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Commentary: Calcium in the pathomechanism of amyotrophic lateral sclerosis - Taking center stage?

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ABSTRACT

Amyotrophic lateral sclerosis is a rare and fatal neurodegenerative disorder characterized by the progressive loss of motor neurons in the central nervous system and neuromuscular junctions in the periphery. The pathomechanism behind the disease, except from some familiar cases associated with genetic mutations, remains unclear, however, numerous mechanisms contributing to the disease have already been disclosed. The key components are the oxidative stress, excitotoxicity, mitochondrial dysfunctions and inflammatory processes. In addition, increased intracellular calcium, which is another identified pathological event, could merge these individual toxic mechanisms into a single, escalating and self-perpetuating cycle of neuronal degeneration. Our previous results suggest that calcium homeostasis might be preserved by modulating the transmembrane calcium flux with therapeutic compounds or via altering the calcium binding protein content to maintain an enhanced calcium buffer capacity. The scope of this commentary is to accentuate the reciprocal calcium dependence of the pathological events associated with amyotrophic lateral sclerosis and to discuss possible therapeutic strategies based on the restoration of calcium homeostasis.

Introduction

French neurologist, Jean Martin Charcot, was the first who defined amyotrophic lateral sclerosis (ALS) as *„la sclérose latérale amiotrophique"* which is a French expression for a pathological manifestation of the disease. Pioneering work of Professor Charcot was the autopsy report of the scar tissue in the anterolateral fasciculus of the spinal cord which manifested as spasticity and paralysis in the patients[1]. Nowadays amyotrophic lateral sclerosis is known as a non-cell autonomous[2], multifactorial[3] and multisystem disease[4], however its exact origin and all the details of the development of the disorder, relentlessly leading to death, are still unclear. Several pathophysiological processes contributing to the progression of the disease have been disclosed in the last five decades, for instance genetic mutations in more than a dozen of genes[5], excitotoxicity[6], oxidative stress[7], immune/inflammatory processes[8], mitochondrial dysfunctions[9] and disruption in calcium homeostasis[10]. Significance of calcium ions in different physiological and pathological conditions is a well-known phenomenon, since it has got a prominent biological property in reversible complex formations[11] and second messenger function. As a rule in biochemical reactions, a limited concentration range characterizes the optimal conditions of the calcium-mediated processes: either too low or too high concentration values are irreconcilable with life. At low concentration values, the vital

role of calcium was first demonstrated on isolated hearts[12], reported by Sidney Ringer more than 130 years ago. At the other end of the concentration range, excess elevation in the intracellular calcium might lead to cell death[13]. Focusing on the state of the art concept of calcium mediated neuronal degeneration, in a recent manuscript, which appeared in the special issue of Biochemical and Biophysical Research Communications devoted to Neurodegeneration, we discussed the possible central role of the elevated calcium level in the pathomechanism of ALS[14]. In this commentary, we would like to corroborate that hypothesis with recent studies, furthermore, our neuroprotective trials and descriptive contribution to this special scientific field are also introduced.

Reciprocal calcium mediated processes in the pathomechanism of ALS

Involvement of elevated calcium concentration has been observed in chronic neurodegenerative diseases for instance in Alzheimer's disease[15], Parkinson's disease[16], Huntington's disease[17] and ALS[18], furthermore, role of elevated calcium level was confirmed in acute neuronal lesions, as well[19]. Interestingly, most of the known factors of pathological processes are capable to interfere with the calcium homeostasis. Thus, although increased intracellular calcium might be located downstream within the complex pathomechanism of ALS, impaired calcium homeostasis is considered a final common pathway leading to injury of motor neurons through a calcium-dependent positive feedback loop. This is further supported by the recent observation that elevated calcium acts as a driver of transactive response DNA binding protein (TDP-43) mediated neuronal toxicity[20], because TDP-43 was identified as a main component of the cytoplasmic inclusions of the neurons in the majority of ALS patients[21].

Excitotoxicity is a major pathological event in a wide variety of degeneration[22], moreover, its crucial role in ALS was also supported by documenting a two-fold increase in the glutamate level in the sera[23] and cerebrospinal fluid[24] of ALS patients. Molecular basis of this glutamate elevation might be based on a reduced number of excitatory amino acid transporter 2, since this receptor is responsible for a swift reuptake of glutamate[25]. In view of the fact that specific alterations in the subunit composition of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors, namely, reduced number of the glutamate receptor 2 (GluR2) subunit was documented in ALS patients, which makes AMPA receptors permeable to calcium, the increased glutamate level may lead to an excess calcium influx and amplification of excitotoxicity[26]. Another well-characterized pathological pathway in ALS is a reactive oxygen species (ROS) mediated degeneration[27], partially due to the gain of function mutation in the Cu/Zn superoxide dismutase enzyme (SOD1) observed in a subset of the patients[28]. Importantly, elevated calcium may induce conformational change of wild type SOD1 which facilitates its amorphous aggregation[29], thus contributes to the oxidative stress[30]. These data accentuate the role of calcium-mediated protein misfolding also in non-SOD1, sporadic ALS[31]. ROS may change plasma membrane properties, target membrane embedded ion channels[32], which may result in increased activity of P/Q type voltage gated calcium channels[33], and a consequent increase of intracellular calcium. Increased cytosolic calcium may further elevate its intracellular level by impairing endoplasmic reticular calcium buffers[34], furthermore, may augment mitochondrial ROS production[35]. Since the major victims are the motor neurons in ALS, when injured, they signal to neighboring microglia, the resident macrophages of the nervous system. This signal could be chemokine ligand 2 (CCL2)[36], or other signaling molecules, yet to be identified. Microglia are equipped with the appropriate receptors and showed activation pattern in the same time frame as the expression of CCL2 by motor neurons after axonal transection[36]. Microglial activation was directly visualized by [^{11}C](R)-PK11195 positron emission tomography in the central nervous system of ALS patients[37]. Activated microglia, by releasing peroxynitrite, may induce membrane perturbations of the neighboring cells, and are capable to inhibit the function of membrane proteins, like glutamate transporters[38], contributing to elevated glutamate levels and excitotoxicity. Furthermore, they can trigger a phenotypic transformation of astrocytes[39], thus mount a full-blown cellular immune response. Besides cellular immunity, recent observations suggest that humoral immunity has a crucial role in disease progression by documenting the presence of more than 20 ALS specific antibodies in the sera of ALS patients[40]. ALS antibodies may also interact with L-type[41], or N-/P-/Q-type calcium channels[42], as well, resulting in increased intracellular calcium in a motor neuron cell line[43]. The first direct evidence of increased calcium level paralleled with mitochondrial disruption in the pathology of ALS is based on electron microscopic observation of neuromuscular synapses in muscle biopsies obtained from ALS patients[44]. These findings got further support from transgenic animal model of ALS, based on SOD1 G93A mutation, where identical morphological changes and increased calcium could be observed not only in the motor axon terminals, but in the spinal motor neurons, as well[45]. These observations suggest, that while the pathomechanism is rather complex in ALS, calcium elevation may be a key component of the pathogenesis, thus neuroprotective trials should focus on this aspect of the disease.

Potential therapeutic possibilities based on the alleviation of calcium burden

Since sustained disruptions in the mechanism of

physiological calcium homeostasis trigger malicious changes in neuronal functions, furthermore, induce apoptotic and other death-related signaling pathways, stabilization of such ionic balance might be a promising therapeutic possibility. Hints for such approaches might be obtained from the observations that not all neuronal populations are equally susceptible during the disease, namely, the oculomotor and the Onufrowicz nucleus are considered to be resistant regions in ALS[46]. The different resistance of these cells might be based on their unique properties, such as cell size or axonal length, size of the motor unit, network connections, etc, or special calcium homeostasis. Besides the number and composition of the ion channels in their plasma membranes, another relevant factor in shaping cellular calcium homeostasis is related to their calcium buffer capacity. The main component of the calcium buffers in the cytosol is comprised of calcium binding proteins with EF-hand motifs[47]. Indeed, systematic studies of brain and spinal cord autopsy samples from ALS patients led to the conclusion that some of these proteins, such as calbindin-D_{28k} or parvalbumin might be used as marker of resistant cell types[48]. Based on such observations, *in vitro* and *in vivo* studies showed that elevation of parvalbumin or calbindin-D_{28k} level in vulnerable cells provide an enhanced resistance against calcium-mediated acute injury[49,50,51]. In a chronic motoneuron degeneration model, based on transgenic mutant SOD1 animals, by creating double transgenic mice with upregulated parvalbumin, significant neuroprotection could also be achieved, but the progression of the disease could not be stopped[52]. Also in the mSOD1 transgenic mouse strain, an alternative way to reduce calcium burden of motor neurons has been tried by applying AMPA receptor antagonist, talampanel[53]. During these experiments, calcium increase in spinal motor neurons of transgenic animals could be successfully prevented only if the treatment was started prior to the appearance of the symptoms of the disease[53]. Considering the universal role of calcium in the pathomechanism, the meager results of protective attempts in the chronic ALS models were unexpected. The reason behind the moderate success might be based on the fact, that calcium buffer capacity merely prolongs the proper homeostasis but loses its effectiveness due to the inevitable saturation of the buffer system. Furthermore, if the therapeutic attempt with AMPA receptor antagonists is started too late, the dialog between the glial cells and motor neurons might have switched from neuroprotective to neurotoxic mode[8], which phase might be identified in the temporal trends of oxidation, respiration, and calcium regulation[54].

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