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General mechanisms of axonal damage and its prevention

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Abstract

Axonal degeneration is a prominent pathological feature in multiple sclerosis observed over a century ago. The gradual loss of axons is thought to underlie irreversible clinical deficits in this disease. The precise mechanisms of axonopathy are poorly understood, but likely involve excess accumulation of Ca ions. In healthy fibers, ATP-dependent pumps support homeostasis of ionic gradients. When energy supply is limited, either due to inadequate delivery (e.g., ischemia, mitochondrial dysfunction) and/or excessive utilization (e.g., conduction along demyelinated axons), ion gradients break down, unleashing a variety of aberrant cascades, ultimately leading to Ca overload. During Na pump dysfunction, Na can enter axons through non-inactivating Na channels, promoting axonal Na overload and depolarization by allowing K egress. This will gate voltage-sensitive Ca channels and stimulate reverse Na–Ca exchange, leading to further Ca entry. Energy failure will also promote Ca release from intracellular stores. Neurotransmitters such as glutamate can be released by reverse operation of Na-dependent transporters, in turn activating a variety of ionotropic and metabotropic receptors, further exacerbating overload of cellular Ca. Together, this Ca overload will inappropriately stimulate a variety of Ca-dependent enzyme systems (e.g., calpains, phospholipases), leading to structural and functional axonal injury. Pharmacological interruption at key points in these interrelated injury cascades (e.g., at voltage-gated Na channels or AMPA receptors) may confer significant neuroprotection to compromised central axons and supporting glia. Such agents may represent attractive adjuncts to currently available immunomodulatory therapies.

Keywords: Axonal degeneration; ATP; Ca

1. Introduction

Myelinated axons in the mammalian central nervous system are uniquely designed to support rapid and efficient saltatory impulse propagation. The highly inhomogeneous segregation of ion channels, with high densities of nodal Na channels conducting inward depolarizing current, and internodal/juxtaparanodal K channels maintaining polarization and electrical stability [1], together with an insulating myelin sheath covering 99% of the surface of the axons, constitutes a very elegant solution to the problem of packing billions of fibers into a relatively compact space. A number of prevalent diseases frequently disrupt the architecture of central myelinated fibers, resulting in varying degrees of clinical disability. Common examples include stroke, brain, and spinal cord injury, and multiple sclerosis, the latter being a prototypical white matter disorder. Although MS has traditionally been considered to be a chronic inflammatory disorder of central white matter [2,3], recent data suggest that mitochondrial dysfunction may play an important role [4] (Trapp, this issue), and could be linked to the observed axonal degeneration. Moreover, it is known that glutamate release and nitric oxide generation are prominent features of inflammatory lesions; these mediators play a central role in anoxic/ischemic CNS injury as well. Taken together, accumulating data are beginning to suggest a convergence between mechanisms of anoxic/ischemic injury of central white matter, and the mechanisms of tissue damage that occur in MS. This article will briefly review current knowledge of the molecular mechanisms of white matter ischemic injury, and emphasize the potential relevance to what is known about axonal injury in MS. The interested reader is referred to more comprehensive recent reviews [5–11].

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2. Anoxia/ischemia causes conduction failure in CNS white matter

The mammalian CNS has very limited intrinsic energy reserves and therefore requires a continuous supply of oxygen and glucose for normal function. Given this absolute dependence on a steady supply of ATP, it is not surprising that anoxia/ischemia rapidly leads to depolarization and failure of conduction in both neurons and central white matter tracts. Fig. 1 shows a typical example of a recording of compound action potentials (CAPs) recorded from rat optic nerve subjected to in vitro anoxia. Conduction along adult rat optic nerve is abolished within minutes of anoxia, in parallel with a strong depolarization of resting membrane potential [12–14]. Glycolytic inhibition (e.g., using iodoacetate) in isolation also causes a marked depolarization and failure of electrogenesis, but the onset is delayed compared to anoxia alone by ≈20 min [15–17], probably because of continued utilization of alternate substrates such as amino acids by the Krebs cycle and ATP generation by oxidative metabolism [13,18]. Aglycemia shows an even longer delay, likely due to supply of lactate to axons by astrocytes [19,20]. Taken together, these in vitro data emphasize the absolute reliance of white matter on oxygen and glucose for continued function.

A deficiency of energy substrates leads to accumulation of axoplasmic Na and Ca, and a loss of K [21,22]. This early ionic deregulation is the key initial step that in turn entrains a variety of damaging cascades. As expected, axoplasmic [K] falls while [Na] rises from ≈20 to ≈100 mM. There is also a parallel four- to fivefold rise in total [Ca] after 60 min of in vitro anoxia. Recent evidence using confocal microscopy suggests that ischemia-induced ionized [Ca], most relevant to biological (including pathological) processes, rises from a presumed baseline of ≈100 nM to the tens of micromolar [23–25]. Ischemic CNS axons also exhibit a rise in free (but of course not total) [Ca] in Ca-free perfusate, indicating a release from intracellular stores (see below). In some white matter tracts, there is sufficient intracellular Ca to severely damage axons without any influx of this ion from the extracellular space [26]. Loss of intracellular K, together with accumulation of axonal Na and a rise in extracellular [K] [27], will promote inappropriate operation of a variety of ion-coupled and electrogenic (i.e., influenced by membrane depolarization) transporters of other ions (notably Ca) and organic molecules, such as neurotransmit-

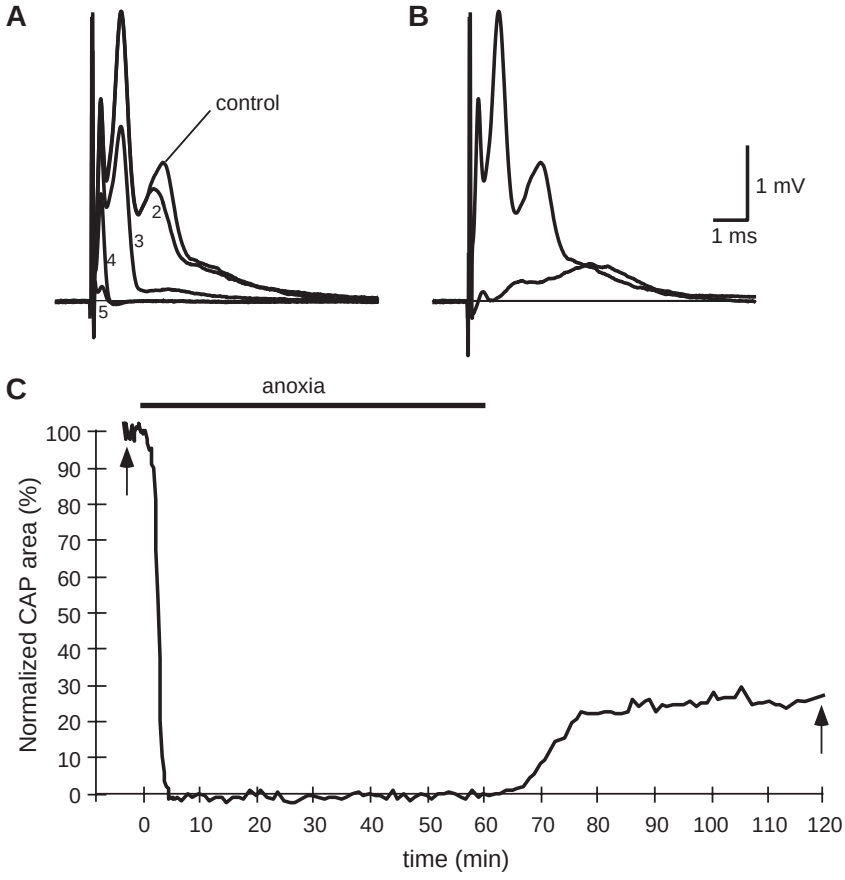

Fig. 1. Effect of in vitro anoxia on rat optic nerve excitability. (A) Representative compound action potentials (CAPs) plotted at 1 min intervals following initiation of anoxia showing a rapid loss of excitability. (B) Comparison of control, pre-anoxic CAP and the same nerve recorded after 60 min of anoxia/60 min re-oxygenation (arrows in panel C). (C) Normalized CAP area plotted against time illustrating a rapid loss of CAP amplitude and recovery to only ≈20–30% of pre-anoxic CAP after re-oxygenation (modified from Ref. [12], with permission).

ters (e.g., glutamate, and likely others). An early proposed mechanism of injury to central axons therefore involved (1) Na–K ATPase failure; (2) Na accumulation through persistent Na channels [28,29] and K efflux; leading to (3) Ca accumulation via reversal of the Na–Ca exchanger (steps 1, 3, and 4 in Fig. 5). By this scheme, it was found that removal of Ca or Na, or blocking of voltage-gated Na channels, was highly protective in anoxic rat optic nerve and spinal white matter tracts [30–32]. This Na-dependent Ca overload was shown to be heavily dependent on transport by reverse Na–Ca exchange, and could be blocked by Na–Ca exchange inhibitors, with a parallel improvement in functional recovery [30,32,33]. As will be discussed below, more severe insults in the form of in vitro ischemia recruit additional Ca-dependent injury mechanisms.

3. White matter excitotoxicity

White matter possesses no synaptic machinery, therefore implicating neurotransmitter-dependent physiological or pathological processes may be counterintuitive. Nonetheless, a number of studies have shown that several neurotransmitters have effects in normal or injured white matter tracts; these include GABA, adenosine, norepinephrine, and nicotine [19,34–39]. Glutamate, the main excitatory neurotransmitter in the mammalian CNS, also plays a very significant role in many modes of white matter injury.

Many of the components of white matter possess receptors for glutamate, and may therefore be targets for excitotoxicity during pathological conditions. Oligodendrocytes express GluR3 and GluR4 AMPA receptor subunits (notably lacking GluR2), as well as all kainate subunits except GluR5 [40–42]. The absence of GluR2 in oligodendrocytes may render them particularly susceptible to Ca flux through these Ca-permeable receptors. AMPA and kainate receptor expression is particularly robust in oligodendrocyte progenitors [43–45], which may explain the sensitivity of these cells to ischemia [46,47]. White matter astrocytes express all AMPA and kainate receptor subunits except GluR4, and although astrocytes are more resistant to excitotoxicity, they can be severely injured when exposed to AMPA receptor agonists [48], particularly when desensitization is blocked [49] (for reviews, see Refs. [7,40,50]). Notably, mature glia lack NMDA receptors, although these receptors are transiently expressed in immature oligodendrocytes and astrocytes [51]. Interestingly, the myelin sheath itself may be directly vulnerable to excitotoxicity. We detected biochemical breakdown of myelin proteins after exposure to glutamate in vitro [48] and, more recently, using two-photon microscopy, we showed that ischemia induces an increase in Ca within the compact myelin of adult dorsal columns and optic nerves. Although the precise routes of Ca influx into myelin are not yet known, ionotropic glutamate receptors are strongly implicated [52]. Indeed, myelin in central axons contains GluR4 (but not GluR2) subunits [48,53], suggesting that Ca-permeable glutamate receptors may render this structure directly vulnerable to elevated ambient glutamate levels. Evidence of axonal protection by NBQX in an in vivo model of experimental allergic encephalomyelitis [54] and in an in vitro model of central white matter ischemic injury [55] raises the question of whether or not AMPA/kainate receptors may be present on axons per se. However, more recent data from Underhill and Goldberg [56] indicate that the axonal protection by glutamate antagonists in their cell culture model may be secondary to sparing of oligodendroglia, thereby reducing the generation of free radicals that could be toxic to neighboring axons. At the moment, the presence of ionotropic glutamate receptors on central myelinated axons is a strong possibility but has not been proven conclusively.

Fig. 2A shows an example of glutamate toxicity in an in vitro white matter model. Exposure of adult rat dorsal column slices to glutamate or kainate (a non-desensitizing agonist at AMPA receptors) causes significant physiological injury with CAP amplitudes irreversibly depressed to $\approx$50% of control. In contrast, even high concentrations of NMDA (with glycine and with Mg removed) did not cause appreciable injury [48], consistent with observations obtained in optic nerve a decade earlier (Stys, Waxman, Ransom, unpublished) and from other laboratories [57,58]. Metabotropic glutamate receptors may also contribute to white matter damage, possibly coupling back to the potentially important mechanism of release from internal Ca stores, via a phospholipase C-dependent mechanism acting on IP3 receptors [59,60]. Now that the toxic role of glutamate in white matter has been confirmed, the key question of course is: How is this neurotransmitter released in tissue devoid of synapses? Injured white matter suffers marked ionic deregulation mainly involving axons, leading to depolarization, loss of K, and accumulation axoplasmic of Na. These conditions will favor reverse operation of a number of important homeostatic mechanisms including the Na–Ca exchanger, Na–H antiport, and Na-dependent glutamate transporters. The latter represents an important mechanism for terminating excitatory synaptic transmission in gray matter under normal conditions, coupling the movement of glutamate or aspartate with three Na ions and one proton in exchange for one K ion [61,62]. The electrogenic nature of this transport cycle implies that depolarization and collapse of Na and K gradients will promote release of glutamate through this mechanism, in a manner similar to that proposed in immature optic nerve after electrical stimulation [63]. Cytoplasm, including axoplasm, is known to contain millimolar concentrations of glutamate that far exceed the low micromolar levels in brain extracellular space [64,65]; therefore there exist ample quantities of this transmitter in intracellular compartments to potentially cause serious damage if released inappropriately. Fig. 2B shows an example of experiments performed on anoxic dorsal columns treated with two specific Na-dependent glutamate transport blockers (dihydrokainate and L-

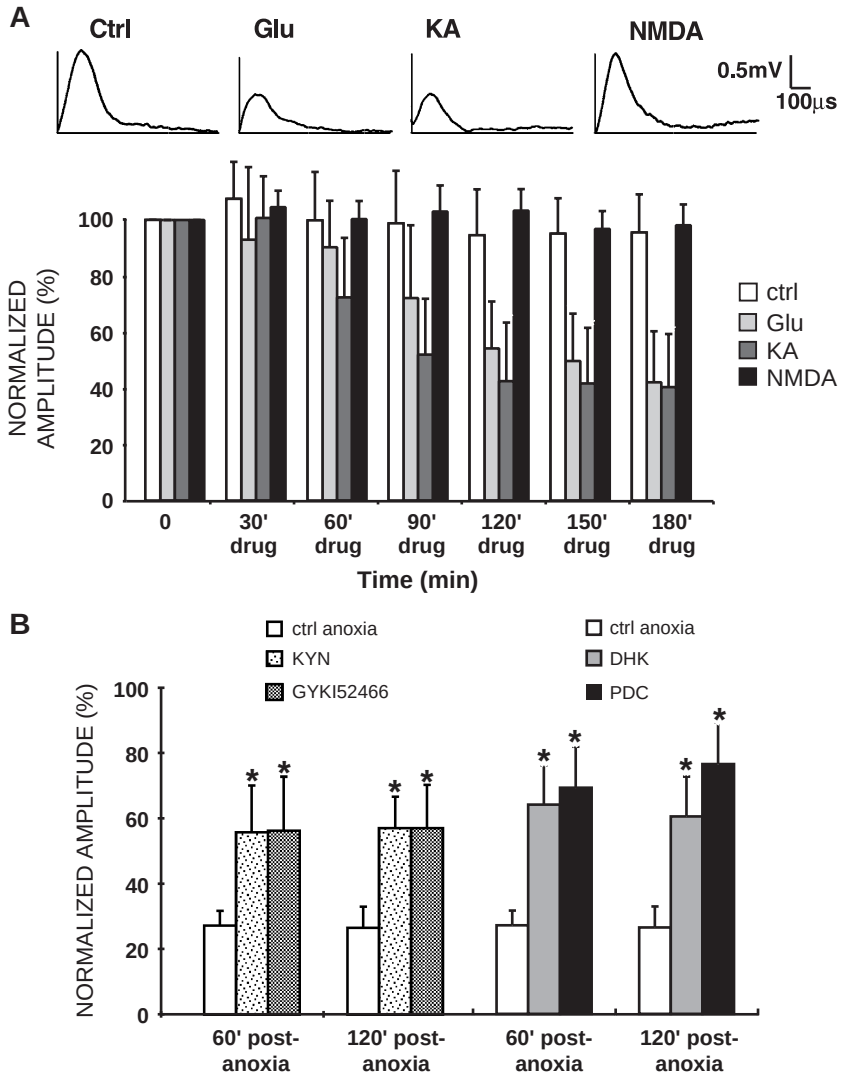

Fig. 2. (A) Effect of glutamate (Glu), kainate (KA), or NMDA on in vitro rat dorsal columns. Glutamate (1 mM) or kainate (500 μM) irreversibly reduced CAP mean amplitudes to ≈40–50% of control after 180 min, whereas NMDA (500 μM, with 20 μM glycine and in the absence of Mg) had no measurable effect on single propagated CAPs after 3 h. (B) Protective effects of ionotropic glutamate receptor antagonists or glutamate transport blockers against dorsal column anoxic injury. Kynurenic acid (1 mM), a broad spectrum (NMDA, AMPA, kainate receptor) antagonist, and the more selective AMPA receptor antagonist, GYKI 52466 (30 μM), are both protective against anoxic injury in rat dorsal columns. Similarly, the Na-dependent glutamate transport blockers, dihydrokainate (DHK) or L-*trans*-pyrrolidine-2,4-dicarboxylic acid (PDC; 1 mM each), were also protective, indicating that endogenous glutamate is released from dorsal columns during anoxia/ischemia via reverse Na-dependent glutamate transport, causing damage by activation of AMPA receptors (A, modified from Ref. [48], with permission, copyright 2000 by the Society for Neuroscience; B, modified from Ref. [68], with permission, copyright 1999 by the Society for Neuroscience).

trans-pyrrolidine-2,4-dicarboxylic acid [66,67]), illustrating the significantly protective effect against anoxic injury. Semi-quantitative immunohistochemistry for glutamate showed that most of this transmitter was released from axon cylinders during anoxia [68], consistent with the expected vulnerability of white matter components (axon > oligodendrocyte > astrocyte). During ischemia, however, with a more severe cellular energy deficit, immature oligodendrocytes also release substantial quantities of glutamate that in turn activates receptors on these same cells to cause death, in effect creating a "fatal glutamate release feedback loop" [46]. Other modes of glutamate release have not been investigated in injured white matter, but may also include anion channel-mediated efflux, exocytotic release during more severe injury where astrocytic [Ca] would be expected to rise, or movement through astrocytic hemichannels [69].

4. Release of Ca from intracellular stores

The majority of cellular Ca is bound to Ca binding proteins and sequestered in organelles such as ER and mitochondria. Axons contain substantial amounts of total Ca (approaching 1 mM [22,70]), but free ionized [Ca] is likely maintained at ≈100 nM as in most neural cells. As one

might expect anoxia and ischemia increase axonal Ca levels substantially [22]. More recently, we have shown that axoplasmic [Ca] increases in response to in vitro ischemia in myelinated axons of both rat optic nerve and spinal dorsal columns in the absence of extracellular Ca [25,26], indicating that there exist intracellular sources of this cation. The precise sources of this intracellular Ca have not all been identified, but possibilities include: (1) mitochondria, which may source Ca from the matrix [71,72]; and (2) endoplasmic reticulum, which can release Ca through four distinct receptors (ryanodine receptors, IP3 receptors, nicotinic acid adenine dinucleotide phosphate (NAADP)-sensitive receptors, and "sphingolipid Ca release-mediating protein of the ER" (SCaMPER) [73–76]), and cytosolic Ca-binding proteins such as parvalbumin, calbindin-D28K, calmodulin, and others [77–79]. Preliminary data from our laboratory suggest that many of the above sources come into play: (i) ryanodine receptor-mediated release from ER in dorsal column axons [26]; (ii) probably release via IP3 receptors in response to IP3 generation by phospholipase C; and (iii) release from mitochondria [25].

An important question is: Is such release from intracellular compartments a minor epiphenomenon, or can it contribute to a significant degree to the ultimate injury produced by ischemia? We explored this question using the in vitro ischemic dorsal column model. A summary of the electrophysiological results is shown in Fig. 3. Recall that removal of extracellular Ca or Na, or blocking of voltage-gated Na channels with TTX, is each highly protective against anoxic injury in both optic nerves and dorsal columns of the adult rat [30,32]. However, if the injury is intensified by exposure of dorsal columns to in vitro ischemia (OGD) for an amount of time equivalent to the anoxic studies (60 min), the responses change radically. Predictably, CAP recovery after 60 min of OGD is worse. Moreover, neither removal of bath Ca nor blocking of Na flux through Na channels is able to rescue the tissue, with CAP recoveries remaining near zero (Fig. 3). This suggests that the more severe ischemic injury is Ca-independent, or that Ca is sourced from intracellular compartments. To rule out the former possibility, we pre-treated the animals with the membrane-permeable Ca chelator, BAPTA-AM, intravenously [80]. Dorsal columns from animals so treated recovered very well following in vitro ischemia, indicating that this injury nevertheless remains dependent on cellular over-accumulation of Ca ions. The key experiment involved ischemic dorsal columns maintained in 0Ca perfusate (which by itself is not much more protective than Ca-replete bath solution) but with the addition of the L-type Ca channel blocker, nimodipine. Here dorsal column CAPs recovered to over 60% of control, compared to just 2% without nimodipine [26]. Clearly the action of nimodipine was not by inhibition of Ca influx through the channel, as all Ca was removed from the extracellular space. How then did this Ca blocker confer its very robust protection?

Dihydropyridines such as nimodipine and nifedipine block Ca channels by interfering with the channel's voltage sensor [81], not by blocking its pore. In skeletal muscle, depolarization sensed by L-type Ca channels is used to activate ryanodine receptors to release Ca from the sarcoplasmic reticulum, in turn promoting contraction. We speculated that a mechanism similar to such "excitation–contraction coupling" also operates in ischemic spinal axons. Indeed, blocking ryanodine receptors directly using

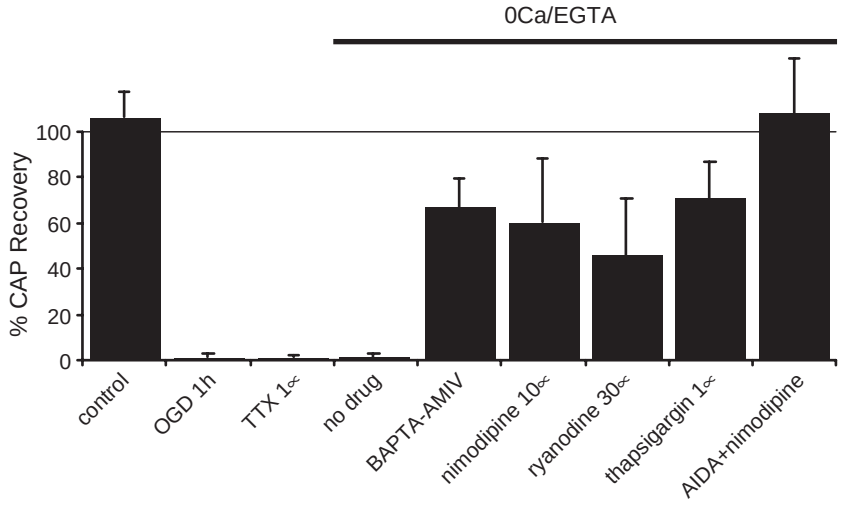

Fig. 3. Compound action potential recovery following in vitro ischemia in rat dorsal columns as a function of various treatments. 60 min of ischemia simulated by oxygen–glucose deprivation (OGD) causes severe functional injury, which could not be improved by either Na channel block (TTX) or Ca-free perfusate (in contrast to anoxia alone where these treatments were very protective). Prior systemic administration of the membrane-permeable Ca chelator, BAPTA-AM, was protective, indicating that a large component of the injury is Ca-dependent. Blocking the voltage sensor on L-type Ca channels (nimodipine), blocking Ca release from stores by inhibition of ryanodine receptors (ryanodine), or depletion of Ca stores (thapsigargin) were all very protective, but only in 0Ca bath. Adding a blocker of group I metabotropic receptors (AIDA), which presumably reduces Ca release from IP3-dependent stores via a phospholipase C-dependent pathway, together with nimodipine and zero external Ca, completely protects dorsal columns from a severe ischemic injury [60].

ryanodine, or depleting ER Ca stores by pre-application of the ER Ca-ATPase inhibitor, thapsigargin, was very protective, to a degree similar to nimodipine. Direct measurements of axoplasmic [Ca] using fluorescent dyes and confocal microscopy revealed that Ca rises substantially during ischemia even in 0Ca perfusate, and that this Ca increase can be greatly reduced by ryanodine [26] or nimodipine [82]. Finally, ultrastructural studies demonstrate the existence of subaxolemmal cisternae [26,83] (Fig. 4A), and triple-label immunofluorescence (Fig. 4B) and co-immunoprecipitation confirm a spatial and physical association between voltage-gated Ca channels and ryanodine receptors near the axolemma of central myelinated axons [26].

Notably, functional recovery, although greatly improved by interference with this "excitation–contraction coupling"-like Ca release mechanism, is still incomplete in dorsal column axons after 60 min of ischemia, suggesting other Ca sources. Preliminary experiments from our laboratory indicate that additional Ca release from IP3 receptors may play a role. Adding the class I metabotropic glutamate receptor antagonist, 1-aminoindan-1,5-dicarboxylic acid (AIDA), to nimodipine/0Ca-treated dorsal column slices completely protected the tissue against a severe 1-h ischemic insult (Fig. 3) [60]. We speculate that mGluR-I receptors activate phospholipase C, leading to IP3 production and Ca release through this pathway. Support for this comes from the additional observation that phospholipase C inhibition is also significantly protective against in vitro ischemia in dorsal columns, presumably by interference with IP3 production and therefore reduction of Ca release from this compartment. Moreover, directly blocking IP3 receptors by intra-axonal heparin also reduces ischemic Ca rise [25]. Taken together, these results indicate that in some white matter tracts, under more severe injury conditions such as ischemia, additional Ca-dependent mechanisms are recruited, involving release from intracellular pools. These experiments raise the strong possibility that controlling extracellular Ca influx is necessary but not sufficient to protect this tissue against more severe insults, with additional control of Ca release from intracellular stores required for optimal protection.

5. Synthesis of injury mechanisms and protective strategies

From work on anoxia/ischemia in white matter carried out over the last decade in several laboratories has emerged a surprisingly complex framework of inter-related events triggered by cellular energy failure. The details are summarized in Fig. 5. Failure of ion-pumping ATPases appears to be a proximal event, with impairment of the plasmalemmal Na–K ATPase promoting Na influx and K loss, and SERCA (sarcoplasmic–endoplasmic reticulum Ca ATPase; step 1b in Fig. 5) failure contributing to release of intracellular Ca. Na overload occurs largely by flux through non-inactivating Na channels, likely the Nav1.6 isoform in mature myelinated fibers. A pathological rise in axoplasmic [Na], coupled with strong ischemic depolarization, will drive a number of Na-dependent, electrogenic transporters to operate in their "reverse" modes. For example, the Na–Ca exchanger will import damaging quantities of Ca from the extracellular space, and the Na-dependent glutamate transporter (and possibly other Na-coupled neurotransmitter co-transporters) will release glutamate from axons and glial

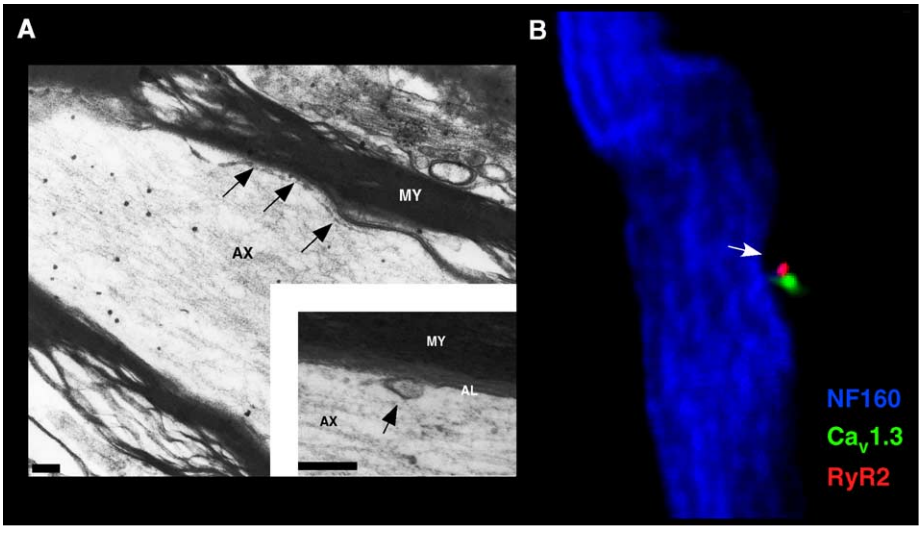

Fig. 4. (A) Distribution of the "axoplasmic" reticulum in dorsal column axons. Ultrastructural examination of dorsal column axons reveals endoplasmic reticulum profiles in the cortical as well as the central axoplasm. Circular, elongated, or irregular cisternae frequently abutted the axolemma. MY: myelin; AX: axoplasm; AL: axolemma. Scale bars, 200 nm (reproduced from Ref. [26], with permission). (B) Immuno-localization of Cav and RyR in spinal dorsal columns. Triple labelled sections (Cav1.3/RyR2/neurofilament) showing adjacent clusters of Cav and RyR proteins at the surfaces of axon cylinders. Such clusters frequently overlaid neurofilament-free "lacunas" (arrow), likely representing ER cisternae that do not contain structural axonal proteins such as neurofilaments [26].

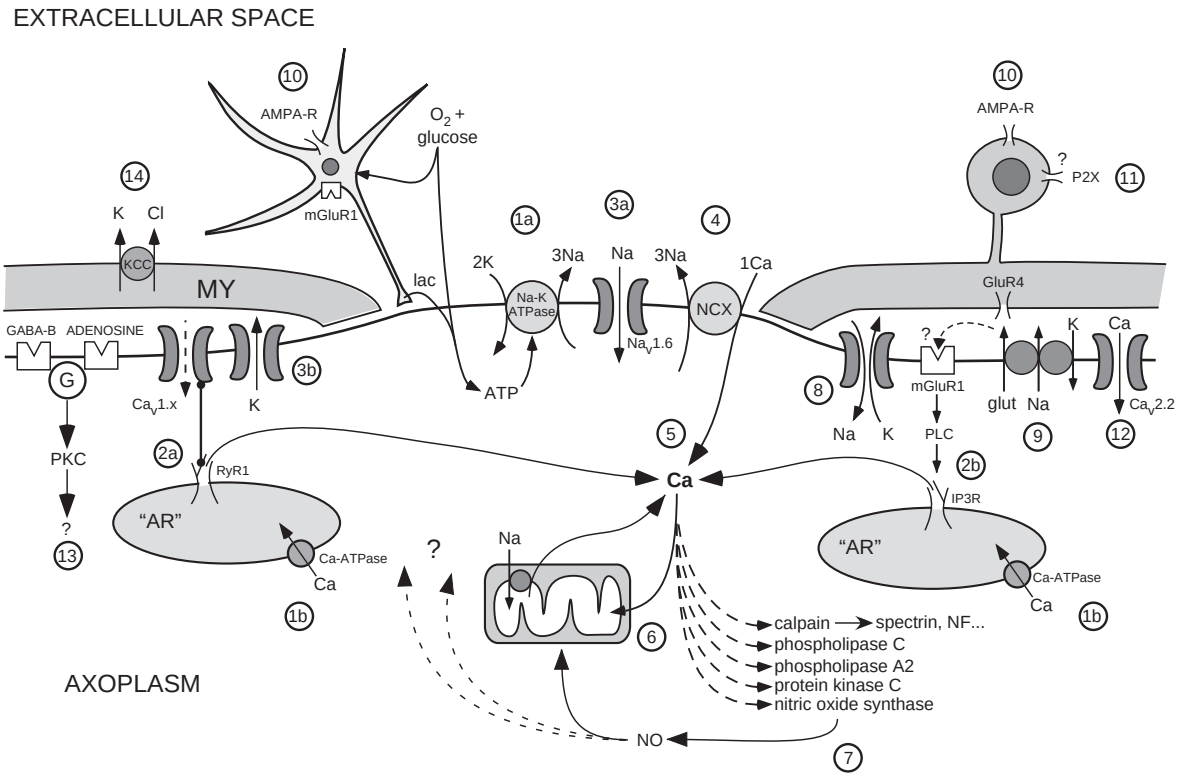

Fig. 5. Events triggered during acute injury of CNS white matter. An energy deficit and/or excess demand impairs ATP-dependent pumps such as the Na–K ATPase (1a) and Ca ATPase (1b), including those located on the "axoplasmic reticulum". Internal stores of Ca may contribute significantly to axonal Ca accumulation, triggered by depolarization via L-type Ca channels (2a) and/or generation of IP3 (2b). The rise in flux through non-inactivating Na channels (3a) will increase $[Na]_i$ and, together with depolarization caused by K efflux through a variety of K channels (3b), stimulates the Na–Ca exchanger to operate in the reverse Ca import mode (4). This Ca accumulation (5) promotes destructive events including mitochondrial Ca overload (especially during reoxygenation) (6), and over-activation of several Ca-dependent enzyme systems (7). NO will inhibit mitochondrial respiration and alter other cellular proteins. Some Na influx may occur through Na/K permeable inward rectifier channels (8) [15,102]. Glutamate is also released through reversal of Na-dependent glutamate transport (9), causing cellular injury from activation of ionotropic glutamate receptors (10). Very recently, ATP-activated P2X purinergic receptors were suggested to cause Ca-dependent oligodendroglial injury (11) [103]. A component of Ca influx into damaged axons directly through voltage-gated Ca channels is also likely (12). GABA and adenosine release may play an "autoprotective" role (13). Anion transporters such as the K–Cl co-transporter participate in volume dysregulation in glia and the myelin sheath, contributing to conduction abnormalities (14). The locations of the various channels and transporters are drawn for convenience and do not necessarily reflect actual distributions (modified from Ref. [11] with permission from Bentham Science Publishers Ltd.).

cells, which will then over-activate a variety of ionotropic (mainly AMPA/kainate) and metabotropic receptors. Voltage-gated Ca channels will exacerbate Ca loads by permeating ion directly, or serving to gate ryanodine receptors. This injury cascade has become remarkably complex over the years and, aside from a few key differences such as lack of vesicular transmitter release, bears a surprising resemblance to mechanisms known to operate in gray matter. By deciphering these fundamental steps, we may now be in a better position to propose rational protective strategies (see below).

A key question relevant to the current symposium is: To what extent are anoxic/ischemic mechanisms responsible for axonal degeneration in MS? On the surface, this chronic neuroinflammatory disorder may exhibit little mechanistic overlap with the acute events of ischemia during stroke. A convincing mechanistic connection emerges if we consider that a mismatch between cellular energy supply and demand occurs in the context of an MS lesion. Concerning the supply side of the equation, it is known that inflammatory lesions generate substantial quantities of nitric oxide (NO) [84], which, together with its metabolite peroxynitrite, inhibits mitochondrial electron transport and therefore oxidative phosphorylation [85]. In vitro exposure of white matter tracts to NO produces irreversible anoxic-like injury [86], and MS lesions in humans display pathological features resembling those found in hypoxia/ischemia [87]. Finally, a recent study reported impaired activity of mitochondrial complexes I and III in the brain and spinal cord of MS patients [4], raising the intriguing possibility of inherent defects in these organelles in MS, which, together with extrinsic inhibitors of mitochondrial respiration such as NO, may seriously compromise their energy-producing capacity.

Concerning energy demand, demyelinated fibers exhibit increased requirements to support conduction. Normally myelinated fibers have high densities of Na channels largely restricted to nodes, and most K channels are located in internodal/juxtaparanodal regions masked by the myelin sheath [1]. The low internodal capacitance and restricted

current leaks allow rapid and efficient saltatory conduction. In contrast, demyelinated axons suffer large current leaks through newly exposed K channels and the large capacitance of the denuded internodal axolemma. In an attempt to restore conduction, these fibers express Na channels more uniformly along their surface, allowing continuous conduction as seen in unmyelinated axons [88]. However, action potential propagation under these conditions exacts a high price in ion movements compared to highly efficient saltatory conduction, which in turn places increased demands on energy-consuming ion-motive ATPases (in particular, the Na–K ATPase). This, together with a potentially impaired ability of mitochondria to synthesize ATP, may produce a state of chronic "virtual hypoxia" [89], leading to deregulation of Ca homeostasis and ultimately structural failure of the fiber, manifested as spheroid formation and finally transection [90]. This hypothetical scenario of an unbalanced energy supply–demand equation in axons altered by demyelinating disease is illustrated in Fig. 6. Whereas normal axons are vulnerable only under pathological conditions such as ischemia, demyelinated axons from MS lesions, particularly those that are more electrically active [91], may suffer a "hypoxic" demise due to extrinsic and intrinsic factors.

The mechanistic convergence between hypoxic/ischemic white matter damage and pathology observed in neuroinflammatory disease presents an opportunity for neuroprotective intervention, which would likely be used as an adjunct to currently available immunomodulatory therapies [92]. Although there are many steps in the injury cascade, a few hold critical positions in the sequence, upon which other events are dependent. One prominent example is the voltage-gated Na channel and, in particular, the persistent, non-inactivating component that fluxes deleterious Na during injury. Event a small absolute permeability of these channels that fails to completely inactivate may promote large Na loads and depolarizations because of the long "injury times" (minutes–hours during ischemia, indeed potentially years in MS) compared to brief action potentials that see large, albeit brief, activations of axonal Na permeabilities. Coupled with a large surface-to-volume ratio in small-diameter axons, these channels will readily load axons with Na, in turn promoting reverse plasmalemmal and mitochondrial Na–Ca exchange, release of glutamate,

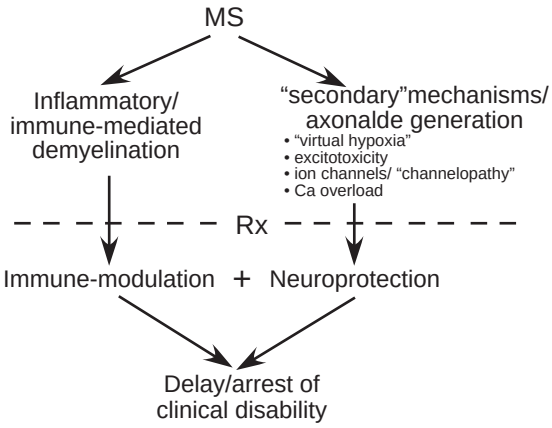

Fig. 7. Proposed dual treatment for MS. Known inflammatory/immune mechanisms will continue to be treated with established disease-modifying agents in present clinical use. The additional secondary mechanisms responsible for axonal degeneration, which may or may not be dependent on the immune mechanisms in the left arm, may benefit from rationally designed neuroprotective therapies modeled on mechanisms derived from white matter anoxic/ischemic paradigms (see text for details).

activation of Ca channels, etc. The key role of Na channels was confirmed by the robust protective effect of TTX against anoxic white matter injury. This highly selective Na channel blocker would not be practical in vivo because it potently blocks normal action potential propagation. However, certain local anesthetics, anti-arrhythmics, and anti-convulsants exhibit "use dependence" (i.e., a predilection for blocking active Na channels; at a molecular level, those that spend more time in their open or inactivated, rather closed states). For instance, in vitro, QX-314, a quaternary analog of lidocaine that preferentially blocks the open conformation of the Na channel (and presumably the persistent component) [93–95], is able to completely protect optic nerves against 1 h of anoxia with very little anesthetic effect on the pre-anoxic response [96]. Interestingly, recent studies using the anti-convulsant, phenytoin [97,98], and the anti- arrhythmic, flecainide [99], both "use-dependent" Na channel blockers, reported significant beneficial effects in animal models of EAE. These agents improved conduction in central tracts, reduced histological damage, and improved clinical scores. Similarly, interference with AMPA/kainate receptor-dependent signalling was also protective in animal models of EAE [54,100,101].

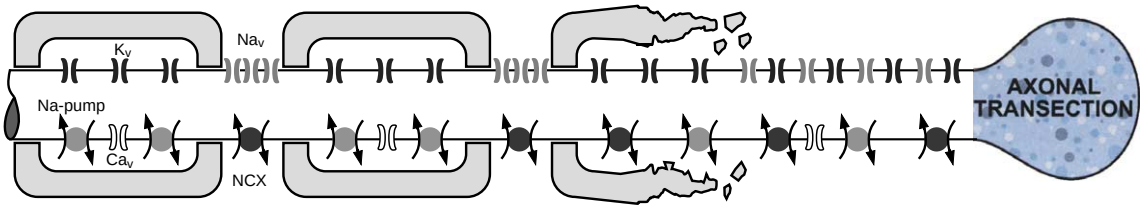

Fig. 6. Normally myelinated axons (left) support efficient saltatory conduction and become vulnerable only when energy supply is compromised such as during ischemia. In contrast, demyelinated axons may exist in a state of imbalance with respect to cellular energy supply vs. demand, leading to chronic "virtual hypoxia." Such energy deficit may lead to deregulation of Ca homeostasis and finally irreversible structural damage to the axon. Nav, Kv, Cav: voltage-gated ion channels; NCX: Na–Ca exchanger (modified from Ref. [89] with permission from Wiley-Liss Inc.).

Together, these data present evidence that mechanisms of injury to white matter in anoxia/ischemia and neuroinflammatory diseases may be surprisingly similar, and neuroprotectants designed for ischemia may be effective adjuncts in neuroinflammatory disorders such as MS. Such a dual approach to treatment of this disorder is illustrated in Fig. 7 for discussion purposes.

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