Role of Voltage-Gated K$^+$ Channels in the Pathophysiology of Spinal Cord Injury

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Introduction

Spinal cord injury (SCI) is a devastating condition afflicting over 13,000 people annually in North America and is an important cause of mortality and neurological morbidity. Although early pharmacological intervention after SCI with methylprednisolone$^{2,3}$ or GM-1 ganglioside$^4$ results in modest neurological improvement, the overall impact of these treatments remains minimal. Therefore, novel therapeutic approaches are required to improve the neurological outcome of these patients.

Mechanisms of cellular injury after neurotrauma

Posttraumatic degradation of white matter is an important aspect of the pathophysiology of SCI. Axonal degeneration after traumatic SCI evolves over minutes to days after injury.$^{5,6,7,8}$ The pathophysiology of neurotrauma involves an initial or "primary" mechanical insult followed by a complex series of molecular and cellular events termed "secondary injury", which include ischemia, rises in intracellular Na$^+$ and Ca$^{2+}$, glutamate toxicity, free-radical mediated cell damage and apoptosis.$^{1,6,7,9-17}$

Relevance of axonal dysfunction to spinal cord injury

The spinal cord is rarely totally transected even after severe SCI associated with complete paralysis.$^{18}$ The injury site is characterized by central cavitation and a subpial rim of surviving axons with varying degrees of demyelination.$^{8,19-23}$ We examined the relationships among the severity of SCI, the number of surviving axons at the injury site and the extent of neurological recovery of rats.$^{24}$

A logarithmic relationship was found between the number of myelinated axons surviving in the subpial rim of the spinal cord after SCI and recovery of neurological function. Persistence of approximately 45,000 axons after SCI (~12% of normal number) was associated with recovery of significant hindlimb locomotor function. This finding suggests that relatively small changes in neuroanatomical integrity or restoration of function of a small number of axons in the CNS can impact substantially in clinical neurological recovery.

Our laboratory and others have shown that axons that persist in the subpial rim after SCI display dysfunctional conduction properties including prolonged refractory periods, high frequency conduction failure, conduction block at subphysiological temperatures, increased threshold of activation and reduced conduction velocity.$^{25-30}$
Figure 1. Changes in pharmacological sensitivity of injured spinal cord white matter to potassium channel blockers. Chronically injured dorsal columns (6–8 weeks post 20g SCI) showed enhanced sensitivity to 200 µM 4-AP (in comparison to noninjured controls) as evidenced by increased amplitude and widening of compound action potentials (CAP) (A,B). Injured dorsal columns also had an enhanced sensitivity to 500 nM \( \alpha \)-Dendrotoxin (\( \alpha \)-DTX) shown by an increase in CAP amplitude and delay in repolarization of the CAP response (C,D). However, there was no change in pharmacological sensitivity of chronically injured dorsal columns to either 10 mM TEA (E,F) or 2 mM CsCl (G,H) as compared to noninjured controls.\(^7\)
Role of Kv⁺ channels in axonal dysfunction after SCI

Experiments by Chiu and Ritchie demonstrated that Kv⁺ channels are localized in the myelin covered paranodal or internodal regions of peripheral mammalian myelinated axons. They showed that there was little Kv⁺ conductance in intact myelinated nerves and that there was a significant increase in outward Kv⁺ current upon acute myelin disruption. Several functions ascribed to the “fast” Kv⁺ channels include action potential repolarization, stabilizing the node to prevent re-excitation of the node after a single stimulus, limiting excessive axonal depolarization and inactivation of nodal Na⁺ channels, and increasing the security of axonal conduction by contributing to the nodal potential.

There is evidence supporting the concept that “fast” or rapidly activating Kv⁺ channels are concealed by myelin in the paranodal or internodal regions. Following disruption of the myelin there is an increased activity of these channels which “clamp” the membrane potential close to the equilibrium potential of Kv⁺ and result in axonal conduction blockade. Our laboratory and others have shown that altered activity of 4-aminopyridine (4-AP) sensitive Kv⁺ channels is associated with axonal dysfunction after SCI. The results from our laboratory are congruent with those of Blight and Kocsis who reported that traumatic disruption of paranodal myelin loops of spinal cord white matter was associated with enhanced sensitivity to 4-AP. Blight and Gruner observed that intravenous administration of 4-AP to cats with chronic thoracic SCI augmented the vestibulospinal free fall responses.

Furthermore, preliminary clinical studies with 4-AP have shown enhanced central axonal conduction and modest degrees of functional improvement in patients with chronic SCI. It is noteworthy that 4-AP blocks a variety of Kv⁺ channels at various PNS and CNS sites so the specific mechanism of this drug is still unknown. While the effect of 4-AP after SCI has been assumed to be on the basis of blockade of rapidly activating, voltage-gated Kv⁺ channels in juxtaparanodal regions of axons, Kv⁺ channels are also located on astrocytes and oligodendrocyte precursor cells.

Molecular structure, electrophysiological properties and pharmacological sensitivity of Kv1.1 and Kv1.2 Kv⁺ channels

Both Kv1.1 and Kv1.2 encode Kv⁺ channel proteins having 6 transmembrane (S1-S6) domains and one intervening pore (P or H5) sequence. Kv1.1 in both rat (RCK1) and mouse (MK1 or MK1) consists of 495 amino acids. The length of the cDNA sequence varies from 3822 bp for the rat RCK1 channel to 2222 bp for the mouse MK1 channel. Kv1.2 encodes a 498 amino acid length sequence for the rat (RCK5) and a 499 amino acid protein for the mouse (MK2). The lengths of cDNA encoding for RCK5 are 2409 bp and 3383 bp for MK2. The Kv⁺ channel is tetrameric, requiring the co-assembly of four α subunit proteins to form one functional channel. Kv1.1 and Kv1.2 can co-assemble to form heteromultimeric Kv⁺ channels.

In general, Kv1.1 and Kv1.2 encode voltage-gated Kv⁺ channels that express outward currents with depolarizing pulses. Both Kv1.1 and Kv1.2 activate rapidly and inactivate slowly. Detailed biophysical parameters are listed for rat and mouse from the following references. A variety of pharmacological blockers inhibit Kv1.1 and Kv1.2 Kv⁺ channel currents with different rank and order of affinities. Kv1.1 is blocked by 4-AP at affinities ranging from IC₅₀=89 mM to IC₅₀=1mM. Kv1.2 is also sensitive to 4-AP blockade mainly in the submillimolar concentration range from IC₅₀=74 mM to IC₅₀=0.8mM 1mM. Kv1.1 has a high affinity for TEA blockade (IC₅₀=0.6mM, Kd=0.3mM), while Kv1.2 is insensitive to TEA (IC₅₀=129mM, Kd=560mM). Both Kv1.1 (IC₅₀=12nM, Kd=20mM) and Kv1.2 (IC₅₀=4nM, Kd=17mM) are highly sensitive to nM blockade by Dendrotoxin (or α-Dendrotoxin).
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Western blot analysis to examine changes in Kv1.1 and Kv1.2 protein expression following spinal cord injury. (A) Absence of microtubule associated protein (MAP2) staining indicates the exclusion of grey matter in the dorsal column preparation (DC) (WC represents the whole cord). (B) There is a reduction of neurofilament (NF200) after SCI, while actin levels are preserved. Western blots for Kv1.1 (C) and Kv1.2 (E) show changes in immunostaining intensity of bands from sham to injured spinal cord white matter in rostral, central and caudal dorsal column segments (hippocampus used as positive control; liver used as negative control). Optical densities of both Kv1.1 and Kv1.2 proteins are normalized to the axonal marker neurofilament (NF200) to assess changes in expression of Kv1.1 and Kv1.2 in axons. Immunostaining intensities for both Kv1.1 (D) and Kv1.2 (F) normalized to NF200 are increased following injury at all three sites (n=5 for each group). Means ± standard errors are plotted. Asterisks denotes significant changes (p < 0.05). Figure taken with permission from reference 77.
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Figure 3. Immunofluorescence microscopy to examine changes in Kv1.1 potassium channel subunit localization along axons following spinal cord injury. (A) Transverse section of noninjured spinal cord showing that Kv1.1 (green) is localized on axons (neurofilament (NF200), red) (data shown only for ventral column). Longitudinal sections of noninjured spinal cord showing discrete highly localized paired staining pattern of Kv1.1 on axons from the lateral column at low (20X, B) and high (60X, C) power. Noninjured dorsal column axons also expressed Kv1.1 with similar paired localized staining patterns (G). Longitudinal sections of chronically injured (6 weeks post injury) spinal cord showing an altered distribution of Kv1.1. On some injured axons Kv1.1 displayed dispersed staining in both lateral (D,E) and dorsal columns (H) that were examined. Injured axons also showed punctate staining of Kv1.1 which were shorter in length of distribution and did not have the orderly structure of the paired localized staining in noninjured axons (data shown only for lateral column, F). Specificity of immunolabelling was indicated by the absence of immunostaining when the antibody was preincubated with its corresponding peptide (data not shown). Asterisks denotes significant changes (p < 0.05). Figure taken with permission from reference 77.
In CNS white matter, Kv1.1 and Kv1.2 colocalize in the juxtaparanodal regions of myelinated axons, for example the corpus callosum, brain stem and spinal cord.\textsuperscript{64,69,70} Co-immunoprecipitation reactions also showed that Kv1.1 forms heteromultimeric K\textsuperscript{+} channels with Kv1.2.\textsuperscript{71} Kv1.1 and Kv1.2 also colocalize in juxtaparanodal regions of peripheral myelinated axons.\textsuperscript{72,74}

Defects in the myelination of axons may play an important role in determining the expression patterns of K\textsuperscript{+} channels on axons. Wang et al.\textsuperscript{70} examined the expression patterns of Kv1.1 and Kv1.2 in the Shiverer mutant mice, which have hypomyelinated CNS and PNS axons. In the brain stem, subcortical and cerebellar white matter, and corpus callosum axons of wild-type mice had punctate staining of both Kv1.1 and Kv1.2. However, axons in Shiverer mice had diffuse staining and elevated expression of both Kv1.1 and Kv1.2 protein. mRNA levels of both Kv1.1 and Kv1.2 were elevated in forebrain, hindbrain and cerebellar regions.

In demyelination lesions of the rat sciatic nerve Rasband et al.\textsuperscript{73} observed a redistribution of Kv1.1 and Kv1.2. In the intact nerve Kv1.1 and Kv1.2 had paired juxtaparanodal localization on axons. At six to nine days following demyelination there was a heterogeneous effect on K\textsuperscript{+} channel distribution. Some axons had diffuse staining at nodes, some axons were devoid of K\textsuperscript{+} channel staining, but none had paranodal localization of K\textsuperscript{+} channels. During remyelination (>12 days following demyelination) Kv1.1 expression was found at the node and over time redistributed to the paranodal/juxtaparanodal sites but were never completely contained in their original juxtaparanodal sites.

Studies on injuries to peripheral nerves have also shown changes in K\textsuperscript{+} channel expression on axons. Nerves from patients with neuromas showed dispersed labelling in the internodes of myelin ensheathed axons.\textsuperscript{75} Ishikawa et al.\textsuperscript{76} reported a decrease in Kv1.1 and Kv1.2 K\textsuperscript{+} channel proteins in 16 to 24 hr cultured dorsal root ganglion cells 14 days after peripheral axotomy.

**Figure 4.** Noninjured axons showing that α-Dendrotoxin (α-DTX, red) colocalizes with Kv1.1 (green) as a paired localized staining pattern (A) (axons stained with NF200, blue) (B). Injured spinal cord axons (NF200, blue) showing a more dispersed distribution of α-DTX (red) (C) and Kv1.1 (green) (D) which colocalize (E). Figure taken with permission from reference 77.
Evidence for involvement of Kv1.1 and Kv1.2 potassium channels in posttraumatic axonal dysfunction

With chronic SCI, surviving axons showed a variety of electrophysiological abnormalities including reduced conduction velocity, increased threshold of activation, high frequency conduction failure and an increased refractory period. These changes were associated with increased sensitivity to 4-AP, which blocks "fast" K⁺ channels, α-DTX which selectively targets K⁺ channels of the Shaker gene family, but not TEA or CsCl which block "slow" and inward rectifier K⁺ channels respectively. Based on these observations, we examined the hypothesis that upregulation and/or altered distribution of Kv 1.1 and 1.2, which form 4-AP and α-DTX sensitive K⁺ channels, and have been reported to be on CNS axons, is associated with the electrophysiological abnormalities seen after chronic SCI. Quantitative confocal immunofluorescence microscopy demonstrated increased axonal expression of Kv1.1 and 1.2 (confirmed by quantitative immunoblotting normalized to the axonal protein NF200). In addition, there was a markedly altered distribution of these K⁺ channel subunits after SCI with dispersed labeling along the internodes (in contrast to uninjured axons where labeling was concentrated in the juxtaparanodal regions). Triple-labelled immunofluorescence microscopy using fluorescently tagged (Texas Red) α-DTX showed that α-DTX colocalized with Kv1.1 and Kv1.2 on neurofilament positive axons. This result suggests that it is the increased activity of the α-DTX sensitive "fast" axonal potassium channels, Kv1.1 and Kv1.2, that contributes to axonal dysfunction after chronic SCI. These results strongly support the hypothesis that increased expression and altered distribution of Kv1.1 and 1.2 K⁺ channel subunits on axons contributes to posttraumatic axonal dysfunction. Therefore, the K⁺ channel subunits Kv1.1 and Kv1.2 may represent clinically important targets for gene therapy or molecular targeted pharmacological approaches to treat spinal cord injured patients.

References