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Calcium in the pathomechanism of amyotrophic lateral sclerosis – Taking center stage?

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ABSTRACT

Amyotrophic lateral sclerosis is an incurable, relentlessly progressive disease primarily affecting motor neurons. The cause of the disease, except for the mutations identified in a small fraction of patients, is unknown. The major mechanisms contributing to the degeneration of motor neurons have already been disclosed and characterized, including excitotoxicity, oxidative stress, mitochondrial dysfunction, and immune/inflammatory processes. During the progression of the disease these toxic processes are not discrete, but each facilitates the deleterious effect of the other. However, due to their common reciprocal calcium dependence, calcium ions may act as a common denominator and through a positive feedback loop may combine the individual pathological processes into a unified escalating mechanism of neuronal destruction. This mini-review provides an overview of the mutual calcium dependence of the major toxic mechanisms associated with amyotrophic lateral sclerosis.

Contents

1. Introduction

Amyotrophic lateral sclerosis (ALS), first described by Ref. [1], is a fatal neurodegenerative disease which, according to the classical definition, affects primarily upper and lower motor neurons in the

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motor cortex, brainstem and spinal cord [2]. In view of the most recent findings however, ALS is a highly heterogeneous condition, often accompanied with cognitive impairment and, though extrapyramidal, cerebellar, autonomic and sensory system involvement is rare, it is now considered a multisystem disorder [3]. ALS patients are traditionally sorted into categories of familial (5–10%; with mutations of more than a dozen different genes, discovered to date) or sporadic form (90–95%), depending on whether they have affected family members [4]. Nevertheless, the discovery of an expansion of the intronic hexanucleotide repeat sequence in chromosome 9 open reading frame 72 (C9orf72) in patients without family history blurred the distinction between these, clinically basically indistinguishable, classes [5], or even the difference between the borders of different neurodegenerative diseases [6]. Since the underlying pathological condition involves multiple cell types, such as astrocytes [7], microglial cells [8], infiltrating immune/inflammatory cells [9], and due to the complex interaction between the identified genetic factors and basic molecular pathways of its pathophysiology, ALS is recognized as a non-cell autonomous [10,11], and multifactorial disease [12]. Several excellent reviews are available which overview the reciprocal relationships of the major individual toxic mechanisms leading to motoneuronal degeneration, such as excitotoxicity, oxidative stress, mitochondrial dysfunction and immune/inflammatory processes [4,12–17]. In this review, however, we will focus on the role of calcium ions, showing that regardless of the site and type of the primary lesion, increased intracellular levels of calcium ions could merge the identified individual toxic mechanisms into a single self-perpetuating cycle of motor neuron degeneration. In relation to these pathological processes, some properties of motor neurons predisposing them to calcium mediated injury, like low calcium buffering capacity and specific composition of glutamate receptors, will also be discussed.

2. Pathological processes inducing motoneuronal calcium increase

2.1. Excitotoxicity

Early pioneering work in the late 60's by Olney, who coined the term excitotoxicity, led to the general appreciation that toxicity induced by excess exposure to excitatory amino acids was not restricted to retinal neurons exposed to glutamate, the experimental system in which the effect was first described. In fact, excitotoxicity might well be responsible for a wide variety of brain damage observed in acute injury and slowly evolving neurodegenerative diseases (reviewed by Ref. [18]). The role of excitotoxicity in ALS was then proposed [19], based on the original observations that glutamate levels in the plasma of ALS patients were two-fold higher than controls [20]. Likewise, high levels of glutamate and aspartate have also been detected in cerebrospinal fluid of ALS patients [21]. These data were further supported by reports of reduced levels of the excitatory amino acid transporter 2 (EAAT2) in the motor cortex and spinal cord of the majority of ALS patients [22], since a rapid uptake of glutamate is accomplished by the EAAT1 and EAAT2 glutamate transporters, as was timely reviewed by Mitsumoto et al. [23]. In parallel studies it was also documented that some ALS patients possess alterations to the structure of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type glutamate receptor. Normally, AMPA receptors are impermeable to calcium. However, if one of their subunits (GluR2) is missing, the receptors become permeable to calcium ions [24]. During neurotransmission, this alteration induces an increased calcium influx into the postsynaptic motor neuron, which may trigger injury. The low expression of the GluR2 protein subunit [25], or the mRNA for GluR2 [26], was indeed seen in ALS patients, which could provide a molecular basis for the increased calcium influx, further amplifying the effect of the high glutamate level.

2.2. Oxidative stress

The presence of tissue damage due to oxidative stress in ALS has been shown by several postmortem studies. Elevated carbonyl derivatives were detected in the spinal cord and motor cortex of ALS patients [27,28], due to direct oxidation of lysine, arginine, tyrosine, proline and threonine residues [29]. Additionally increased 3-nitrotyrosine level was found in spinal motor neurons of patients [30], which is a marker of peroxynitrite-mediated damage [29]. These autopsy findings were later corroborated with data obtained from cerebrospinal fluid or blood of ALS patients, demonstrating increased levels of 8-hydroxy-2′-deoxyguanosine, a marker of oxidized DNA [31,32], and 4-hydroxynonenal, a marker of lipid peroxidation [33,34].

Plasma membranes and embedded ion channel proteins are distinct targets of free radicals involved directly in changing the intracellular ionic milieu [35]. The action of free radicals on membrane fatty acids may either change membrane fluidity, resulting in secondary clustering or oversensitization of voltage-gated calcium channels [36], or may directly alter ion channel properties resulting in increased P/Q-type calcium channel activity, as was shown in calcium channels expressed in *Xenopus* oocytes [37]. Alternatively, reactive oxygen species (ROS) may interact with 1,4,5-trisphosphate (IP_3) and ryanodine receptors and sarco/endoplasmic reticulum Ca^{2+} ATPase transporters (SERCA) present in the membrane of the endoplasmic reticulum, leading to increased calcium release and decreased uptake by the organelle under oxidative stress, resulting in increased intracellular calcium levels [38].

Reactive oxygen species are continuously generated during physiological cellular processes, mainly due to leakage of electrons from the respiratory chain in the mitochondria, thus the cells are equipped with robust ROS detoxifying machinery. Cu/Zn superoxide dismutase (SOD1) is the major enzymatic defense system in the cytosol, catalyzing the conversion of superoxide radicals to hydrogen peroxide, which is converted by glutathione and catalase to harmless oxygen and water. Thus, the discovery of mutations in SOD1 in patients in 1993 [39] led to expectations that the degenerative mechanism for motor neurons in ALS would soon be interpreted as impaired oxidative protection in motor neurons. On the basis of the identified mutations more than 20 rodent models were generated which recapitulated several aspects of the human disease, and are being widely used in ALS drug studies [40]. However, despite more than 2 decades of intensive studies, the detailed mechanism of the toxicity of the mutant SOD1 remains elusive [41]. While the importance of reactive oxygen species mediated mechanisms in the pathophysiology of ALS is not questioned, it is now generally accepted that ROS mediated toxicity is multifactorial [14,42], and is, at least partially, based on reactive oxygen species produced by microglia [43].

2.3. Mitochondrial dysfunction

Motoneuronal mitochondrial abnormalities in ALS were first identified as structural alterations in the intramuscular nerves [44] and in the anterior horn neurons [45] in autopsy samples from patients. This was later corroborated by electron microscopic images also from autopsy cases by describing alterations in the intermembrane space and the inner compartments of mitochondria [46]. Altered mitochondrial functions were also described in

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