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Mechanisms of neuronal damage in multiple sclerosis and its animal models: role of calcium pumps and exchangers

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Abstract

Multiple sclerosis is an inflammatory, demyelinating and neurodegenerative disorder of the central nervous system. Increasing evidence indicates that neuronal pathology and axonal injury are early hallmarks of multiple sclerosis and are major contributors to progressive and permanent disability. Yet, the mechanisms underlying neuronal dysfunction and damage are not well defined. Elucidation of such mechanisms is of critical importance for the development of therapeutic strategies that will prevent neurodegeneration and confer neuroprotection. PMCA2 (plasma-membrane Ca^{2+}-ATPase 2) and the NCX (Na^{+}/Ca^{2+} exchanger) have been implicated in impairment of axonal and neuronal function in multiple sclerosis and its animal models. As PMCA2 and NCX play critical roles in calcium extrusion in cells, alterations in their expression or activity may affect calcium homoeostasis and thereby induce intracellular injury mechanisms. Interventions that restore normal PMCA2 and NCX activity may prevent or slow disease progression by averting neurodegeneration.

Keywords

calcium dyshomoeostasis; experimental autoimmune encephalomyelitis; multiple sclerosis; neurodegeneration; plasma-membrane Ca^{2+}-ATPase (PMCA)

Introduction

Multiple sclerosis is a disease of unknown aetiology affecting over 2 million people worldwide. It is believed that an interplay between susceptibility genes and environmental factors contributes to the pathogenesis of multiple sclerosis [1]. The clinical symptoms include paresthesias, optic neuritis, diplopia, fatigue, paralysis and cognitive dysfunction. The disease course is variable among affected subjects and prognosis is unpredictable. The vast majority of multiple sclerosis patients experience RRMS (relapsing–remitting multiple sclerosis) during which an episode of clinical symptoms is followed by complete or partial recovery. Over time, disability may progress and become permanent, a disease form called secondary progressive. Approx. 20% of patients are afflicted by primary progressive multiple sclerosis during which deficits gradually increase without any remissions. Inflammation, demyelination, oligodendrocyte death, gliosis, axonal damage and neurodegeneration are the main histopathological hallmarks of multiple sclerosis [2,3].

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Molecular events and cellular interactions underlying neural damage in multiple sclerosis have often been addressed by use of animal models. EAE (experimental autoimmune encephalomyelitis) is an animal model of multiple sclerosis that shares some pathological, histological and clinical features with the human disease. It is induced in some animal species and strains by immunization with myelin components or passive transfer of encephalotigenic T-cells. EAE actually encompasses several models that are believed to mimic different aspects of multiple sclerosis [4]. For example, inoculation of Lewis rats with MBP (myelin basic protein) induces acute EAE during which a short episode of clinical symptoms is often followed by recovery. In contrast, C57Bl/6J mice immunized with MOG (myelin oligodendrocyte glycoprotein) develop chronic disease that lasts several weeks. Additional models include relapsing-remitting EAE in SJL mice immunized with proteolipid protein. Regardless of the type of EAE, the disease is characterized by progressive ascending paresis and paralysis. Whereas demyelination occurs in some EAE models, inflammation, axonal damage and neurodegeneration appear to be features common to most forms. In general, EAE has been extremely useful to unravel important cellular mechanisms and establish therapeutic treatments for multiple sclerosis patients [5].

GM (grey matter) pathology, neuronal dysfunction and axonal injury in multiple sclerosis and EAE

Evidence of neuronal and axonal pathology in post-mortem multiple sclerosis brain has been reported as early as 1868 by Jean-Martin Charcot [6]. Yet, for most of the next century, this finding received relatively little scientific attention. However, in recent years, a revitalized interest in this topic was spurred, in part, by detection of transected axons in both acute and chronic brain lesions, using more modern histological approaches [7–9]. The advance in MRI (magnetic resonance imaging) and MRS (magnetic resonance spectroscopy) techniques incited further investigations which established a correlation between axonal loss and neurological deficits, with particular emphasis on permanent disability [10–12]. Originally, it was hypothesized that the early demyelination and repeated inflammatory attacks lead to axonal damage at late stages of multiple sclerosis. However, the concept of a two-stage disease has recently been challenged [13]. The lack of good correlation between GM injury and inflammatory demyelination indicated that these deleterious processes take place concomitantly, rather than sequentially. In fact, MRS studies on metabolites primarily associated with neurons, such as NAA (*N*-acetylaspartate), has provided novel insights into the course and pattern of neuronal dysfunction and axonal damage in multiple sclerosis. These investigations reported a decrease in NAA not only in lesions but also in normal-appearing WM (white matter) and GM, suggesting widespread neuronal and axonal pathology already at the onset of clinical symptoms or early in the course of the disease [14–20]. Diffuse GM and WM pathology is observed in all multiple sclerosis phenotypes, and GM atrophy, potentially due to neurodegeneration, is also evident at early stages of multiple sclerosis [21]. The reduction in NAA may either reflect permanent neuronal and axonal loss or transient neuronal dysfunction. In support of the former concept, Wylezinska et al. [22] suggested that the correlation between a decrease in NAA in the thalamus and atrophy of this brain region reflects neuronal degeneration at early stages of RRMS. These results are in agreement with previous findings showing a significant neuronal loss in the thalamus of post-mortem multiple sclerosis brain [23]. Alternatively, reductions in NAA may be the consequence of a transient and reversible neuronal dysfunction [19].

The importance of GM pathology in multiple sclerosis is further highlighted by longitudinal studies on subjects initially presenting with clinically isolated syndromes suggestive of multiple sclerosis. These investigations indicated that the early development of multiple sclerosis is associated with progressive GM but not WM atrophy [24]. It is worth noting that a correlation between clinical disability and metabolic changes in normal-appearing WM,

diffuse GM pathology and GM atrophy has been reported [21,25–28]. In particular, decreases in both GM and WM volumes have been associated with cognitive deficits [26–28].

Several important histological and molecular abnormalities associated with neuronal and axonal dysfunction in multiple sclerosis are also present in EAE. Similarities in axonal damage as revealed by impaired transport of APP (amyloid precursor protein) have been shown in both multiple sclerosis and EAE [29]. Early axonal damage in EAE has been linked to compromised neuronal microtubule integrity [30], and elevated levels of non-phosphorylated NFH (neurofilament H), an important element of the neuronal cytoskeleton, have been detected even before demyelination [31]. Non-phosphorylated NFH-positive axons are found in multiple sclerosis lesions as well [8], and increased NFH in cerebrospinal fluid of multiple sclerosis patients correlates with disease disability and poor clinical outcome after initiation of therapeutic treatment [32]. Also consistent with human studies is the finding that loss of axons is an important component of permanent disability in EAE [33,34].

Despite extensive information regarding the importance of neuronal damage in multiple sclerosis pathology, little is known about the underlying molecular mechanisms. A number of studies indicated that PMCA2 (plasma-membrane Ca^{2+}-ATPase 2), NCX (Na^{+}/Ca^{2+} exchanger) and some ion channels may play a critical role in neuronal pathology and axonal injury and therefore might be targets for therapeutic interventions.

Role of ion channels, calcium pumps and exchangers in axonal injury and neuronal dysfunction in multiple sclerosis and its animal models

Ion homoeostasis plays a critical role in the function of neurons. In particular, calcium, which mediates many intracellular events under normal conditions, can cause neurodegeneration in pathological circumstances due to excess accumulation within the cell [35–36]. Cellular calcium homoeostasis is maintained by several mechanisms including influx, extrusion, buffering and sequestration. The malfunction of any of these processes may cause calcium dyshomoeostasis, initiating injury mechanisms that lead to neuronal dysfunction and death. Calcium extrusion is mediated by PMCAs and NCXs. Both have been implicated in neuronal and axonal injury in multiple sclerosis and EAE.

PMCAs are a family of P-type ATPases that comprise four isoforms encoded by different genes, with additional variants generated by alternative splicing [37]. The cellular and tissue distributions of these isoforms suggest that they play distinct roles [38,39]. PMCA isoforms 1 and 4 are ubiquitously expressed, whereas PMCA2 and PMCA3 show a more restricted distribution [40]. PMCA2 is enriched in some central nervous system regions and found primarily in neurons. Knockout mice deficient in PMCA isoforms display distinct phenotypes [41]. In particular, PMCA2-null mice exhibit hindlimb weakness consistent with a reduction in the number of spinal cord motor neurons as compared with the wild-type [42]. Abnormal gait and balance, ataxia and hearing deficits are also characteristics of the PMCA2-null mouse phenotype, indicating that the lack of PMCA2 cannot be compensated for by the presence of other isoforms [43]. Emerging evidence supports the notion that alterations in PMCA expression or activity are linked to a number of pathological conditions [44].

Indication of a potential involvement of PMCA2 in spinal cord neuronal pathology during acute EAE in the Lewis rat was provided by the studies of Nicot et al. [45] who reported a significant decrease in the levels of PMCA2 in GM cells at the onset of symptoms. In contrast, the levels of other ion pumps including the NCX and SERCA (sarcoplasmic/

endoplasmic-reticulum Ca^{2+}-ATPase) were reduced only at later disease stages, whereas mRNA levels of PMCA isoforms 1, 3 and 4 were not altered [45,46]. PMCA2 levels were restored to almost control values before clinical recovery, suggesting that changes in PMCA2 expression may be reversible [46]. The decrease in PMCA2 mRNA and protein levels was also observed at the onset of symptoms in MOG-induced chronic EAE in the mouse [46]. In contrast with acute EAE, PMCA2 levels remained low throughout the course of chronic disease. These results taken together suggested a correlation between PMCA2 expression in the spinal cord and EAE disease course.

Further studies were undertaken in order to establish a causal relationship between a decrease in PMCA2 and neuronal pathology. This issue was addressed in neuronal cultures, which offer the possibility of investigating mechanisms in a controlled environment. Inhibition of PMCA activity by use of 5-(6) carboxyeosin diacetate delayed the clearance of depolarization-induced calcium transients and exposed neurons to higher intracellular calcium concentrations for an extended period of time, a trigger that could induce injury mechanisms [42]. In fact, the delay in calcium clearance was followed by neuritic beading and swelling and cytoskeletal abnormalities as indicated by an increased number of non-phosphorylated NFH-immunoreactive neurons. It is worth noting that an increase in non-phosphorylated NFH-immunoreactive axons, as assessed by use of the SMI-32 antibody, has been shown in post-mortem multiple sclerosis brain [8] and in various EAE models [47,48]. Motor neurons appeared to be particularly vulnerable to inhibition of PMCA activity. It is possible to hypothesize that reductions in PMCA2 expression and activity may cause injury due to activation of calcium-dependent proteases or protein phosphatases. One consequence of abnormal protein phosphatase activation would be the dephosphorylation of cytoskeletal proteins such as neurofilaments. This would affect neuronal stability and increase the vulnerability of cytoskeletal proteins to proteasemediated degradation [49]. In fact, some of these proteases, including calpains, have been implicated in multiple sclerosis and EAE [50,51]. Overactivation of calpain as a result of calcium dyshomoeostasis and the consequent degradation of its substrates have been described in many neurodegenerative diseases [52]. Increased calpain expression has been shown in multiple sclerosis plaques [51] and in spinal cord neurons during EAE [53]. Degradation of calpain substrates has also been reported in EAE [54]. Interestingly, activation of calpain leads to degradation of PMCA in platelets [55]. If such a mechanism also occurs in neurons, it can exacerbate damage by further reducing PMCA-mediated calcium extrusion.

Axonal damage in multiple sclerosis can also be the consequence of a reversal in the activity of NCX and the consequent increase in intracellular calcium. Under normal circumstances, the NCX extrudes calcium from cells. However, an abnormal increase in Na^+ within the neuron or axon can lead to reversal of NCX activity, causing entry rather than expulsion of calcium which can initiate injury mechanisms. The increase in Na^+ may be due to a failure in the function of Na^+/K^+ ATPase, following a decrease in ATP in affected cells as well as aberrance in Na^+ channel expression, distribution and function [56–58]. In normal myelinated axons, Na^+ channels are mostly clustered in the Nodes of Ranvier and less frequently in myelinated segments of the axons. Waxman and co-workers [59,60] have shown that the Na^+ channels $Na_v1.6$ and $Na_v1.2$ are increased all along demyelinated axons in post-mortem multiple sclerosis spinal cord and the optic nerve in EAE. Moreover, $Na_v1.6$ co-localizes with NCX in the injured axons in the spinal cord of mice with EAE [61]. $Na_v1.6$ produces a large, persistent sodium influx that can lead to a substantial increase in intracellular sodium concentration, thereby mediating the reversal of NCX in multiple sclerosis. It has been suggested that the increased expression and redistribution of Na_v 1.2 along demyelinated axons compensates for conductance failure, whereas the co-localization of Na_v 1.6 with NCX may be a mechanism underlying axonal injury. In agreement with these findings, inhibition of NCX protects axons during inflammation [62], and sodium

channel blockers, such as phenytoin and lamotrigine, prevents axonal degeneration and significantly improve clinical scores in EAE [63–66].

Conclusions

Whereas some axonal injury may be the consequence of demyelination, other triggers may be responsible for induction of neuronal pathology and neurodegeneration independent of demyelination. Irrespective of the underlying mechanisms or the causes, it is clear that neurodegenerative processes play a prominent role early in multiple sclerosis pathogenesis and are linked to irreversible disability in later stages of the disease. This evidence prompted experts to recommend early treatment with current disease-modifying therapeutic agents and to urge other researchers to investigate the efficacy of putative neuroprotective treatments [67,68]. In fact, early intervention with approved therapies during an initial demyelinating episode delays the conversion into clinically definite multiple sclerosis [69] and promotes axonal metabolic recovery in RRMS [70]. Consequently, the discovery of novel therapeutic agents targeting specific pathways involved in axonal injury and neurodegeneration, and their combinatorial use prior to occurrence of irreversible damage, may significantly alter the course and outcome of multiple sclerosis. Therefore understanding the intracellular events leading to neuronal pathology and death is of critical importance for the design and implementation of therapeutic strategies to fully prevent disease progression. In this regard, the role and contribution of calcium pumps and exchangers to multiple sclerosis pathology warrant further investigations.

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Abbreviations used

EAE	experimental autoimmune encephalomyelitis
GM	grey matter
MOG	myelin oligodendrocyte glycoprotein
MRS	magnetic resonance spectroscopy
NAA	*N*-acetylaspartate
NCX	Na^+/Ca^{2+} exchanger
NFH	neurofilament H
PMCA	plasma-membrane Ca^{2+}-ATPase
RRMS	relapsing–remitting multiple sclerosis

WM	white matter