach 1 m long). Even within the axon, cargos must be delivered to specific compartments, thereby increasing the importance of axonal transport in motoneurons. Several works demonstrated the accumulation of neurofilaments in motoneuron cell bodies in human patients, suggesting that axonal transport is impaired in these cells [41–44]. Additionally, abnormalities of organelle axonal trafficking occur in ALS patients [45]. Axonal transport has been widely studied in animal models mimicking ALS. It has been demonstrated that transgenic mice overexpressing SOD1 transgene develop neuronal cytoskeletal pathology resembling human ALS [46]. Controversially, recent evidence suggests that axonal transport deficits may evolve independently from motoneuron degeneration in mutant SOD1 mice [47]. Marinkovic et al. [47] demonstrated that mutant SOD1 axons are able to survive despite long-lasting transport deficits since these are present soon after birth, months before the first signs of muscle denervation [48–50].

Protein aggregates or inclusions have long been recognized as a pathological hallmark of several neurodegenerative disorders, including ALS, in which protein aggregates are common in spinal motoneurons [1]. Ubiquitin-positive inclusions are characteristic of ALS histopathology. Nevertheless, it remains unclear whether inclusion formation is responsible for cellular toxicity and ALS pathogenesis, if aggregates may be innocuous neurodegeneration-derived products, or if they may represent a protective reaction of the cell to reduce intracellular concentrations of toxic proteins. Several proteins are found forming the intracellular inclusions in ALS, including neurofilaments [42–44], SOD1 [51–53], TDP-43 [9, 54], FUS [55, 56], ubiquilin 2 [57] and C9OFF72 [12, 13].

Physiologically, accumulation of misfolded proteins elicits the endoplasmic reticulum (ER) stress response. ER-resident chaperones sense the accumulation of misfolded proteins and activate the Unfolded-Protein Response (UPR), which leads to the suppression of general translation and ER-associated protein degradation. However, prolonged UPR activation may trigger apoptotic signaling [58]. The addition of CSF from ALS patients induces ER stress on cultured NSC-34 cells and primary rat spinal motoneuron cells [59]. Considerable evidence implicates ER stress as an important feature of motoneuron degeneration in ALS. UPR markers are upregulated in sALS patients [60] as well as in mutant SOD1 rodent models [61, 62]. Interestingly, a longitudinal gene expression profile in mutant SOD1 mice revealed early upregulation of several UPR markers prior to muscle denervation in vulnerable motoneurons (innervating fast fatigable muscles, e.g. extensor digitorum longus) compared to resistant motoneurons (innervating slow muscle fibers, e.g. soleus). Similar changes eventually occurred in disease-resistant motoneurons but 25–30 days later [62], suggesting a role for ER stress in determining the susceptibility of motoneurons in ALS.

Neighboring glial cells also play a crucial role in the motoneuron degeneration occurring in ALS [1, 63, 64]. Clement et al. [65] generated chimeric mice expressing mSOD1 in specific cell lines and demonstrated that normal motoneurons developed ALS signs when surrounded by mutant SOD1-expressing glia. To further explore the contribution of microglia in ALS, double transgenic mice were generated expressing the Cre–Lox recombination system to selectively suppress the mutant SOD1 expression in motoneurons or microglia. Mutant SOD1 deletion in motoneurons lead to delayed disease onset but no modifications of disease progression once initiated. On the other hand, mutant SOD1 suppression in microglia and macrophages did not alter disease onset but significantly prolonged mice survival. These findings suggest that disease onset and

progression might be related to different mechanisms [66, 67]. It is also accepted that astrocytes play a role in ALS. Astrocytes derived from postmortem tissue of familial and sporadic ALS patients are toxic to motoneurons but not to GABAergic neurons. Blocking mSOD1 expression produced significant neuroprotective effects on ALS-derived astrocytes [68].

Neuroinflammation is a common pathological event of neurodegenerative disorders [69] and its modulation has been proposed as an important potential therapeutic target [70]. In ALS, motoneuron damage leads to the activation of microglia, astrocytes and the complement system, further contributing to neurodegeneration [71, 72]. Spinal cord tissue and CSF from sporadic and familial ALS cases present increased microglial activation and T cells infiltration [73, 74] as well as higher concentration of some proinflammatory mediators, including monocyte chemoattractant protein 1 (MCP-1) and IL-8 [75]. Gene array analysis of mutant SOD1 mice revealed an enhanced expression of inflammatory-related molecules especially at late stages of the disease [76, 77].

RNA processing abnormalities were first related to MND by the description of Spinal Motor Neuron protein 1 (SMN1) mutations as a cause of SMA [78]. The SMN proteins play a role in the assembly of small ribonucleoproteins, which participate in pre-mRNA splicing [79]. Later identification of TDP-43, a RNA-DNA binding protein, as a major component of the ubiquitinated protein inclusions in ALS patients [9] focused the attention to RNA processing alteration as an important pathophysiological mechanism of the disease. TDP-43 is predominantly localized in the nucleus where it is implicated in several events for RNA processing, including transcriptional regulation, alternative splicing and microRNA processing. ALS-related TDP-43 positive cytoplasmic inclusions are present in neuronal and non-neuronal cells, excluding those based on mSOD1 and FUS mutations [54, 80]. Recent studies have evaluated the RNA-binding targets of TDP-43 [81–83] and revealed that TDP-43 binds to several RNA target molecules (about 30 % of the mouse transcriptome). Such high level of intronic binding suggests a nuclear function for TDP-43. In fact, blocking tardbp43 expression using antisense oligonucleotides in adult mouse striatum altered the expression levels of 601 mRNA and changed the splicing pattern of 965 mRNA transcripts, including some relevant to neurodegeneration, such as progranulin, choline acetyltransferase or FUS [81]. TDP-43 alteration might potentially alter the transcriptional process of crucial genes for motoneuron homeostasis. Additional evidence about dysregulated RNA processing as motoneuron injury contributor in ALS arises from the detection of RNA oxidation biomarkers in human ALS and mSOD1 mice [84]. Since the discovery of C9ORF72 hexanucleotide (G_4C_2) repeat expansions as a frequent cause of ALS and FTD, efforts have been conducted for investigating linked pathophysiological abnormalities. Repeat containing RNA foci in these patients suggested a deleterious gain of function. Repeats are able to form G-quadruplexes, which may be able to facilitate the binding and sequestration of different RNA binding proteins to the repeat [85]. Subsequently, these proteins are not able to execute their normal functions. Another mechanism is the possible occurrence of repeat-associated non-ATG (RAN) translation along the hairpin-forming repeat. This results in aggregates containing different dipeptide repeat proteins in patients with the C9ORF72 repeat expansion [15, 86]. Recently, two independent studies used engineered drosophila to express high repeat expansions of G_4C_2 [87, 88], and established a strong connection between defective nuclear trafficking and neurodegeneration in these flies.

16.3 Structure and Functions of Sigma-1 Receptor

The Sigma-1R is a transmembrane protein found in the ER [89, 90], which is highly expressed in motoneurons and other cells in the spinal cord [89, 91–94]. Although it was initially classified as an opioid receptor, further experiments showed that its properties were distinct from known opioid receptors [95]. A 223 amino acids Sigma-1R

protein has been cloned from several mammals, and contains 90 % identical and 95 % similar amino acid sequences across species, with both the N- and C-termini on the same side of the membrane facing the cytoplasm [90, 96], and can be present in monomeric or oligomeric forms even in the absence of ligand [97, 98]. The N-terminus, of approximately 110 amino acids, determines the diversity of intracellular interactions of Sigma-1R with a variety of proteins [99–102]. Many synthetic compounds have been characterized as selective modulators of the Sigma-1R [103], and several endogenous molecules have been proposed to be Sigma-1R ligands as well, including lipid steroids (DHEA, progesterone and pregnenolone sulfate) [104], lipid sphingosine derivatives [105] and N,N-dimethyl tryptamine (DMT) [106]. It is plausible that these compounds regulate Sigma-1R function in different tissues according to their availability.

This receptor has the ability to translocate from the ER to the plasma membrane and mitochondria-associated membranes [90, 107, 108]. In the nervous system, Sigma-1R mediates regulation of a wide range of processes, such as neuritogenesis [109], modulation of K+ channels [110] and N-methyl-D-aspartate (NMDA) receptors activity [111], ER-mitochondria communication [90], modulation of G-protein couples receptors (GPCRs), Ca2+ homeostasis [90], and microglial activity [112]). The Sigma-1R appears as a pluri-functional target involved in a broad range of cellular processes and, thereby, its modulation might provide better translational outcomes than drugs acting selectively on one of these multiple aspects.

Langa et al. [113] developed homozygous Sigma-1R knock out mice, which showed to be fully fertile and with no obvious behavioral alterations. However, further careful analyses revealed alterations of hippocampal neurogenesis [114, 115], ethanol consumption [116], retinal function [117, 118], anxiety, memory impairments [119] and, most relevant, motor dysfunction and loss of neuromuscular junctions [120, 121].

16.4 Sigma-1 Receptor and Motoneurons

To fully understand the mechanisms underlying Sigma-1R role in motoneurons, it is important to know its subcellular localization in the cells. It has been shown that Sigma-1R is enriched in the subsurface cisternae in postsynaptic C-terminals of motoneurons [120]. Synaptic innervation onto motoneurons is complex, with synapses involving all the major neurotransmitters, that have been classified as S, M, T F, P and C-boutons/terminals (referring to the pre- or postsynaptic structure, respectively) [122]. C-terminals are large cholinergic postsynaptic sites with a unique ultrastructure seen at the electron microscopy level. They are referred to as “C” because of the subsurface cisternae of smooth endoplasmic reticulum adjacent to the plasma membrane, and are large synapses found only on soma and proximal dendrites of motoneurons [123]. Presynaptic C-boutons originate from a group of cholinergic interneurons located near the spinal cord central canal, which have been shown to increase motoneuron excitability and, thus, potentiate muscle contraction [124]. Interestingly, Sigma-1R is specially enriched in the subsurface cisternae underlying the postsynaptic membrane of C-boutons in motoneurons. Diverse alterations of C-boutons have been reported in animal models of ALS and spinal cord injury [125–128]. The postsynaptic membrane of C-boutons is rich in numerous proteins, including m2-type muscarinic receptors (m2AChR) [129–131], voltage-gated Kv2.1 [132], Kv1.4, Kv1.5 [110] and Ca^{2+}-activated K^+ (SK) channels [133], connexin 32 [134], VAMP-2 [129], Sigma-1R [135] and neuregulin-1 [136]; whereas the presynaptic element contains, at least, neuregulin-1 receptors ErbB2 and ErbB4 [136]. In contrast, the role of subsurface cisternae in postsynaptic densities where Sigma-1 receptors are located is still unknown, but believed to couple the electrical activity of the plasma membrane with intracellular signaling involving the ER [137, 138].

Cholinergic innervation onto motoneurons plays a role in modulating the excitability of the cells during locomotion [124, 139]. Interestingly, Sigma-1R, m2AChR and SK channels have special relevance regarding motoneurons excitability. Indeed, it has been proposed that differential expression of SK2.2 and SK2.3 channels in neurons is a marker for α-motoneurons innervating fast or slow muscle fibers modifying the hyperpolarization properties of the plasma membrane [133]. Miles et al. [139] described how cholinergic innervation on motoneurons increases excitability during fictive locomotion by acting on m2AChR, whereas motoneurons lacking Sigma-1R have increased excitability [140]. Sigma-1R has been also shown to interact with diverse potassium channels, thereby shaping neuronal excitability [99, 110, 141, 142] (Fig. 16.1).

Sigma-1R co-localizes with neuregulin-1 expressed at the motoneuron C-boutons postsynaptic membrane [136]. Neuregulin-1 is a neurotrophic factor essential for the normal development and function of the nervous system [143]. Neuregulin-1 ErbB receptors are also located in the presynaptic terminals of C-boutons. Neuregulin-1/ErbB system alterations have been related to ALS, with reduced neuregulin-1 type III expression in the spinal cord of ALS patients and mouse models [144]. Loss-of-function mutations on the gene encoding for ErbB4 receptor produce late-onset ALS in patients [145]. Although no link between Sigma-1R and neuregulin-1 has been established yet, it is likely that Sigma-1R serves as a chaperone for neuregulin-1 at subsurface cisternae of motoneurons, as it has been shown to participate in the post-translational processing of other neurotrophic factors [146] (Fig. 16.1).

Little is known about endogenous ligands for Sigma-1R. It has been shown that N,N-dimethyltryptamine (DMT) is an endogenous agonist for the Sigma-1R [147] and that Indole(ethyl)amine N-methyltransferase (INMT), the enzyme that converts the amino acid tryptophan into DMT, co-localizes with Sigma-1R at C-terminals of motoneurons [135]. Endogenous steroids have been shown to act as Sigma-1R agonists, including dehydroepiandrosterone (DHEA) sulfate and pregnenolone sulfate [148]. Nevertheless, further studies are needed to elucidate the mechanisms by which Sigma-1R function is endogenously modulated and how this affects motoneuron physiology (Fig. 16.1).

16.5 Evidences of Sigma-1 Receptor Contribution in Motoneuron Disease

There is a body of evidence suggesting that Sigma-1R alterations lead to motoneuron dysfunction and degeneration [121, 140]. Mutations in a highly conserved region of the transmembrane domain of the Sigma-1R were described in ALS patients. The mutation produces an aberrant subcellular distribution of the receptor in NSC34 cells, and cells expressing the mutant protein are more prone to undergo apoptosis induced by ER stress [149]. Sigma-1R was found to abnormally redistribute in alpha-motoneurons of ALS patients and form ubiquitinated aggregates that lead to UPR. Additionally, Sigma-1R levels were found reduced in samples of ALS patients [150]. Other mutations in the 3′-untranslated region (UTR) of the Sigma-1R gene were described in affected individuals with the FTD-ALS pedigree [151].

Interestingly, Sigma-1R KO mice display locomotor deficits associated with muscle weakness, axonal degeneration and motoneuron loss [121, 140]. Altered Sigma-1R function in motoneurons has been also shown to disrupt ER-mitochondria contacts and affect intracellular calcium signaling, leading to activation of ER stress and to defects in mitochondrial dynamics and transport [121]. Crossing Sigma-1R KO mice with mutant SOD1 mice ($SOD1^{G93A}$) exacerbated the motor phenotype and accelerated the end stage of the disease [140]. Conversely, stimulating Sigma-1R function using the agonists PRE-084 or SA4503 has been shown protective in both *in vitro* and *in vivo* models of mutant SOD1 ALS [94], as well as in non-SOD1 linked MND [152].

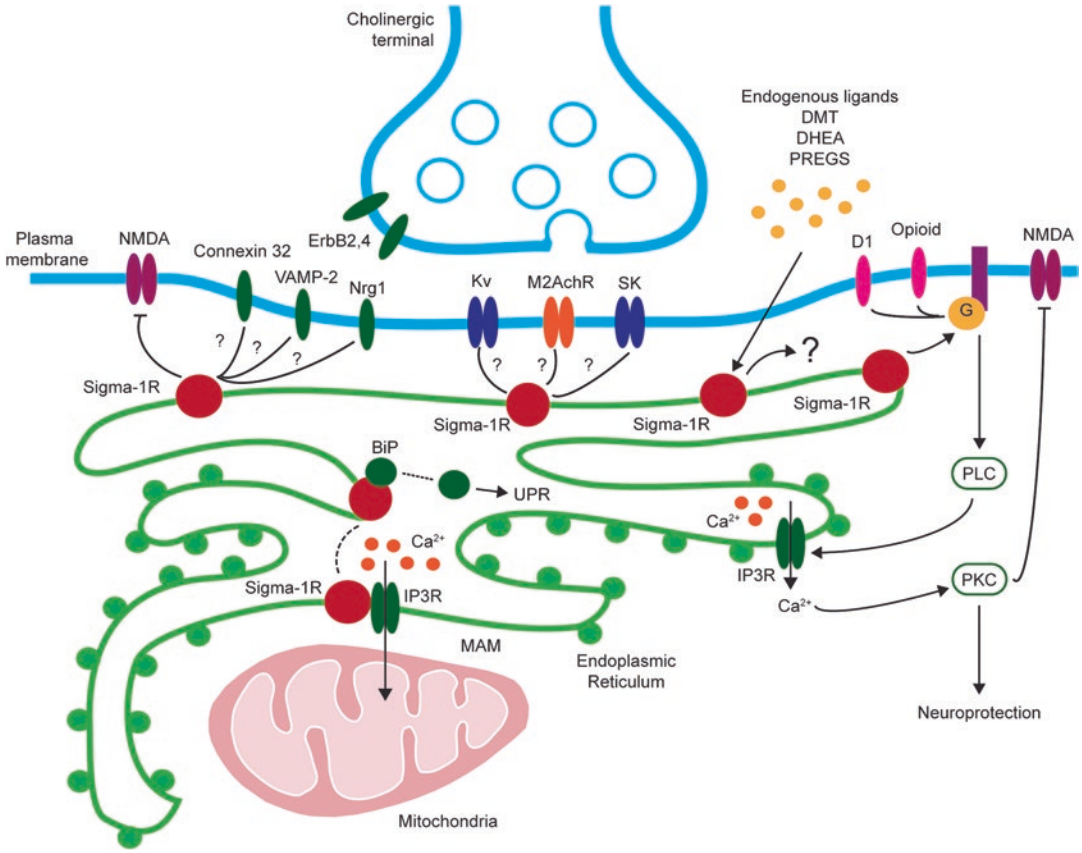

Fig. 16.1 Sigma-1 receptor localization at the C-boutons and its pleiotropic role in the motoneuron. Sigma-1 receptor is located at the endoplasmic reticulum subsynaptic cisterna of the cholinergic synapses, from where it may interact both with elements of the plasma membrane (e.g. ion channels) or the cytoplasm (e.g. mitochondria). Sigma-1 receptor modulates the activity of several ionotropic and metabotropic receptors, including M2AchR, NMDA, dopaminergic D1 and opioid receptors. Further studies are needed to elucidate how Sigma-1 receptor interacts with ion channels (Kv or SK) and other elements present at the C-boutons, such as Connexin32, VAMP-2 and Neuregulin1. The Sigma-1 receptor is also able to interact with BiP, a chaperone of the endoplasmic reticulum, and to participate in the interactions between the endoplasmic reticulum and the mitochondria. For further details, see the Sect. 16.4 text

A note of caution must be taken since there is controversy about the expression profile of Sigma-1R in mutant SOD1 ALS models. Analysis of Sigma-1R in protein extracts from lumbar anterior spinal cord showed no changes in the amount of Sigma-1R expressed [94], whereas immunohistochemical analysis revealed decreased labeling of Sigma-1R at the C-boutons of SOD1 lumbar motoneurons at early presymptomatic stages of the disease [127].

16.6 Potential Mechanisms on Sigma-1 Receptor-Mediated Therapeutic Actions

The Sigma-1R has been shown to be a target for the treatment of a variety of chronic neurological diseases, including pain [153–155], depression [148], Alzheimer's [156–158], Parkinson's [159], and Huntington [160] diseases, schizophrenia

[161], stroke [162, 163], ischemia [164], degeneration of retinal neurons [117, 118], and selective cholinergic lesions [156]. The administration of Sigma-1R ligands has promoted neuroprotection after several types of insults, including excitotoxic damage [165], hypoxia-mediated neurotoxicity [166], oxidative stress-induced cell death [167] and glucose deprivation [164].

Regarding motoneurons, the selective Sigma-1R agonist PRE-084 has been reported to exert positive effects on motoneuron death. PRE-084 administration promotes neuroprotection and neurite elongation through activation of protein kinase C (PKC) on motoneurons in an *in vitro* organotypic model of excitotoxic lesion [168]. Moreover, administration of PRE-084 significantly prevented the marked death of spinal motoneurons after spinal root avulsion in adult rats, an effect that was associated with attenuating ER stress within motoneurons and promoting the expression of GDNF by surrounding glial cells [93]. Remarkably, treatment of SOD1 mice with Sigma-1R agonists resulted in significantly improved motoneuron function and preservation, and increased animal survival [94, 152, 169, 170]. Several mechanisms have been hypothesized to underlie motoneuron protection in ALS models (Fig. 16.2). Sigma-1R agonists administration resulted in increased PKC-specific phosphorylation of NR1 subunits present in spinal motoneurons, likely reducing the calcium permeability of NMDA receptors and its influx into motoneurons, thereby attenuating excitotoxicity [94, 111]. Sigma-1R agonists, such as SKF10097 and PRE-084, have been reported to also suppress NMDA currents in rat retinal ganglion cells and cortical neurons through a PKC-dependent mechanism, leading to reduction of calcium influx into the cytoplasm [111, 166]. Sigma-1R agonists administration also reduced microglial and astroglial reactivity in the mutant SOD1 and in the wobbler ALS mouse models, and enhanced glial expression of neurotrophic factors, such as BDNF [94, 152]. In this sense, Sigma-1R activation has been linked to modulation of multiple aspects of microglial activation *in vitro* [171, 172], as well as to increase the glial expression of neurotrophic factors after spinal root avulsion [93].

Overall, two main interconnected mechanisms are likely to underlie the direct effect of Sigma-1R manipulation on motoneurons: the modulation of the neuronal excitability and the calcium homeostasis. The Sigma-1R is located in C-terminals in close proximity to Kv2.1 and SK channels, which appear as two suitable candidates for the Sigma-1R modulation of postsynaptic excitabil-

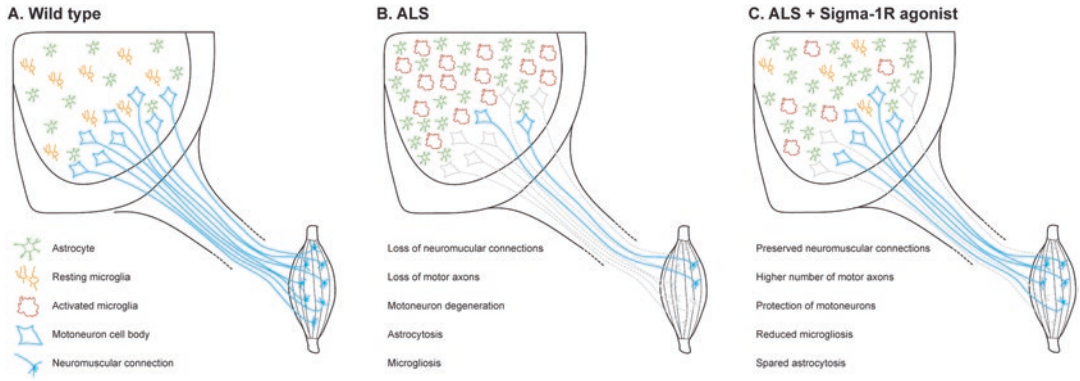

Fig. 16.2 Schematic representation of the effect of agonizing the Sigma-1 receptor in ALS mouse models. (**a**) Wild type spinal cord motoneurons project their axons from the anterior horn of the spinal cord through the anterior root to reach the skeletal muscles. (**b**) ALS spinal cord suffers a dramatic death of motoneurons, accompanied by loss of neuromuscular connections and ventral root motor axons. In addition, non-neuronal cells proliferate and become activated across the spinal cord, contributing to the disease progression. (**c**) Sigma-1 receptor agonists are able to prevent the loss of neuromuscular connections and motor axons, as well as the death of motoneuron cell bodies in the spinal cord. Furthermore, Sigma-1R agonists reduce microglial reactivity, despite no changes are observed in astrocytosis

ity of motoneurons. A body of evidence indicates that inhibition of m2AChR and/or activation of Kv2.1 and/or SK channels in C-terminals contribute toward reduction of motoneuron excitability [124, 133, 139]. Although the mechanisms by which Sigma-1R activates Kv2.1 and/or SK channels and thus decreases motoneuron excitability are still unclear, it has been shown within other systems that Sigma-1R can modulate activities of SK channels and a variety of Kv type channels [99, 142, 173]. Sigma-1R can form complexes with a variety of G-protein coupled receptors (GPCRs) that can subsequently alter ionotropic receptors including opioid and dopaminergic D1 receptors [174, 175] (Fig. 16.1).

As previously mentioned, Sigma-1R is located in the subsurface cisternae of C-terminals underlying the plasma membrane of motoneurons [89, 120]. Such physical proximity between the plasma membrane and the subsynaptic cisternae in C- terminals (less than 10 nM) makes direct molecular interaction possible for proteins located in adjacent membranes. Indeed, the Sigma-1R is characterized by a unique mode of action in regulating both the calcium entry at the plasma membrane level (e.g. via potassium channels, voltage-sensitive Ca^{2+} channels, etc.) and calcium mobilization from the endoplasmic stores (e.g. via IP_3 receptors). The ER supplies calcium directly to mitochondria via inositol 1,4,5-triphosphate receptors (IP_3 receptors) at close contacts between the two organelles referred to as mitochondrial-associated ER membranes (MAM). Sigma-1R is a calcium-sensitive and ligand operated chaperone at MAM, normally forming a complex with another chaperone, binding immunoglobulin protein (BiP), which normally prevents the Sigma-1R from translocation. Upon ER calcium depletion or via ligand stimulation, Sigma-1R dissociates from BiP, leading to prolonged calcium signaling into mitochondria via IP_3 receptors. Sigma-1R translocation has been shown to occur under chronic ER stress conditions. Indeed, increasing Sigma-1R in cells counteracts ER stress response, whereas decreasing its expression enhances apoptosis [90]. Subsequently, activity of both Kv2.1 and SK channels has been shown to be modulated by calcium, either directly or indirectly through Ca/calmodulin/calcineurin dependent mechanisms [176, 177] (Fig. 16.1).

In addition, Sigma-1R also contributes to maintenance of protein quality by regulating protein degradation and stability. Indeed, abnormal Sigma-1R accumulation is found in neuronal nuclear inclusions in neurodegenerative diseases [151, 178]. Sigma-1R participation in the degradation of misfolded protein via the ER machinery linked to the ubiquitin-mediated UPR suggests that Sigma-1R may function to counteract this pathological mechanism and promote survival in affected motoneurons. Ligand activation may promote and stabilize Sigma-1R oligomers, thus conferring improved chaperone functionality to the receptor [90].

Finally, modulation of Sigma-1R may also contribute to neuroprotection by reducing oxidative stress. It was shown that depletion of Sigma-1R leads to increased oxidative stress and abnormal mitochondrial membrane potential, thus triggering cytochrome C release and elevated caspase-3 cleavages [179].

16.7 Bases of Motoneuron Vulnerability

Understanding the bases of motoneuron vulnerability is crucial for developing novel strategies to cope with MND. In this section we focus on those aspects of motoneuron vulnerability that are related to mechanisms in which Sigma-1R plays a relevant role: the alteration of excitability properties of motoneurons and calcium homeostasis. As previously mentioned, ALS is a degenerative disease in which lower and upper motoneurons are selectively vulnerable, but interestingly some groups of motoneurons are relatively resistant to the disease process. It has been hypothesized that the differential susceptibility of motoneuron populations might be related to their excitability properties. Indeed, a consistent clinical feature of ALS is the preservation of eye movements and the external sphincters function. Pathological studies confirmed that there is relative sparing of the cranial motor nuclei of the

oculomotor, trochlear and abducens nerves, and of the Onuf's nucleus in the sacral spinal cord, which innervates the external sphincter of the pelvic floor [180]. Although neuronal numbers are relatively well-preserved in these resistant motor nuclei, some pathological changes resembling those observed in ventral spinal cord motoneurons are present, but to a lesser degree [181, 182]. Oculomotor nuclei are also relatively spared in mutant SOD1 mouse models [183]. The pattern of innervation of extraocular muscles is different from other skeletal muscles. Neuromuscular junctions are distributed throughout the fiber length at a high density [184], and show some structural peculiarities [185]. About 20 % of the extraocular muscles fibers are innervated by multiple neuromuscular junctions [186]. Oculomotor motor units are amongst the smallest seen in any skeletal muscle [187], with high firing discharge rates. Even in the primary position of gaze, 70 % oculomotor neurons are active, commonly discharging at 100 Hz [188]. In contrast, there is strong experimental evidence of a special susceptibility of large, phasic motoneurons in the degenerative process of ALS. Electromyographic analysis performed in ALS patients revealed that the larger and stronger motor units are clearly more affected by the disease [189], and histopathological studies have described a preferential degeneration of large motoneurons in ALS [190]. In mutant SOD1 models, selective vulnerability of large fast-fatigable hindlimb motor units before the onset of clinical symptoms was reported, followed by affectation of fast fatigue-resistant motor units at symptoms onset, but with sparing of slow motor units [191]. This is consistent with the rapid denervation of extensor digitorum longus muscle (rich in fast fatigable motor units) and the resistance of soleus muscle (with mainly slow motor units) described along disease progression in $SOD1^{G93A}$ mice [192, 193].

Understanding the differences in properties of vulnerable vs. resistant motoneurons may provide insights into the mechanisms of neuronal degeneration, and identify targets for therapeutic manipulation. In an interesting study Brockington et al. [194] performed a microarray analysis to compare the gene expression profile of isolated motoneurons from the ALS-resistant oculomotor nuclei and ALS-vulnerable spinal cord motoneurons from post-mortem ALS patients tissue. They found nearly 2000 genes differentially expressed by the two motoneurons subtypes, participating in synaptic transmission, ubiquitin-dependent proteolysis, mitochondrial function, transcriptional regulation, immune system functions and the extracellular matrix. They further focused on glutamate and GABA neurotransmission. The AMPA glutamate receptor consists of four subunits, GluR1–GluR4, and the presence of the GluR2 subunit determines the calcium permeability of the receptor. In the absence of GluR2, the AMPA receptor–ion channel complex becomes permeable to calcium. Gene array results showed up-regulation of the GluR2 subunit in resistant oculomotor motoneurons relative to the vulnerable lumbar motoneurons, thus reducing calcium influx into the cells. On the other hand, GABA is the most widely distributed inhibitory neurotransmitter in the CNS and acts through the interaction with GABA-A (ligand-gated chloride channel) and GABA-B (metabotropic) receptors. In oculomotor motoneurons, there is up-regulation of six GABA-A receptor subunits and of GABA-B receptor subunit 2 relative to spinal motoneurons, leading to an increased inhibition. Other studies performed in mSOD1 models confirmed these findings, revealing an excitatory/inhibitory imbalance affecting synaptic inputs into spinal motoneurons [23]. To test the hypothesis that inhibitory interneuron innervation of motoneurons was abnormal in ALS, Chang and Martin [195, 196] measured GABAergic, glycinergic and cholinergic immunoreactive terminals on spinal motoneurons of $SOD1^{G93A}$ mice. They found reduction of glycinergic innervation from pre-symptomatic age (8 weeks), before loss of choline acetyltransferase-positive boutons, whereas no significant differences in GABAergic boutons density were found along age.

Interestingly, the increased excitation and reduced inhibition onto motoneurons has been hypothesized as a protective compensatory reaction rather a detrimental phenomenon [197]. As

above mentioned, oculomotor nucleus motoneurons are strongly resistant to degeneration, but have particular physiological characteristics, including high discharge rates [188]. In turn, vulnerable fast-fatigable spinal motoneurons are those with larger cell bodies and more phasic activity pattern. Surprisingly, early administration of an AMPA receptor agonist protected spinal motoneurons whereas an AMPA receptor antagonist enhanced motoneurons pathology in SOD1^{G93A} mice [197]. Furthermore, the authors proposed that reduction of gephyrin (an inhibitory synapse marker), increase of serotonin labeled area in the ventral spinal cord and increased C-boutons size and number are protective compensatory reactions that promote motoneuron survival. In agreement with these findings an abnormal response of the potassium-chloride co-transporter 2 (KCC2) in mutant SOD1 motoneurons in response to axonal damage and deafferentation [198] was recently described. KCC2 is a transmembrane chloride extruder that maintains low intracellular chloride levels, thereby allowing GABA and glycine to exert inhibitory transmission during adulthood [199–201]. Under normal conditions, KCC2 is down-regulated after motoneuron insults thus promoting increased excitability needed for axonal regeneration [202, 203]. In contrast, mutant SOD1 motoneurons were unable to down-regulate their KCC2 and thus did not become hyperexcitable even when already disconnected from their muscles [198]. Further studies revealed that functional overload is able to rescue motor units in mutant SOD1^{G93A} mice [204], supporting the hypothesis of hypoexcitability as one potential factor underlying selective motoneuron damage.

In vitro studies of motoneuron excitability also show discrepancies regarding whether hypo- or hyperexcitability is a susceptibility factor for motoneurons in ALS. Changes in excitability have been reported to occur very early in mutant SOD1 mice [205]. Motoneurons from mutant SOD1 embryos recorded in culture show signs of hyperexcitability [206, 207], as well as motoneurons in *in vitro* preparation of mutant SOD1 embryonic spinal cords [208] or from the hypoglossal nucleus in the brainstem [209]. Contrarily, Pambo–Pambo et al. [210] did not observe any change in spinal motoneurons excitability properties, whereas Bories et al. [211] and Leroy et al. [212] reported spinal motoneurons to be hypoexcitable. A note of caution must be taken within this context since most of these studies were performed at developmental stages, when the maturation of the spinal circuitry is not yet completed.

Motoneurons express low levels of cytosol calcium-binding proteins compared to other neuronal populations, with motoneuron populations that are typically lost earlier during the disease course showing the lowest expression levels, suggesting that reduced cytosol calcium buffering contributes to the selective vulnerability of motoneurons [213, 214]. In fact, ALS-vulnerable spinal and brainstem motoneurons display low endogenous Ca^{2+} buffering capacity, 5–6 times lower than that of ALS-resistant motoneurons (i.e. oculomotor motoneurons), making them more susceptible to excitotoxic insults [215]. However, this view may not agree with the above mentioned oculomotor motor units properties since, although this motoneuron population is highly active, it is not vulnerable to ALS.

Interestingly, novel evidence has recently pointed out the potential contribution of C-boutons as participating in ALS pathophysiology [127, 140]. As described in Sect. 16.4, the postsynaptic membrane of C-boutons is rich in numerous proteins, including Sigma-1R [135], M2 muscarinic receptors [129–131], voltage-gated Kv2.1 [132] and Ca2+−activated K (SK) channels [133], connexin 32 [134], VAMP-2 [129], and neuregulin-1 [136]; whereas the presynaptic element contains, at least, neuregulin-1 receptors ErbB2 and ErbB4 [136]. Several alterations that may be related to C-bouton have been reported in ALS. It has been shown that mutations in Sigma-1R cause juvenile ALS [149, 150]. In agreement with this observation, knocking down Sigma-1R in mutant SOD1 mice leads to reduced lifespan [140], whereas treatment with a Sigma-1R agonist is neuroprotective [94]. Other morphological alterations appear to be present in ALS-linked mutations of VAMP-associated pro-

tein B, which is abnormally aggregated in C-boutons altering their function (VAPB, ALS8) [216]. The neuregulin1/ErbB system is also involved in ALS pathogenesis since ErbB4 mutations leading to a reduced autophosphorylation of ErbB4 receptors are associated with a hereditary late onset form of ALS [145], and neuregulin1/ErbB signaling alterations have been also observed in SOD1^{G93A} mice [144].

16.8 Conclusions

Overall, mutations of Sigma1-R have been reported in ALS in human patients, and sigma-1R modulation has proven to protect motoneurons *in vitro* and in *in vivo* models of traumatic injury to motoneurons and neurodegeneration. Although the exact molecular mechanisms underlying such effect have not been elucidated yet, Sigma-1R is a pleiotropic target, involved in several functions, many of them related to the pathophysiology of MND, including modulation of neuronal excitability, calcium homeostasis, and ER and mitochondrial activity. Thus, the multi-functional nature of the Sigma-1R provides an attractive target for treating ALS. Further human trials will be needed to assess whether pharmacologically targeting Sigma-1R is a suitable tool to protect motoneurons in MND.

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