ABSTRACT

Excitotoxic injury to neurons may be common to many degenerative neurologic disorders. Although excitotoxicity is well established in central nervous system gray matter, relatively few studies have examined the importance of excitotoxicity in white matter injury. A series of studies conducted in isolated white matter tracts have demonstrated that the movement of Ca\(^{2+}\) and Na\(^{+}\) across cell membranes is of particular importance in the development of anoxic or ischemic injury. Excitotoxic effects of glutamate, likely including the activation of AMPA-type glutamate receptors, damage the myelin sheath and impair the ability of neurons to propagate action potentials. These studies suggest several potential therapeutic targets in degenerative neurologic conditions, possibly including multiple sclerosis.


In the central nervous system (CNS) gray matter, it is generally accepted that anoxic or ischemic injury develops, at least in part, as a result of Ca\(^{2+}\) entry into neurons following the stimulation of glutamate receptors by excessively high extracellular levels of this excitatory neurotransmitter. Until recently, it was not known whether white matter injury could be caused by a similar excitotoxic mechanism. Although anoxia and multiple sclerosis (MS) are very different conditions, emerging evidence suggests many of the mechanisms that cause axonal degeneration in anoxia may also be significant in the pathophysiology of MS.

The mechanisms of neurologic injury in white matter tracts subjected to anoxia and reoxygenation have been examined in a series of experiments using an isolated rat optic nerve model. The effect of anoxia on nerve conduction in this model (measured using the propagated compound action potential) is illustrated in Figure 1. Anoxic neurons quickly lose the ability to conduct action potentials, and the compound action potential remains seriously disordered even 60 minutes after reoxygenation. Anoxia rapidly decreases the intracellular concentration of K\(^{+}\) to about 10% of baseline levels, which is accompanied by a parallel rise in the concentration of Na\(^{+}\) and a more gradual increase in Ca\(^{2+}\) (Figure 2).

To better understand how the movement of various ions across the cell membrane contributes to neurologic injury, a series of studies was conducted in which ion currents were manipulated by altering the ion concentrations of the bathing medium or by the use of pharmacologic agents that block the movement of ions across the cell membrane. These experiments suggested that the movement of Ca\(^{2+}\) and Na\(^{+}\) across the cell membrane was especially important in the development of anoxic injury. The removal of Ca\(^{2+}\) from the bathing medium produced nearly complete protection from even relatively long periods of anoxia. However, Ca\(^{2+}\) apparently does not enter the neurons via voltage-gated Ca\(^{2+}\) channels, because Ca\(^{2+}\)-channel blockers did not protect against anoxia. Removing Na\(^{+}\) from the extracellular medium also provided protection against anoxia. This protection appeared to be related to the movement of Na\(^{+}\) through voltage-gated channels, as the sodium-channel blocker tetrodotoxin also protected the neurons against ischemia to approximately the same degree.

One way neurons regulate the movement of Ca\(^{2+}\) and Na\(^{+}\) ions is via a Na\(^{+}/Ca\(^{2+}\) exchanger. This mem-
brane-bound protein normally exports Ca\textsuperscript{2+} from the cell while importing Na\textsuperscript{+} ions on a 3-Na\textsuperscript{+}-for-1-Ca\textsuperscript{2+} basis. Although these exchangers are important in the normal homeostatic processes of the cell, under pathologic conditions such as anoxia, they may reverse the direction in which they transport ions, causing an accumulation of Ca\textsuperscript{2+} within neurons. Blocking this exchanger pharmacologically (eg, using bepridil) also produced a significant degree of recovery from anoxia.

**WHITE-MATTER EXCITOTOXICITY**

The concept of white-matter excitotoxicity may appear to be counterintuitive: because white matter lacks synapses, there is no obvious mechanism by which glutamate is released. A series of experiments conducted over the past several years, however, has confirmed that excitotoxic injury occurs in white matter tracts subjected to anoxia, and initial studies have begun to characterize the sources of glutamate release, the role of the various glutamate receptors and ion channels, and the effects of excitotoxicity on neuronal and glial elements in white matter tracts.

**AMPA-TYPE GLUTAMATE RECEPTORS**

One set of experiments examined the effects of glutamate receptor blockade on intracellular ion concentrations and anoxic injury to neurons in isolated spinal white matter tracts.\textsuperscript{5} The magnitude of the compound action potential of tissue slices was first recorded from nerve fibers isolated from the rat dorsal column that were exposed to drugs that block the action of glutamate at different glutamate receptor subtypes. In control tissue slices that were not exposed to anoxia or glutamate antagonists, the magnitude of the compound action potential remained constant over 3 hours. Anoxia and reoxygenation produced a permanent decrease in the magnitude of the compound action potential to approximately 25% of normal control values. Anoxia and reoxygenation produced a permanent decrease in the magnitude of the compound action potential to approximately 25% of normal control values. Exposure of the cells to the broad-spectrum glutamate receptor antagonist kynurenic acid (which blocks both N-methyl-D-aspartate receptor [NMDA] and non-NMDA glutamate receptors) or exposure to the specific AMPA-receptor antagonist GYKI52466 provided significant (although incomplete) protection against the effects of ischemia and increased the magnitude of action potential recovery following anoxia and reoxygenation. These results suggested that endogenous glutamate released from the axonal cytoplasm of spinal white matter tracts injured the
myelin sheath. The source of this glutamate appears to be a Na⁺-dependent glutamate transporter, similar to the Na⁺/Ca²⁺ exchange pump described previously. This glutamate transporter normally moves glutamate into the cell, but anoxia reverses the direction of transport. Drugs that block this Na⁺/glutamate transporter significantly reduced the magnitude of the loss of the action potential after anoxia and reoxygenation. Finally, confocal microscopy demonstrated that the concentration of glutamate in axon cylinders and oligodendrocytes decreased with anoxia and that this decrease was largely prevented by glutamate transporter inhibitors.

These observations were extended in a second study using the same model system. In this study, the application of glutamate (without anoxia) permanently reduced the magnitude of the compound action potential by approximately 50%. Glutamate-induced damage was reduced to a similar degree when the tissue slices were incubated with the broad-spectrum glutamate receptor antagonist kynurenic acid, with a specific AMPA/kainate receptor antagonist (NBQX), or with a specific AMPA/receptor antagonist (GYKI52466) but not when they were incubated with MK-801, a selective NMDA-receptor antagonist. This study also examined the sites of excitotoxic injury in the dorsal columns. Tissue slices were stained using antibodies that detect degenerated myelin basic protein and a product of cellular structural damage (spectrin). Confocal microscopy revealed that exposure of the tissue slices to glutamate for 3 hours produced significant damage to the myelin sheath, whereas no myelin injury was observed in control slices that were not exposed to glutamate. Exposure to glutamate also produced significant structural damage to oligodendrocytes and astrocytes but not to axon cylinders. One particular glutamate receptor subunit (the GluR4 receptor subunit), which is a component of AMPA-type glutamate receptors, was identified in large numbers on the myelin sheath and on oligodendrocytes and astrocytes. Together, the results of these studies suggest that white matter tracts are susceptible to excitotoxic injury that is at least in part due to activation of AMPA receptors on neurons, myelin, oligodendrocytes, and astrocytes.

**Calcium Is Also Derived from Intracellular Stores**

As discussed previously, removal of Ca²⁺ from the bathing medium protected spinal column tissue slices from anoxia. Surprisingly, removal of Ca²⁺ did not improve the recovery from oxygen-glucose deprivation (an in vitro model system of ischemia) in these dorsal column axons. This suggested that either oxygen-glucose deprivation produced neurologic injury independent of Ca²⁺ or that Ca²⁺ was available from intracellular stores. Pretreating the animals with a Ca²⁺ chelator to lower intracellular Ca²⁺ levels significantly reduced the effects of ischemia. In addition, confocal microscopy using Ca²⁺ sensitive dyes indicated that ischemia increased the intraneuronal Ca²⁺ concentration even when Ca²⁺ was removed from the bathing medium. These findings suggest that ischemic nerve injury in white matter is the result of Ca²⁺ release from an intracellular source.

**Intervention in White Matter Injury**

The complexity of the processes that contribute to anoxic injury of axons, with a large number of ion channels, transporters, and Ca²⁺ sources, might suggest that it would be difficult to intervene to improve white matter recovery. However, there are points in the injury cascade that may provide good therapeutic targets, including the Na⁺ channel. As described previously, studies of isolated rat optic nerves found that Na⁺ entry into the axon is an important step in the processes that lead to irreversible neurologic injury, perhaps as a result of the reversal of the Na⁺/Ca²⁺ exchanger. The ability of several local anesthetics (which block Na⁺ entry during ischemia but with less effect on the Na⁺ channel that propagates action potentials) to prevent anoxic injury was examined. Pretreatment of optic nerves with the tertiary amine anesthetics lidocaine or procaine significantly improved recovery from anoxia compared with untreated optic nerve, although both of these agents also significantly depressed the action potential before anoxia. Quaternary amine anesthetics (QX-314 and QX-222), which have less effect on the Na⁺ channel in axons important in the propagation of action potentials, did not affect nerve function before the axons were subjected to anoxia but produced a greater improvement following anoxia and reoxygenation than the tertiary amine anesthetics. At the optimal concentration, QX-314 produced little effect on the compound action potential during normal oxygenation but was associated with an average recovery of the magnitude of the compound action potential to >90% of the control value. This agent is not well suited for clinical use because it is electrically charged and does not cross the blood-brain barrier. However, these findings illustrate the potential effectiveness of selective Na⁺ channel blockade if an appropriate molecule with good blood-brain barrier penetrability could be identified.
As described by Dr Raine, some studies have suggested that excitotoxicity in white-matter tracts may also be important in the pathophysiology of MS. Targeting sodium channels may also be effective in MS. Lo and colleagues examined the effects of phenytoin, an anticonvulsant that blocks sodium channels, in a mouse model of experimental allergic encephalomyelitis (EAE). Three groups of animals were examined: EAE alone, EAE mice that were fed phenytoin (incorporated into pelleted mice chow), and a control group of mice that received phenytoin but did not undergo EAE. This study clearly demonstrated that the number of axons remaining in the cortical spinal tract and the dorsal columns was increased among animals that received phenytoin (Figure 3). Electrophysiologic function of the white-matter tracts was also significantly improved among EAE mice that received phenytoin compared with control EAE animals.

**SUMMARY AND CONCLUSIONS**

Although the pathophysiologic processes that produce neurologic injury in MS and ischemia are not identical, there are parallels between what is fairly well established during ischemia and what may be happening in neuroinflammatory diseases. In ischemia, the sudden loss of the cellular energy supply results in the failure of ion pumps that require metabolic energy to function, causing accumulation of Na⁺ ions, depolarization, release of glutamate and increase in intracellular Ca²⁺. In MS, the loss of myelination causes the axons to lose a great deal of their energy efficiency, creating an energy supply imbalance. Thus, for different reasons, neurons in both anoxia and MS may be faced with an imbalance between energy supply and demand, initiating the cascade of events that leads to Ca²⁺ accumulation and cell death. Therapeutic approaches that target these ion channels or receptor molecules that regulate Ca²⁺ entry may improve clinical outcomes in patients across a wide range of neurologic conditions.

**REFERENCES**