

Ion Channels and Neuronal Dysfunction in Multiple Sclerosis

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What causes the signs and symptoms of multiple sclerosis (MS)? It is almost axiomatic to regard MS as a demyelinating disorder in which axonal conduction block, caused by loss of myelin, produces clinical deficits. In addition, during the past few years, increased attention has focused on axonal degeneration in MS. The available evidence suggests that demyelination provides a structural correlate for relapsing-remitting disease while axonal degeneration, which may be associated with atrophy of the brain and spinal cord, produces nonremitting deficits.

Neurons, of course, are the ultimate mediators of nervous system function and arbiters of neurologic status. Do surviving neurons in MS, nonatrophic and with intact axons, exhibit abnormalities at the molecular level, more subtle than axonal degeneration or neuronal atrophy? If so, are these abnormalities inconsequential molecular oddities or do they contribute to neuronal injury or perturb neuronal function? Among the molecules that make up neurons, ion channels are especially critical because they endow these cells with their signaling capabilities. Thus far, most studies on neuronal ion channels in demyelinating diseases have focused on their adaptive roles; restoration of impulse conduction can occur in chronically demyelinated axons as a result of the insertion of increased densities of sodium channels within the bared axon membrane. Emerging evidence, however, is also beginning to suggest that ion channels within neurons can be involved in maladaptive changes in MS.

Experimental studies in animal models of MS and molecular analysis of autopsy specimens from humans with MS have begun to raise the possibility that, in addition to demyelination and axonal de-

generation, 2 distinct modes of dysregulated ion channel expression can injure neurons or interfere with neuronal signaling in MS. One abnormal mode of ion channel expression suggested by Kornek et al¹ is the ectopic distribution of calcium channels, which they suggest are up-regulated within the axon membrane in experimental allergic encephalomyelitis (EAE) and in MS. They used immunocytochemical methods to examine the distribution of the α_{1B} pore-forming subunit of the N-type voltage-gated calcium channel. Consistent with earlier reports, they observed a pattern of staining in the normal brain, which suggested the presence of substantial numbers of α_{1B} subunits in presynaptic axon terminals (where they participate in synaptic transmission), but they observed only low levels of α_{1B} within the plasma membrane of myelinated axons within normal white matter. In EAE and MS, they observed a different distribution of α_{1B} immunoreactivity, which was present at higher levels in axons and axonal spheroids within demyelinating lesions, in a pattern similar to that of β -amyloid precursor protein (APP), a marker of acute axonal injury and possibly of impaired axonal transport. They interpret these results as indicating that α_{1B} subunits (which in the normal brain are carried within axons by axoplasmic transport en route to the presynaptic terminal) are inserted into the demyelinated axon membrane in EAE and MS.

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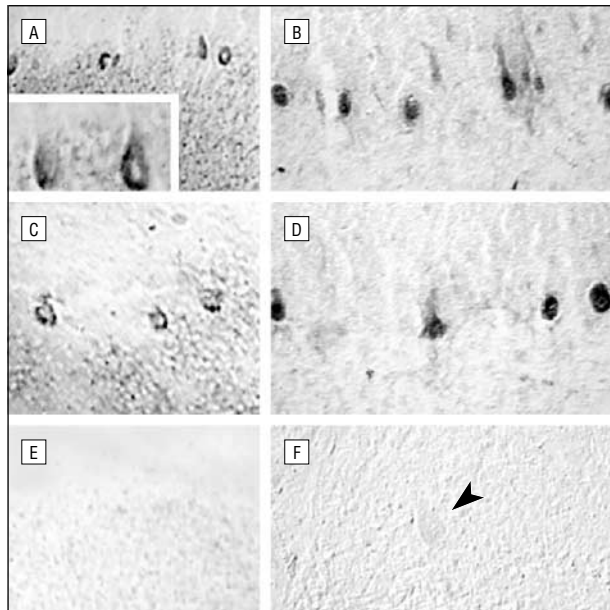


Figure 1. $Na_v1.8$ sodium channel is aberrantly expressed in cerebellar Purkinje cells in patients with multiple sclerosis (MS). In situ hybridization demonstrates $Na_v1.8$ messenger RNA in Purkinje cells from patients with MS (A and C) but not in controls without neurologic disease (E). The inset shows Purkinje cells at higher magnification. Immunocytochemical analysis with $Na_v1.8$ -specific antibodies demonstrates $Na_v1.8$ protein within Purkinje cells in patients with MS (B, D) but not in controls (F). The arrowhead indicates unlabeled Purkinje cell. This figure was modified with permission from Black et al.⁷

Since the expression of α_{1B} was studied only at the protein level (and not at the messenger RNA [mRNA] or functional level), further studies are needed to confirm and extend these results. Nonetheless, it is worth considering the consequences of abnormal calcium channel deployment in demyelinated axons. Kornek et al¹ propose that increased intracellular calcium levels due to activity of voltage-gated calcium channels may activate calcium-dependent proteases (calpains) that can degrade important axonal proteins,^{2,3} thus contributing to axonal injury. Action potentials traveling along the axon or depolarization triggered by an initial insult, such as inflammation, could act to activate these channels. Consistent with the hypothesis that calcium channel activity can contribute to axonal injury, it has been shown that the blockade of calcium channels (including subtype-specific blockade of N-type calcium channels⁴) can protect a subpopulation of myelinated axons from axotomy-induced and anoxia-induced degeneration.^{4,5}

A second type of molecular abnormality, termed *transcriptional channelopathy*, has been described in cerebellar Purkinje neurons and may perturb the ability of these cells to encode information in animal models of MS and in humans with MS.^{6,7} The timing of action potentials and the occurrence of complex bursts of action potentials within Purkinje cells are critical for the proper functioning of the cerebellum in motor control and motor learning.⁸⁻¹⁰ It is well established that these aspects of Purkinje cell signaling depend in large part on current flowing through sodium channels.^{11,12} It is also now known that there are 10 different genes encoding molecularly distinct sodium channel subtypes, all sharing a common overall structural motif but with different amino acid sequences that endow them

with different voltage-dependences and kinetic properties.¹³ Purkinje cell firing patterns are perturbed in mouse mutants in which sodium channel expression is altered by knockout of specific sodium channel genes,¹⁴ and these changes are responsible for cerebellar ataxia in these mutants.¹⁵ To determine whether different subtypes of sodium channels are expressed in Purkinje cells in demyelinating disorders, Black et al⁶ first studied the taiep rat, a mutant in which myelin is formed normally but subsequently degenerates owing to an abnormality of oligodendrocytes. Using in situ hybridization and immunocytochemistry with subtype-specific antibodies, they examined expression of the $Na_v1.8$ sodium channel, which is normally present only in spinal sensory neurons and trigeminal neurons (and is not detectable within the normal brain); earlier studies had shown that the expression of $Na_v1.8$ within spinal sensory neurons changes following axonal transection and in association with inflammation. Consistent with the selective expression of $Na_v1.8$ in primary sensory neurons, $Na_v1.8$ was not detected in control brains. However, there was enhanced expression of $Na_v1.8$ mRNA and of $Na_v1.8$ sodium channel protein within Purkinje cells following loss of myelin within the brains of taiep rats.

A more recent study examined the expression of the $Na_v1.8$ channel in the brains of mice with chronic relapsing EAE and in postmortem brain tissue from patients with secondary progressive MS who had a history of cerebellar deficits.⁷ As expected, $Na_v1.8$ mRNA was not detectable within the cerebellum of control mice or humans without neurologic disease. In contrast, $Na_v1.8$ mRNA was clearly present within Purkinje cells of mice with chronic-relapsing EAE and in humans with MS (**Figure 1** A, C, and E). Translation of the mRNA had occurred, since $Na_v1.8$ protein was also present (Figure 1B, D, and F). Taken together, these observations in 2 animal models of MS, and in humans with MS, show that the $Na_v1.8$ gene (which is normally inactive in the cerebellum) is aberrantly activated in Purkinje neurons, producing the $Na_v1.8$ sodium channel protein, which is not normally present in these cells.

Is the expression of $Na_v1.8$ in Purkinje cells functionally important? That is, are the $Na_v1.8$ channels functional and, if so, does their presence within Purkinje cells matter in terms of cerebellar activity? Because $Na_v1.8$ channels display a unique physiological signature characterized by depolarized voltage-dependence of inactivation, slow development of inactivation, and rapid recovery from inactivation,^{16,17} the expression of these channels in neurons that normally do not produce them would be predicted to perturb the firing patterns of these cells. Experimental support for the idea that $Na_v1.8$ channels can affect neuronal firing patterns has been provided by observations on 2 model systems. Studies on transgenic mice demonstrate that identical stimuli produce different patterns of action potentials in dorsal root ganglion cells (the cells in which $Na_v1.8$ is normally expressed), depending on whether $Na_v1.8$ channels are present or not; cells expressing $Na_v1.8$ generate larger action potentials and, when depolarized, produce pacemaker-like repetitive trains of impulses that are not seen in cells lacking $Na_v1.8$.¹⁸ More recent studies have begun to extend this type of analysis to Purkinje cells, which under normal circumstances tend to generate complex, stereotyped bursts consisting of mul-

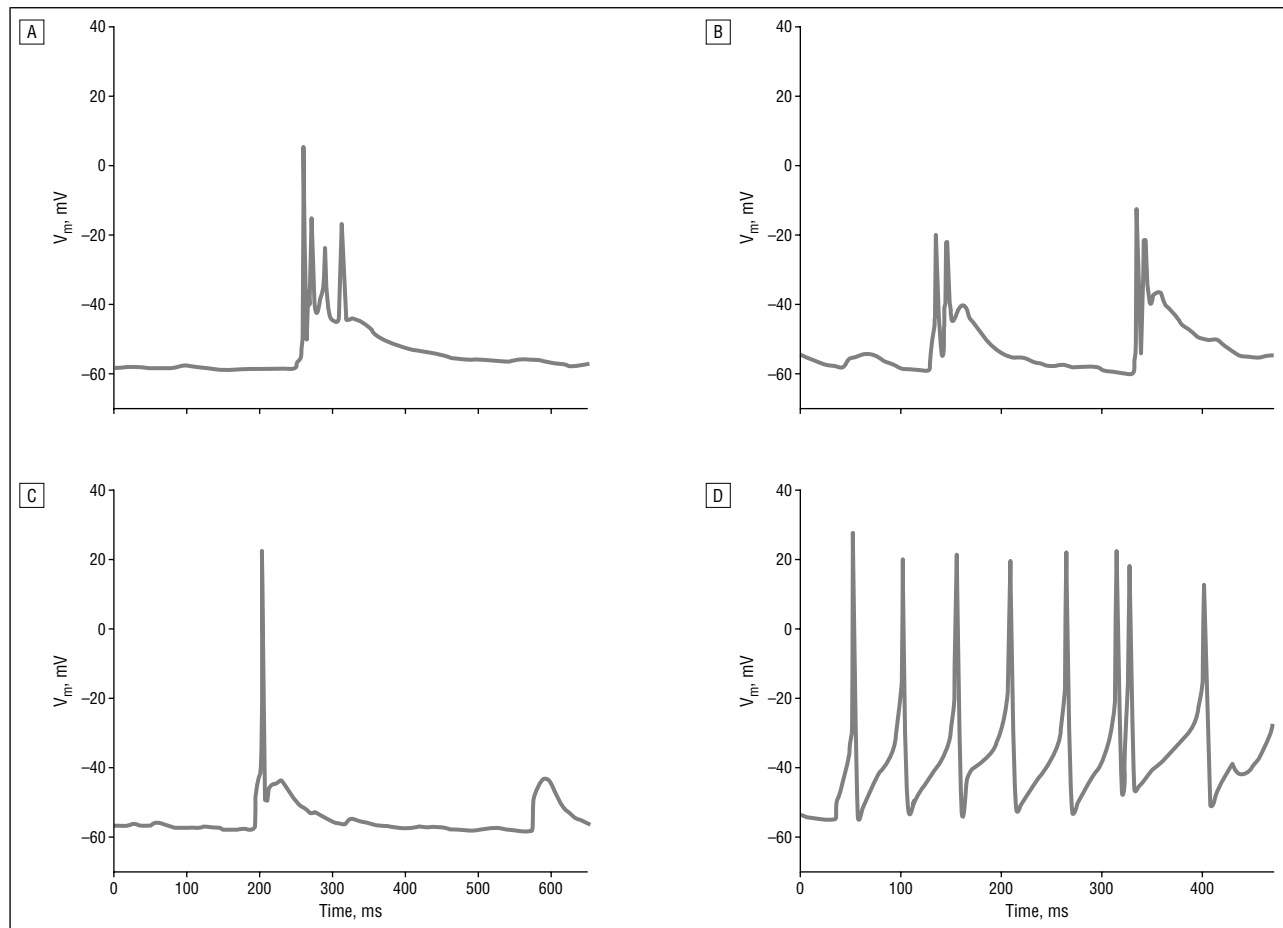


Figure 2. A-D, Expression of $Na_v1.8$ sodium channels perturbs signalling in cultured Purkinje cells. These current-clamp recordings illustrate action potential activity in a control rat Purkinje cell (A, B) and in a Purkinje cell in which $Na_v1.8$ sodium channels were biologically expressed (C, D). Normal Purkinje cells spontaneously generate bursts of action potentials superimposed on a plateau depolarization (A). Following expression of $Na_v1.8$ sodium channels, these bursts are replaced by single action potentials (C). In response to a depolarizing stimulus (120 pA, 1 second), Purkinje cells expressing $Na_v1.8$ produce sustained trains of action potentials (D) that are not seen in normal cells (B).

multiple action potentials superimposed on a plateau depolarization.¹⁴ **Figure 2** shows the effects of $Na_v1.8$ channels on Purkinje cells in vitro, which are similar to the effects observed in dorsal root ganglion cells.¹⁸ When $Na_v1.8$ sodium channels are experimentally expressed within Purkinje cells, the firing pattern changes markedly; Purkinje cells that express $Na_v1.8$ produce larger action potentials compared with the responses in Purkinje cells that lack $Na_v1.8$, and the secondary, tertiary, and subsequent action potentials that contribute to the bursts tend to be eliminated so that bursts are replaced by single-action potentials (Figure 2A and C). The responses evoked by depolarizing stimuli are also different: Purkinje cells expressing $Na_v1.8$ produce pacemaker-like trains of large-amplitude action potentials that are not seen in normal cells (Figure 2B and D). Thus, similar to dorsal root ganglion cells, Purkinje cells in vitro produce substantially different patterns of activity as a result of the expression of $Na_v1.8$.

Since biopsy of cerebellar tissue is not commonly performed in MS, it will not be easy to establish whether these physiologic changes occur in humans with MS. Clinical observations, however, provide some support for this suggestion. The not uncommon clinical observation of patients with MS having cerebellar deficits on examination

but without apparent cerebellar lesions in neuroimaging studies is consistent with the hypothesis that molecular changes that are too subtle to be detected by currently available imaging techniques can lead to dysfunction within the cerebellum. In addition, paroxysmal ataxia has been well described in MS.^{19,20} The temporal profile of the sudden and brief attacks and the therapeutic response to carbamazepine are not easily explained by demyelination or axonal degeneration. However, the similarity of these attacks to the paroxysmal episodes that occur in the episodic ataxias, which are associated with inherited channelopathies, is consistent with the hypothesis that they are the result of a channelopathy.

Although more research will be needed to fully define the extent and to understand the causes and physiologic consequences of molecular changes within neurons in MS, the available information on ion channels may present some new molecular targets and some new therapeutic opportunities. For example, if N-type calcium channels are involved in the degeneration of axons in MS, pharmacologic blockade of these channels might be expected to ameliorate, at least partially, the loss of axons. This hypothesis could be readily examined in animal models.

Participation of $Na_v1.8$ sodium channels in the production of cerebellar deficits in humans with MS, if con-

firmed by additional experiments, may also provide a new therapeutic target. In principle, blockade of Na_v1.8 channels would be expected to restore normal electrogenesis in Purkinje cells, thus improving cerebellar function. Na_v1.8-specific channel blocking drugs are not yet available, but this may change since the deployment of Na_v1.8 channels within nociceptive spinal sensory neurons has made Na_v1.8 an attractive molecular target.²¹ When Na_v1.8-specific blocking drugs are developed, a next step will be to examine the effect of these drugs in animal models of MS, such as EAE.

Finally, are the transcription or deployment of other subtypes of calcium or sodium channels or channels selective for other ions altered in MS, and in how many types of neurons does this occur? There is some evidence for abnormal expression of the Na_v1.2 sodium channel²² and of potassium channels²³ in dysmyelinated axons in animal models. Axons and glial cells are intimately related in myelinated fibers, and both soluble factor-mediated and contact-mediated signaling from myelinating glial cells appear to modulate ion channel expression in axons.^{24,25} Thus, we may learn about other neuronal channels and receptors that are misexpressed in the demyelinating diseases. Far from being molecular oddities, these changes in the deployment of ion channels may be clinically important; although they do not detract from the importance of demyelination or axonal loss, they may suggest new molecular mechanisms and novel strategies for treating patients with MS and related disorders.

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REFERENCES

1. Kornek B, Storch MK, Bauer J, et al. Distribution of a calcium channel subunit in dystrophic axons in multiple sclerosis and experimental autoimmune encephalomyelitis. *Brain*. 2001;124:1114-1124.
2. Schlaepfer WW, Lee C, Lee VM, Zimmerman UJ. An immunoblot study of neurofilament degradation in situ and during calcium-activated proteolysis. *J Neurochem*. 1985;44:502-509.
3. Banik NL, Matzelle DC, Gantt-Wilford G, Osborne A, Hogan EL. Increased calpain content and progressive degradation of neurofilament protein in spinal cord injury. *Brain Res*. 1997;752:301-306.
4. Fern R, Ransom BR, Waxman SG. Voltage-gated calcium channels in CNS white matter: role in anoxic injury. *J Neurophysiol*. 1995;74:369-378.
5. George EB, Glass JD, Griffin JW. Axotomy-induced axonal degeneration is mediated by calcium influx through ion-specific channels. *J Neurosci*. 1995;15:6445-6452.
6. Black JA, Fjell J, Dib-Hajj S, et al. Abnormal expression of SNS/PN3 sodium channel in cerebellar Purkinje cells following loss of myelin in the taiep rat. *Neuroreport*. 1999;10:913-918.
7. Black JA, Dib-Hajj S, Baker D, Newcombe J, Cuzner ML, Waxman SG. Sensory neuron specific sodium channel SNS is abnormally expressed in the brains of mice with experimental allergic encephalomyelitis and humans with multiple sclerosis. *Proc Natl Acad Sci*. 2000;97:11598-11602.
8. Braitenberg V. Is the cerebellar cortex a biological clock in the millisecond range? *Prog Brain Res*. 1967;25:334-346.
9. Miall RC, Keating JG, Malkmus M, Thach WT. Simple spike activity predicts occurrence of complex spikes in cerebellar Purkinje cells. *Nat Neurosci*. 1998;1:13-15.
10. Mauk MD, Medina JF, Nores WL, Ohyama T. Cerebellar function: coordination, learning or timing? *Curr Biol*. 2000;10:R522-R525.
11. Llinás R, Sugimori M. Electrophysiological properties of in vitro Purkinje cell somata in mammalian cerebellar slices. *J Physiol*. 1980;305:171-195.
12. Raman IM, Bean BP. Ionic currents underlying spontaneous action potentials in isolated cerebellar Purkinje neurons. *J Neurosci*. 1999;19:1663-1674.
13. Goldin AL, Barchi RL, Caldwell JH, et al. Nomenclature of voltage-gated sodium channels. *Neuron*. 2000;28:365-368.
14. Raman IM, Sprunger LK, Meisler MH, Bean BP. Altered subthreshold sodium currents and disrupted firing patterns in Purkinje neurons of Scn8a mutant mice. *Neuron*. 1997;19:881-891.
15. Kohrman DC, Smith MR, Goldin AI, Harris J, Meisler MH. Missense mutation in the sodium channel Scn8a is responsible for cerebellar ataxia in the mouse mutant jolting. *J Neurosci*. 1996;16:5993-5999.
16. Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. *Nature*. 1996;379:257-262.
17. Dib-Hajj SD, Ishikawa I, Cummins TR, Waxman SG. Insertion of a SNS-specific tetrapeptide in the S3-S4 linker of D4 accelerates recovery from inactivation of skeletal muscle voltage-gated Na channel μ 1 in HEK293 cells. *FEBS Lett*. 1997;416:11-14.
18. Renganathan M, Cummins TR, Waxman SG. Contribution of Na_v1.8 sodium channels to action potential electrogenesis in DRG neurons. *J Neurophysiol*. 2001;86:629-640.
19. Andermann F, Cosgrove JBR, Lloyd-Smith D, Walters AM. Paroxysmal dysarthria and ataxia in multiple sclerosis. *Neurology*. 1959;9:21-216.
20. Espir MLE, Watkins SM, Smith HV. Paroxysmal dysarthria and other transient neurological disturbances in disseminated sclerosis. *J Neurol Neurosurg Psychiatry*. 1966;29:323.
21. Akopian AN, Souslova V, England S, et al. The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. *Nat Neurosci*. 1999;2:541-548.
22. Westenbroek RE, Noebels JL, Catterall WA. Elevated expression of type II Na⁺ channels in hypomyelinated axons of shiverer mouse brain. *J Neurosci*. 1992;12:2259-2267.
23. Wang H, Allen ML, Grigg JJ, Noebels JL, Tempel BL. Hypomyelination alters K⁺ channel expression in mouse mutants shiverer and Trembler. *Neuron*. 1995;15:1337-1347.
24. Boiko T, Rasband MN, Levinson SR, et al. Compact myelin dictates the differential targeting of two sodium channel isoforms in the same axon. *Neuron*. 2001;30:91-104.
25. Kaplan MR, Cho MH, Ullian EM, Isom LL, Levinson SR, Barres BA. Differential control of clustering of the sodium channels Na_v1.2 and Na_v1.6 at developing CNS nodes of Ranvier. *Neuron*. 2001;30:105-119.