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Perspective role of Substance P in Amyotrophic Lateral Sclerosis: From neuronal vulnerability to neuroprotection

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ABSTRACT

The neuropeptide Substance P (SP) and its preferred Neurokinin1 Receptor (NK1R) are known to participate in the physiopathology of neurodegenerative diseases and mainly exert a neuroprotective role. In the present work, we have described the involvement of SP and NK1R in Amyotrophic Lateral Sclerosis (ALS). This was demonstrated by the detection of altered levels of SP in the brain, spinal cord and cerebrospinal fluid (CSF) of patients and preclinical models of ALS, and by its ability to inhibit excitotoxicity-induced neurodegeneration in ALS animal models. These data are supported by results indicating an excitatory effect of SP at the motor neuron (MN) level, which promotes locomotor activity. ALS patients are characterized by a differential susceptibility to MNs degeneration, since sphincters and extraocular muscles are classically spared. It is hypothesized that SP may play a role in the maintenance of the ocular system and the innervation of the pelvic floor by contributing directly or indirectly to the selective resistance of this subset of MNs.

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron disease in adults, characterized by a rapidly progressive degeneration of the upper motor neurons in the motor cortex and the lower motor neurons in the brainstem and spinal cord (Feldman et al., 2022). The degeneration of motor neurons leads to a rapid onset of progressive motor symptoms with marked weakness of voluntary skeletal muscles and reflex abnormalities leading to muscle atrophy, paralysis and death within 2–5 years of diagnosis.

There are 2 types of ALS: familial ALS (fALS), which is inherited in an autosomal dominant manner and is due to mutations in genes directly associated with motor neuron degeneration, and sporadic ALS (sALS), which is not clearly associated with a family history of the disease but is caused by multiple factors and accounts for approximately 90 % of cases (Pasinelli and Brown, 2006).

The causes of both the sporadic and the familial forms of ALS are still unknown. The two forms are clinically and pathologically similar, suggesting a common pathogenesis (Gruzman et al., 2007). Although ALS remains a relatively rare disease, its incidence appears to be increasing, mainly due to the aging of the population, particularly in developing countries (Arthur et al., 2016).

As there is currently no effective cure for ALS, treatment focuses on the use of disease-modifying therapies and maximizing quality of life. The two currently FDA-approved treatments, the anti-glutamate agent riluzole and the antioxidant edaravone, have only a very modest effect on disease progression (Abe et al., 2017; Fang et al., 2018). In the United States, a combination of dextromethorphan and quinidine is approved for the treatment of symptoms (Feldman et al., 2022).

The difficulty of early diagnosis and the incompletely understood mechanism of selective and progressive degeneration of motor neurons limit the identification of effective treatments and remain a challenge for ALS research.

Various pathogenic mechanisms contribute to the selective death of motor neurons in ALS. Similar to Alzheimer's (AD) and Parkinson's (PD) disease, which are characterized by the deposition of neurotoxic high molecular weight aggregated proteins, insoluble protein complexes of superoxide dismutase (SOD) have been detected in tissues of ALS animal

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models (Johnston et al., 2000). ALS is a multifactorial disease as several mechanisms are involved, including oxidative stress related to the production of nitric oxide (Estévez et al., 1999) and the resulting mitochondrial dysfunction (Grosskreutz et al., 2007), which is confirmed by the elevated levels of markers of free radical damage found in biological fluids from patients with sALS (Mitsumoto et al., 2008).

Furthermore, it is known that altered activity of glial glutamate transporters (Trotti et al., 2001), hyperexcitability and glutamate excitotoxicity (Foran and Trotti, 2009), neuroinflammation (A. McCombe and D. Henderson, 2011), microvascular damage (Schreiber et al., 2023) and altered functionality of ion channels contribute to the pathogenesis of ALS (Carunchio et al., 2010; Kuo et al., 2005; Massimo Pieri et al., 2009).

Although motor neuron degeneration is a hallmark of ALS, today the broad spectrum of non-motor manifestations such as cognitive impairment (Pender et al., 2020) visual impairment (Nepal et al., 2022) and sensory/automatic changes (Vucic et al., 2008) support the concept of a non-motor centered disease. In addition, these non-motor manifestations are relevant for patient stratification, identification of prognostic markers and assessment of treatment response due to their early onset and correlation with the rate of disease progression.

In recent years, the alteration of eye movement and the appearance of retinopathy have been proposed as new clinical markers of the neurodegenerative process in ALS and as experimental paradigms to study the pathological mechanisms and discover new molecular targets.

Among the molecules of interest in neurodegenerative diseases, numerous studies point to the neuropeptide Substance P (SP) as one of the markers for the absence of motor symptoms in Parkinson's disease and as a promising neuroprotective agent for the degeneration of peripheral and central neurons (Tirassa et al., 2021).

The involvement of SP in ALS is less explored, but based on our previous and current studies, we propose in this review that this neuropeptide and its receptor NK1 may represent a neuronal substrate for the symptoms and progression of the disease and exert protective effects on motor and non-motor neurons, which could explain the particular degenerative resistance of functions in ALS.

1.1. Involvement of neuropeptides in ALS

Neuropeptides are small proteins that are widely distributed in the central and peripheral nervous system and act as neurotransmitters, neuromodulators and neuroregulators (Petrella et al., 2019). There is growing evidence that many neuropeptides are involved in both neurophysiological functions and neuropathological diseases, including ALS. The role that neuropeptides play in this pathology is supported by evidence that they are primarily neuroprotective and are pathologically altered in brain regions such as the brain and spinal cord of patients and preclinical models of ALS. Among the neuropeptides involved, the most studied is pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide with beneficial effects in many neurodegenerative and motor neuron diseases, including ALS (Zuccaro et al., 2021). PACAP plays a neuroprotective role by promoting the survival of MNs, especially in the early phase of the disease, as shown by the significant deregulation of mRNAs of PACAP and its receptor PAC1R in the motor cortex of ALS patients (Maugeri et al., 2020) and the ability of this neuropeptide to effectively prevent apoptotic death of MNs (Bonaventura et al., 2018).

Other neuropeptides are involved in ALS and have been shown to play an essential role in the survival and plasticity of MNs, such as neuropeptide Y (NPY) (Pain et al., 2022), neuropeptide VGF-derived peptide LQEQ-19 (Noda et al., 2019) and calcitonin gene-related peptide (CGRP) (Ringer et al., 2017), which emphasizes their potential clinical importance for understanding ALS pathogenesis and the possibility of acting as disease-modifying agents.

1.2. The activity of substance P and its neuroprotective role

Substance P (SP), an 11 amino acid peptide (RPKPEEFFGLM-NH2) belonging to the family of Tachykinins (TKs) and encoded by the gene known as preprotachykinin A (PPT-A or TAC1), has a broad spectrum of biological activities and acts as a neurotransmitter and neuromodulator in the brain, often released together with various peptides, amines, amino acids and other active substances (Severini et al., 2002).

SP, whose activity is related to the proper development of neurons, is widely distributed in the central (CNS) and peripheral (PNS) nervous systems (Otsuka and Yoshioka, 1993). SP and its natural high-affinity G-protein-coupled (Gαq and/or Gαα11) receptor neurokinin-1 (NK1R) are abundant, particularly in areas involved in the central control of various peripheral autonomic functions and higher brain functions such as learning and memory (Severini et al., 2002).

Due to their central role in nervous system, SP and NK1R are also associated with neuromodulation and protection in neurodegenerative diseases such as AD, PD and Huntington's disease (HD), as shown by their lower expression in some brain regions of both patients and animal models (N. W. Kowall et al., 1993; Raffa, 1998).

In addition to these neurological pathologies, SP/NK1 have been studied in the physiopathology of the anterior and posterior segments of the eye, including retinal degeneration (Hong et al., 2015) and the ability of SP to counteract retinal deterioration in animal models has also been reported (D'Alessandro et al., 2014; L. Yang et al., 2014) as outlined and discussed in more detail in the paragraph 5.

The neurotrophic and neuroprotective activity of SP has been studied for a long time. SP can act as a non-specific growth factor for peripheral and central nervous tissue, as established by its ability to stimulate the growth of neurites in various cultured neuron types such as neuroblastoma cells (Narumi and Maki, 1978) and rat sympathetic ganglia (Kozlova et al., 1986) and to promote the proliferation of adult neural progenitor cells under normal and ischemic conditions (Carthew et al., 2012; Park et al., 2007).

During postnatal development of the rat brain, increased expression of SP and its preferred NK1R was detected, suggesting that the SP/NK1R system may play a role in synaptic plasticity associated with morphological and functional development of the mammalian CNS (Hökfelt et al., 2000; Taoka et al., 1996).

In addition, the neurotrophic and neuroprotective activity of SP has been proved in neurons from different areas such as the hippocampus (Whitty et al., 1993), the auditory nerve (Lallemend et al., 2003), the mesencephalon (Salthun-Lassalle et al., 2005) and the cerebellum (Amadoro et al., 2007).

The trophic effect of SP has also been demonstrated in the spinal cord of rats, either directly (Iwasaki et al., 1989) or indirectly via its induction by Transforming Growth Factor beta (TGF beta) (Chalazonitis et al., 1992), suggesting that SP could be a potential therapeutic strategy in ALS. The neuroprotective properties of SP are also confirmed by its ability to counteract apoptosis in neurons as well as in thymocytes and macrophages (Dimri et al., 2000; Kang et al., 2001; Lallemend et al., 2003).

Interestingly, in cultured cerebellar granule neurons, SP has been shown to have an anti-apoptotic effect that does not involve active caspase 3 but impairs calpain activity, suggesting a neuroprotective mechanism that differs from that reported in other systems (Amadoro et al., 2007).

The neuroprotective effect of SP has been established in neurodegenerative diseases such as AD (Severini et al., 2016). The ability of SP to counteract Aβ toxicity has been demonstrated in vitro in cerebellar granule neurons (M. Pieri et al., 2010) and in hippocampal neurons (Campolongo et al., 2013) and confirmed in vivo by antagonism against Aβ-induced neuronal loss and cognitive impairment by SP treatment (Campolongo et al., 2013; Kowall et al., 1991). In addition, SP was able to increase α-secretase activity by activating ADAM9 mRNA expression, thereby decreasing the production of neurotoxic Aβ peptides (Marolda

et al., 2012) and modulate the expression of selective potassium channels (Kv4.2 and Kv4.3 subunits), which were altered by Aβ treatment both in vitro (M. Pieri et al., 2005) and in vivo, preventing Aβ-induced impairment of cognitive processes (Campolongo et al., 2013).

SP also acts predominantly as a neuroprotective factor in PD (Tirassa et al., 2021). The anti-parkinsonian properties of SP and NK1R agonists have been characterized in various in vitro models using neurotoxins such as 6-hydroxydopamine (6-OHDA) or methyl-4-phenylpyridinium (MPP+), which partially simulate the histological and/or biochemical features of PD (Chu et al., 2011; Wang et al., 2015; Zhao et al., 2016). Moreover, the anti-apoptotic effect of the NK1R agonist peptide was demonstrated in vivo in rats with 6-OHDA lesions and exerts its effect via a caspase-independent signaling pathway (Chu et al., 2011).

2. The involvement of SP in ALS

2.1. SP and its receptor distribution in ALS brain and spinal cord

The involvement of SP in ALS has been investigated by several authors who have demonstrated significant changes in the expression of SP in different districts at both clinical and preclinical levels (Table 1).

In ALS patients, when investigating the origin of peptidergic afferents to the spinal cord, an early selective disappearance of SP fibers was found, suggesting a neurotrophic role of SP in the pathogenesis of the disease (J. Schoenen, 1988).

Moreover, a significant decrease in the binding of SP receptors was observed in the postmortem spinal cord, particularly in the ventral horn, which is associated with the loss of motor neurons. This indicates a postsynaptic localization of the SP receptors on the motor neurons and a role of SP for their function (Dietl et al., 1989).

More recently, a marked depletion of axon terminals and an impairment of SP-ergic projections has been demonstrated in the striatum of sporadic ALS patients, suggesting a key role of SP neurons in the degeneration of the efferent system of the striatum (Riku et al., 2016). In addition to ALS, a significant reduction of SP-immunopositive cells was also detected in the striatal efferent terminals in basal ganglia of patients affected by motor neuron disease with basophilic inclusions in adulthood (MND/BI) compared to control subjects (Ito et al., 1995).

Despite the reduced expression of SP and its binding sites in the CNS, increased levels of SP were found in the cerebrospinal fluid (CSF) of ALS patients compared to control groups (Matsuishi et al., 1999). In particular, this higher SP concentration was especially pronounced in patients with disease duration of less than 2.5 years compared to patients with ALS of longer duration. Interestingly, SP levels return to baseline levels as the disease progresses. This may confirm the important role of SP as a neurotrophic factor that is increased to compensate for the degeneration of anterior horn cells that occurs in ALS, suggesting a possible disease-modifying role of SP in ALS.

Elevated levels of SP were found in the spinal cord and brain of an ALS animal model. In the Wobbler mice, a spontaneous mutation model of the disease that exhibits severe and progressive degeneration of motor neurons in the cervical spinal cord, a significant increase in SP expression was found in the spinal cord, hypothalamus and midbrain late in the course of the disease (Yung et al., 1992). The author speculated that the increased concentration of SP around the motor neurons could exert additional trophic influences on the dying target cells in a last ditch attempt to alleviate the progressive motor neuron disease. Moreover, an age-related decrease in SP receptors in the cervical spinal cord of Wobbler mice was found, indicating a strain-related defect in the developmental patterns of SP neurons/receptors (Deng et al., 1996).

In contrast, higher NK1 expression was found in the cortical tissue and cultured neurons of the G93A mouse model of ALS than in the control group, likely leading to a downregulation of glutamatergic transmission (Caioli et al., 2011) (Fig. 1).

Table 1
Changes in SP and its receptors in ALS patients and animal models.

ALS patients		
Region	*Change*	*Reference*
Spinal cord	Reduction of SP	(1989 Schoenen, n.d.)
	Reduction of SP receptors	(Dietl et al., 1989)
Striatum	Reduction of SP	(Riku et al., 2016)
CSF	Increase of SP at early stages	(Matsuishi et al., 1999)
ALS patients		
Spinal cord, hypothalamus and midbrain of Wobbler mice	Increase of SP at later stages	(Yung et al., 1992)
Cervical spinal cord of Wobbler mice	Reduction of SP receptors	(Deng et al., 1996)
Cerebral cortex of G93A mice	Increase of NK1 receptor	(Caioli et al., 2011)

2.2. SP and excitotoxicity

Several studies suggest that excitotoxicity plays a role in the development of sporadic and familial ALS. This is supported by several experimental evidences and by the therapeutic effect of riluzole, which is probably due to its anti-excitotoxic properties (Van Damme et al., 2005).

One of the most important hypotheses investigated on the etiology of ALS concerns excitatory neurotransmitters, in particular the most important one in the CNS, glutamate. Excitotoxicity occurs when extracellular glutamate concentrations increase, due to augmented release from presynaptic terminals or insufficient reuptake from the synaptic cleft, resulting in excessive stimulation of glutamate receptors and degeneration of neurons (Meldrum and Garthwaite, 1990).

Among the ionotropic glutamate receptors, the amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which are permeable to Na+, K+ and, to varying degrees, Ca2+, play a key role in mediating rapid excitatory transmission. The permeability of these receptors is largely determined by the GluR2 subunit (Seeburg, 1993).

One of the mechanisms for the selectivity of motor neuron death in ALS may be due to their sensitivity to excitotoxicity by glutamate as a result of AMPA receptor activation, since the expression of GluR2 and calcium buffer proteins is low in these cells (Carriedo et al., 1996; Rothstein et al., 1992). In support of this hypothesis, cortical hyperexcitability has been found to precede the development of clinical symptoms in familial ALS patients with SOD1 mutation, suggesting that cortical hyperexcitability could be the cause of neurodegeneration (Vucic et al., 2008).

We have previously validated through electrophysiological recordings the involvement of SP and its cognate NK1R in glutamatergic transmission in ALS by investigating their role in mice transgenic for the G93A mutant hSOD1 (G93A mice), a widely used and well-characterized model for this pathology (Caioli et al., 2011).

In cultured cortical G93A neurons, SP perfusion was able to reduce the amplitudes of kainate currents across AMPA receptors in a dose-dependent manner, compared to control cultures. The specificity of the SP effect via the NK1 receptor was further confirmed by complete abolition with a selective antagonist (GR82334).

Downregulation of glutamatergic transmission is associated with neuroprotective properties (Spalloni et al., 2004), and this was confirmed by the ability of SP to protect cortical neurons from death induced by kainate exposure in G93A cortical neurons.

The significant over-expression of NK1 receptors compared to control neurons suggests a postsynaptic activity of SP in modulating AMPA receptor functionality (Caioli et al., 2011).

In general, several studies established that TKs and their receptors can act as satisfactory controllers of neuronal excitability to inhibit

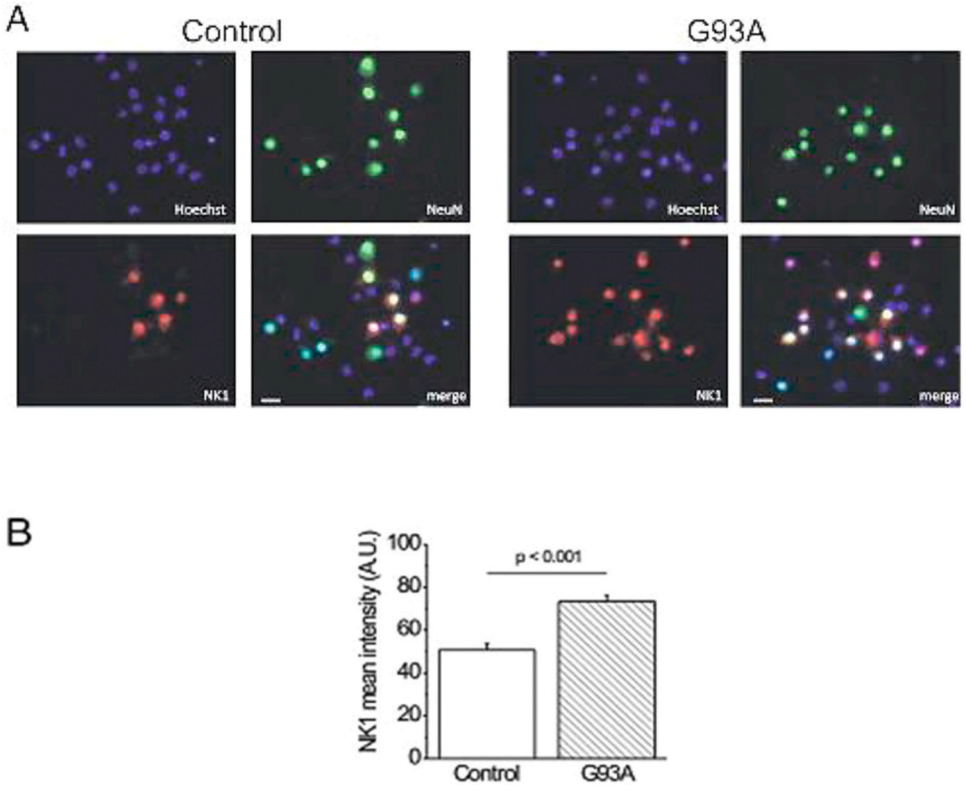

Fig. 1. NK1 expression in Control and G93A cultured cortical cells. A— Triple immunofluorescence for the tachykinin NK1 receptor (red), neuronal nuclei marker NeuN (green) and DNA (Hoechst, blue) in Control (left) and G93A (right) cortical cultures at 8 DIV. Scale bar: 20 μm. B— Bar plot of the NK1 mean fluorescence intensity. The quantification of the fluorescence intensity for NK1 receptors is significantly higher in G93A compared to Control cultures (A.U.: arbitrary unit; $p < 0.001$; independent *t*-test) (From Caioli et al., 2011, with permission).

excitotoxicity-induced neurodegeneration by controlling excitatory glutamatergic and inhibitory gabaergic transmission. Indeed, activation of NK1 receptors in the globus pallidus, nucleus tractus solitarius, entorhinal cortex and hippocampus has been reported to lead to increased release of GABA, supporting the beneficial effects of SP in neurodegenerative diseases (Bailey et al., 2004; Chen et al., 2009; Stacey et al., 2002).

Overall, the results reported here speak in favor of a trophic role of SP in ALS and its involvement in the pathogenesis of the disease. However, other aspects not yet investigated deserve a more in-depth study, considering the ability of SP to exert an excitatory effect at the level of motor neurons and to promote locomotor activity.

3. Excitatory role of SP in motor neuron activity

The involvement of SP and its binding sites in motor neuron and locomotor activity is supported by evidence of dense innervation of all motor neuronal pools by SP-immunoreactive fibers, possibly originating from at least three sources: brainstem raphe neurons, primary afferent neurons or SP-expressing cells intrinsic to the spinal cord (Rekling et al., 2000). Moreover, the distribution of SP binding sites throughout the CNS, including the motor nuclei, has also been extensively demonstrated, particularly in the rat, suggesting that the NK1R is the most representative TKs receptor with variable expression in motor neuron pools (Elde et al., 1990; Manaker and Zucchi, 1998; Nakaya et al., 1994).

SP has been shown to excite motor neurons mainly via NK1R (Rekling et al., 2000). SP exerts an excitatory effect on neonatal rat spinal cord motor neurons and induces depolarization through a direct postsynaptic effect (Konishi and Otsuka, 1974) by inhibiting a relatively voltage-independent K+ resting current (Fisher and Nistri, 1993; E. Yang et al., 2022). The mechanism by which SP may modulate excitatory inputs to motor neurons may be through a reduction in membrane conductance via a postsynaptic mechanism or presynaptically by enhancing the release of 5-idrossitriptamina (5-HT) (Mitchell and Fleetwood-Walker, 1981).

Activation of the NK1R has been shown to improve locomotion in addition to its stimulatory effect on motor neurons. Indeed, it was displayed long ago that injection of SP and related TKs in rats produces a dose-dependent increase in locomotor activity (Elliott and Iversen, 1986; Treptow et al., 1983).

The excitatory effect on motor function has also been demonstrated in in vivo models of neurotoxic parkinsonism. Treatment with the NK1R agonist peptide attenuated the motor deficit in 6-OHDA-lesioned rats, and inhibited the apoptotic caspase-independent signaling pathway (Chu et al., 2011). Furthermore, neurotoxic PD rat models treated with SP through intracaudal (Kryzhanovskii et al., 1989) or intranasal (Kryzhanovskiĭ et al., 1992, n.d.; Zhao et al., 2016) application appeared to increase motor activity and almost completely eliminate muscle stiffness.

4. SP and susceptibility to motor neuron degeneration

The selective target in ALS is the upper and lower somatic motor neurons (MNs), which degenerate retrogradely, leading to a rapid onset of progressive motor symptoms. However, some subsets of MNs are relatively resistant to degeneration, such as the neurons innervating pelvic floor muscles form the sacral spinal cord (SC) (Onuf`s nucleus), and those of the oculomotor system.

4.1. Motor neurons resistance

A number of morphological and functional features that distinguish this subset of MNs from the rest of the MN populations are thought to influence the susceptibility of MNs to ALS. Although several factors play

a role in determining the gradient of MNs susceptibility to neurodegeneration, they are tentatively divided into neuronal (intrinsic and extrinsic) and non-neuronal factors (Ovsepian et al., 2023).

Among the neuronal factors, the size and morphology of the MNs, the multiple neuromuscular innervation sites per single myofibre, the set of ion channels and transporters and their regulation, and the mechanisms controlling intracellular Ca^{2+} dynamics influence the MN excitability and vulnerability (Manuel and Zytnicki, 2019; Nijssen et al., 2017). Accordingly, the MNs that innervate fast-twitch muscles and are characterized by low excitability are most severely affected and degenerate first, while the more excitable and smaller MNs that innervate slow-twitch muscles, such as the neurons of the Onuf's nucleus and those of the oculomotor system, gradually degenerate as the disease progresses (Okamoto et al., 1993; Sasaki, 1991).

Besides differences in intrinsic properties, the balance between excitatory and inhibitory (E/I) efforts is crucial for neurodegeneration in ALS, suggesting that MNs are differentially affected by their synaptic inputs and neurochemical microenvironment, with the E/I ratio being highest in the most vulnerable neuron type (Nijssen et al., 2017; Ovsepian et al., 2023).

In addition, the paracrine/autocrine effects of neuropeptides, hormones and neurotrophic factors contribute to the susceptibility of MNs to ALS, such as dense innervation by enkephalin, somatostatin, CGRP, Neuropeptide Y and neurokinin-positive terminals as well as thyrotropin-releasing hormone-positive axons in ALS-resistant MNs, which are involved in the functional maintenance of sphincter contraction and eye movement in ALS patients (Ovsepian et al., 2023).

4.2. Onuf's nucleus (ON)

Regarding the ON, the dense peptidergic innervation demonstrates the mixed somatic-autonomic properties of this nucleus and could explain the different rate of degeneration in different neurodegenerative diseases (Conti et al., 2020; Gibson et al., 1988). As suggested by Gordon et al., who analyzed the distribution of several peptides, including SP, in the post mortem spinal cord samples of patients with motor neuron disease (MND) and healthy humans, the preservation or loss of MNs in the ON correlates with the presence of peptide innervation (Gibson et al., 1988).

Beside humans, SP has been also found localized in the clusters of ON motoneurons in rats (Charlton and Helke, 1985) and cats (Tashiro et al., 1989b). The SP/NK1 system in the ON controls the external sphincters of the anus and bladder, but the SP-immunoreactive fibers localized in and around the ON have not been clearly understood. A study in cats indicates that possible sources are cells near the central canal, but descending projections from the brain may also contribute (Tashiro et al., 1989b).

The studies on the distribution of SP in the sacral spinal cord of humans and other mammals support the observation in cats (Severini et al., 2002), but further investigations are necessary to better characterize the origin of SP in ON, and to identify its contribution in the maintenance of integrity of this subpopulation of MNs.

Regarding the origin of SP, it is important to know that neuropeptides can act as neuromodulators and reach their receptors at a considerable distance from the site of release. The concept of volume transmission (VT) or extra-synaptic neurotransmission has been proposed for this particular form of endocrine transmission, which is confirmed by the frequently observed discrepancy between the distribution of neuropeptides and their receptors in many brain regions (Petrella et al., 2019).

It is also worth noting that the pudendal nerve reflects the sensory-autonomic and motor components of ON and it is a mixed nerve containing both efferent and afferent component, which can transport SP retrogradely to the sacral motoneuronal cell body (Tashiro et al., 1989a). it is therefore likely that changes in SP at the target levels, including the anal/urogenital muscles, could affect the survival and activities of the MNs of the ON in ALS.

4.3. Oculomotor system

The more excitable and smaller MNs of the nuclei which innervate the eye muscles are largely spared in ALS until the final stage of the disease (Okamoto et al., 1993), allowing the use of eye-tracking devices as a means of communication for fully paralyzed patients (Caligari et al., 2013). A similar role of SP could be hypothesized for the reduced vulnerability of oculomotor system in ALS. Indeed, the Oculomotor nerve (OMn), like the pudendal nerve, is a mixed nerve with somatic motor and autonomic functions that controls and allows pupillary constriction, eyelid positioning and eye movement by innervating the sphincter pupil muscle, and the extraocular muscles (EOM).

The somatic and autonomic components of OMn originate from the oculomotor nucleus (OMN) and the Edinger-Westphal nucleus (EWN), respectively. Many neuropeptides have been localized in the oculomotor system of humans and other mammals (Kozicz et al., 2011; Priest et al., 2023) and SP has been found in neurons in the EWN, where it is co-expressed with CCK (Maciewicz et al., 1983; O'Connor et al., 2004; Skirboll et al., 1983), but not in the OMN.

However, low or moderate numbers of neurokinin (TK) immunoreactive fibers have been reported in the OMN and abducent nucleus (ABN) (Coveñas et al., 2003) which also project to the EOM and contribute to the regulation of eye movements.

It is therefore possible that the SP produced in the EWN or derived from other producing areas of the midbrain, such as the Peri-Aqueductal Gray (PAG) (Li et al., 1991) interacts directly with the NK1 receptors in the OMN and ABN and contribute to the resistance of the MNs to degeneration.

Moreover, considering that different isoforms of the NK1 receptor with atypical antagonist binding properties have been detected in MNs (Rekling et al., 2000), it would be worthwhile to investigate the expression of the different NK1R isoforms in the oculomotor system, also to uncover possible differences from non-ocular motor neurons that could explain the selective resistance associated with the stage-related changes in SP in ALS.

In this context, it has been found that increased expression of certain trophic factors, such as neurotrophins (Benítez-Temiño et al., 2016), vascular endothelial growth factor (VEGF) (Silva-Hucha et al., 2024), insulin-like growth factor 2 (IGF-2) (Allodi et al., 2016), and neuropeptides such as pituitary adenylate cyclase-activating polypeptide (PACAP) (Maugeri et al., 2020) may play a role in increasing the resistance of target ocular muscles to ALS. Since SP is regulated by neurotrophins in nervous system (Skoff and Adler, 2006), and together with PACAP has been demonstrated to stimulates neurotrophins in muscles (Alqudah et al., 2011), promote angiogenic factors including VEGF (Hong et al., 2020), as well as to cooperate with other the cornea (Singh et al., 2022) and retina protection (Pender et al., 2020), the possibility that SP might contribute to the resistance of MNs by acting in synergy with other factor cannot be excluded.

The potential role of SP in selective neuronal resistance to the degeneration of oculomotor and sacral neurons is summarized in Fig. 2.

5. Conclusion and future perspectives

SP and its NK1 receptor are involved in neurodegenerative diseases, including PD, in which SP/NK1R alterations characterize vulnerable cells in the periphery and brain and correlate with prodromal manifestations (Tirassa et al., 2021).

In this article, we report the studies on the involvement of SP in ALS. Due to the rapidly progressive course of the disease, leading to death on average 3–5 years after the onset of the first symptoms, almost all studies reporting changes in SP or NK1R refer to the late stage, when a large proportion of motor neurons have been lost and neuronal functional changes are present at both central and peripheral levels. However,

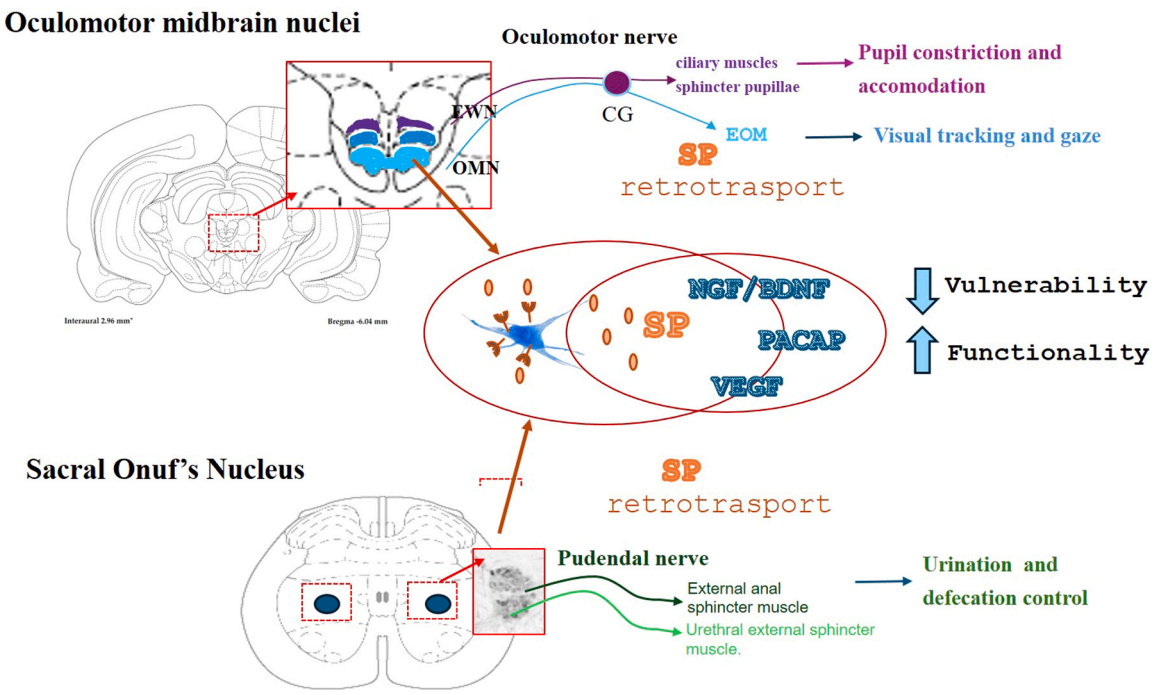

Fig. 2. Integrated picture of the possible role of SP in selective neuronal resistance to degeneration of oculomotor and sacral neurons. SP, mainly of neuronal origin, might directly act on MNs (in blue) of oculomotor nerve and pudental nerve expressing NK receptors. SP produced and released at target level might be also transported retrogradely by the nerve to the motorneuron cell bodies. More, the possibility that SP might cooperate with other factors, including neurotrophins, VEGF and PACAP is also suggested. The oculomotor system, comprising the EWN and OMN spinal nuclei, and CG innervating the EOM and pupilar and ciliary muscle is showed in the upper panel, while the clusters of motoneurons of the Onuf nucleus that control the external sphincters of the anus and bladder is showed in the lower panel. Abbreviations: CC= Ciliary Ganglia; EWN= Edinger-Westphal nucleus; OMN= Oculomotor nerve; EOM= extraocular muscles.

based on the studies in patients and animal models, it is likely that changes in the synthesis and release of SP in the target areas are correlated with disease progression. We hypothesize that the increase in SP observed in the initial phase of motor decline may exert a protective and trophic effect on motor neurons, hypothesizing an autocrine and paracrine SP/NK1 mechanism.

The possible neuroprotective role of SP in the early stages of ALS could be supported by the evidence that SP and its receptors are localized in the EWN and ON, which originate from the oculomotor nerve and the pudendal nerve and regulate the contraction of the iris and the of the anal sphincter, respectively, whose functionality is peculiarly preserved in ALS patients.

As described in the paragraph 4 and summarized in Fig. 2, we suggest that SP may play a role in the maintenance of the ocular system and the innervation of the pelvic floor by contributing directly or indirectly to the selective resistance of this subset of MNs.

In conclusion, we propose that SP/NK1R may play a role in ALS and that, in particular, tissue- and time-dependent changes in its expression may correlate with the peculiar and patient-specific progression of motor neuron degeneration in ALS.

Studies on humans and animal models are currently on going in our laboratory to fully elucidate the potential role of SP in the vulnerability of MNs in ALS and to investigate the expression of SP in the brain and spinal cord, also in correlation with other trophic factors, such as neurotrophins, at different disease stages.

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