

Mechanisms of Disease: sodium channels and neuroprotection in multiple sclerosis—current status

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SUMMARY

Sodium channels can provide a route for a persistent influx of sodium ions into neurons. Over the past decade, it has emerged that sustained sodium influx can, in turn, trigger calcium ion influx, which produces axonal injury in neuroinflammatory disorders such as multiple sclerosis (MS). The development of sodium channel blockers as potential neuroprotectants in MS has proceeded rapidly, and two clinical trials are currently ongoing. The route from the laboratory to the clinic includes some complex turns, however, and a third trial was recently put on hold because of new data that suggested that sodium channel blockers might have multiple, complex actions. This article reviews the development of the concept of sodium channel blockers as neuroprotectants in MS, the path from laboratory to clinic, and the current status of research in this area.

KEYWORDS multiple sclerosis, neuroprotection, sodium channel blockers

REVIEW CRITERIA

PubMed was searched for articles published up to October 2007, including electronic early release publications. Search terms included “multiple sclerosis”, “neuroprotection”, “axonal injury”, “sodium channels” and “sodium channel blockers”. Relevant articles were retrieved and prioritized for inclusion in the Review and their references were checked for additional material when appropriate. In addition, the author used his own files of references.

INTRODUCTION

The past decade has seen increasing interest in the possibility of neuroprotective therapy with sodium channel blockers in multiple sclerosis (MS), as a result of recognition that axon degeneration is a major contributor to disability in MS, and the demonstration of a critical role for sodium channels in degeneration of CNS axons. This area is one of rapid flux—5 years ago sodium channel blockers had not been studied in animal models of MS, and 2 years ago clinical studies had not begun to assess the protective effects of sodium channel blockers in humans with MS. Now, however, data are available from animal models on the effects of four sodium channel blockers, all of which are in routine clinical use for other indications, and two clinical studies of sodium channel blockers in patients with MS are ongoing. This article will review the development of the concept of sodium channel blockers as neuroprotectants in MS, the path of translation from laboratory to clinic, and the current status of this field.

EARLY USE OF SODIUM CHANNEL BLOCKERS IN MULTIPLE SCLEROSIS

Early experience of neurologists in the use of sodium channel blockers in MS centered largely on the use of carbamazepine—and in some cases other sodium channel blockers—to treat positive phenomena such as tonic flexion spasms, Lhermitte's sign and, most commonly, trigeminal neuralgia.^{1,2} Phenytoin, and lidocaine and its orally absorbed derivative mexiletine, have also been used for these applications in patients with MS with some degree of success.³

The pathophysiological basis for positive phenomena such as trigeminal neuralgia in MS is not fully understood. It is known, however, that sodium channels contribute a transmembrane current that can produce oscillations in membrane potential, resulting in ectopic firing in demyelinated axons.⁴ The use of sodium channel blockers as treatments for positive clinical phenomena in MS, therefore,

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has a rational basis. In addition to dampening abnormal ectopic activity, however, the blocking of sodium channels would be expected to decrease the safety factor (the ratio of current available to stimulate a given node of Ranvier versus the current needed to generate an action potential at the node) for transmission of normal action potentials. This drug-induced decrease in safety factor would not be functionally important in healthy myelinated fibers in which the safety factor is 5–6, but it could produce conduction block in some demyelinated fibers in which the safety factor was already reduced by demyelination to around 1.0, with no room for further compromise.⁵ Impaired impulse conduction resulting from the reduction in safety factor produced by sodium channel blockers would be expected to be transient, and to be reversed on unbinding and metabolism of the drug. Consistent with these observations in the laboratory, transient worsening of negative symptoms (for example, weakness) has been noted in a small number of patients with MS during treatment for positive symptoms with carbamazepine but, importantly, it was reported to reverse within a few days after cessation of treatment.⁶ In an attempt to capitalize on this phenomenon, Sakurai *et al.*⁷ demonstrated that lidocaine can unmask silent demyelinating lesions in MS and proposed that this might provide a useful diagnostic test.

A 3-year follow-up study of patients with MS treated with sodium channel blockers for neuropathic pain or other paroxysmal symptoms revealed adverse effects that mimicked a relapse in 12 out of 36 patients treated with carbamazepine. In these patients, clinical status returned to the pretreatment level once the drug was discontinued.⁸ No studies have so far been carried out to determine whether there are long-term effects of treatment with sodium channel blockers in MS, and no case reports of long-term changes have been published.

SODIUM CHANNELS AS DRIVERS OF AXONAL INJURY IN THE CNS

Early evidence that voltage-gated sodium channels have a role in degeneration of CNS axons was gained from an *in vitro* model of CNS anoxia.⁹ It was shown that axon degeneration within white matter could be triggered by a cascade (Figure 1) involving sodium influx through noninactivating sodium channels. This influx overwhelms the ability of the ATP-fueled

Na^+, K^+ -ATPase pump to extrude sodium, thereby promoting calcium-importing 'reverse' operation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, which results in elevated, injurious levels of intra-axonal calcium. There is evidence that the increased intra-axonal calcium levels can activate intracellular mechanisms that increase the amplitude of—and prolong—the sodium current flowing through $\text{Na}_v1.6$ channels situated along myelinated and demyelinated axons, providing a positive feedback loop that imports still more calcium, thereby further amplifying the damage.¹⁰ Injury-induced sodium influx can also trigger release of calcium from intracellular stores within white matter axons.¹¹ The increased intra-axonal calcium concentration that results from these movements of calcium into the axoplasm activates multiple injurious pathways that involve calpain and other degradative enzymes.¹² This injurious cascade can occur in normal myelinated axons (in which sodium channels are clustered at nodes of Ranvier¹³) when they are subjected to energy deprivation (for example, through anoxia). In individuals with MS, it is expected that demyelinated axons, some of which display long expanses of membrane that express sodium channels,¹⁴ would be especially susceptible to this mode of injury. Remyelinated axons, in which nodes of Ranvier can be very closely spaced,^{15,16} might be expected to be at increased risk.¹⁷

Building upon these observations, subsequent experiments demonstrated that the sodium blockers tetrodotoxin (TTX)⁹, lidocaine, procaine,¹⁸ mexiletine,¹⁹ phenytoin, and carbamazepine²⁰ can protect white matter axons from anoxic and ischemic injury *in vitro*. Importantly, protection could be achieved in these *in vitro* experiments with sodium channel blockers at concentrations that did not compromise the conduction of action potentials.^{18,21}

An important link to axonal injury in MS was established with the discovery that nitric oxide (NO) is present at increased levels within MS lesions, where, among other effects, it can trigger axon degeneration similar to the axonal injury produced by anoxia.^{22,23} This action can be attributed in part to deleterious effects of NO on mitochondrial function,^{24,25} which result in a reduction in ATP levels and a rundown of Na^+, K^+ -ATPase, thereby compromising the axon's ability to maintain normal transmembrane sodium

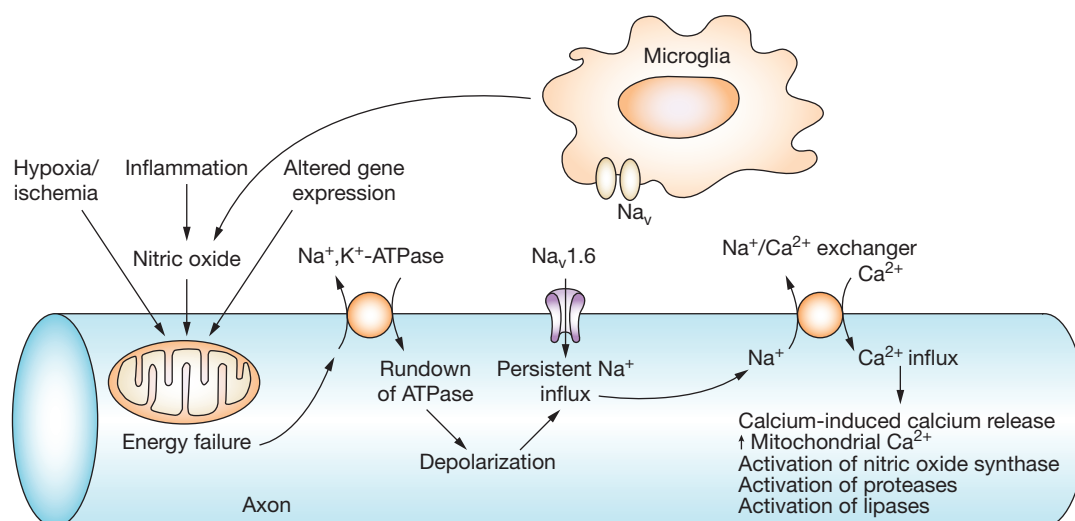


Figure 1 The central role of sodium channels in the axon degeneration cascade. A number of factors (nitric oxide; ischemia that results from inflammation of small blood vessels; and decreased expression of genes that encode mitochondrial redox carriers) contribute to energy failure and subsequent rundown of the Na^+ , K^+ -ATPase pump, with subsequent depolarization and loss of capacity to maintain transmembrane ion gradients. The depolarization activates sodium channels (e.g. $\text{Na}_v1.6$), which provide a route for persistent sodium influx. This process, in turn, drives the Na^+ / Ca^{2+} exchanger to operate in a calcium-importing mode. The rise in intracellular calcium induces a further increase in calcium levels via calcium-induced calcium release. Increased intra-axonal calcium also injures mitochondria, and activates nitric oxide synthase and harmful proteases and lipases. Permission obtained from Nature Publishing Group © Waxman SG (2006) *Nat Rev Neurosci* 5: 932–942.

gradients. Consistent with this mechanism of NO-triggered injury, Smith *et al.*²² showed that impulse activity at physiological frequencies can trigger degeneration of axons exposed to NO. Kapoor *et al.*²⁶ showed that the sodium channel blockers lidocaine and flecainide can protect axons from NO-mediated degeneration. Garthwaite *et al.*²⁷ showed that TTX and another sodium channel blocker, sipatrigine, can protect axons from NO-mediated damage, while concomitantly protecting ATP levels in white matter.

Studies in animal models and patients with MS have provided additional support for participation of noninactivating sodium channels—acting in concert with the Na^+ / Ca^{2+} exchanger—in axon degeneration in neuroinflammatory disorders. It was shown that one particular sodium channel subtype, $\text{Na}_v1.6$, which produces noninactivating as well as rapidly inactivating sodium currents,²⁸ is colocalized with the Na^+ / Ca^{2+} exchanger across extensive regions (much longer than nodes of Ranvier) along degenerating axons in mice with experimental autoimmune encephalomyelitis (EAE),²⁹ and in acute lesions in patients with

MS.¹⁴ The diffuse distribution of $\text{Na}_v1.6$ sodium channels in demyelinated axons might, in itself, be expected to subject substantial lengths of demyelinated axons to sustained sodium influx, which would increase the energy demand. When colocalized with the Na^+ / Ca^{2+} exchanger in axon regions lacking myelin, $\text{Na}_v1.6$ would contribute to a molecular machine that is critically well poised to load demyelinated axons with damaging levels of calcium.

NEUROPROTECTION IN MULTIPLE SCLEROSIS

Major impetus for studies on neuroprotection in MS was provided in the late 1990s by increased recognition of the frequency of axon degeneration in acute MS lesions,^{30,31} together with increasing evidence that axonal damage can occur at very early stages in MS³² and that it can cause persistent, nonremitting deficits.^{33–36} Advances in these areas focused attention on the therapeutic objective of protecting axons so that they would not degenerate in MS, as a strategy for preventing disability. Additional interest in this approach arose from several findings. It was

shown that transcription of genes that encode mitochondrial respiratory chain complexes was impaired in postmortem brains from patients with MS.³⁷ Histopathological observations indicated that hypoxia-like tissue injury could occur in MS lesions, possibly owing to inflammatory compromise of microvasculature.^{38,39} In addition, Stys⁴⁰ proposed that the widespread distribution of sodium channels along at least some parts of the demyelinated axon membrane^{14,29} should result in a mismatch between energy supply and demand in demyelinated axons, creating a state of 'virtual hypoxia'. As these lines of evidence matured, they underscored the potential relevance to MS of the demonstrations, described above, of attenuation of anoxia-induced and NO-triggered injury to axons by treatment with sodium-channel-blocking agents. This provided impetus for studies on the neuroprotective effects of sodium channel blockers, initially in animal models of MS and later in humans with MS.

Neuroprotection in animal models of multiple sclerosis

Using EAE as an animal model of MS, a number of studies have been carried out to address the question of whether sodium channel blockers might be neuroprotective in this condition. Lo *et al.*^{41,42} showed that phenytoin protects CNS axons during a 28–30-day period of oral administration, at doses that achieve plasma levels in the human therapeutic range, to C57Bl mice with myelin oligodendrocyte glycoprotein (MOG)-induced EAE. In this EAE model, phenytoin treatment ameliorated loss of axons within the corticospinal tract from 63% to 28%, and enabled axonal conduction to be maintained in a substantial fraction of the surviving axons. Importantly, treatment with phenytoin also resulted in substantially improved clinical outcome, as assessed during a 28–30-day treatment period (Figure 2).^{41,42} In another study on DA rats with chronic relapsing EAE induced by injection of syngeneic spinal cord homogenate, Bechtold *et al.*^{43,44} showed that flecainide has a similar protective effect (reducing axonal degeneration from 40% to 2–17%, depending on when treatment was started, in rats that exhibited severe disease during the trial), and improved functional outcome, when studied at the end of a 28–30-day period of administration. In a third study, lamotrigine was reported to reduce the degree of spinal cord axon degeneration

from 33.5% to 10.4% in rats with chronic relapsing EAE, and was observed to provide a small but statistically significant reduction in neurological deficit at the termination of these experiments at 27–29 days.⁴⁵ So, by the year 2005, a neuroprotective effect of three sodium channel blockers had been observed in rodent models of MS.

Interestingly, the initial studies on the effects of phenytoin in EAE showed that it also protected against the reduction in conduction velocity that occurs in untreated EAE, suggesting that phenytoin treatment might have reduced the degree of demyelination.⁴² Similarly, it was noted that flecainide administration reduced the severity of neurological symptoms early in the course of chronic relapsing EAE, raising the possibility of immunomodulatory effects.⁴⁴ Subsequent studies demonstrated that treatment with phenytoin ameliorated the inflammatory cell infiltrate in EAE by 75%.⁴⁶

Additional evidence for an inflammatory connection

Additional evidence that sodium channels have a role in immune cell function in animal models of MS and in MS itself was provided by Craner *et al.*,⁴⁶ who noted that Na_v1.6 sodium channels are present in murine macrophages and microglia, and that there is a robust increase in sodium channel expression in activated microglia and macrophages in EAE. Further support for a functional role for sodium channels in the regulation of cells of the macrophage–microglia lineage (macrophages/microglia) was provided by this group's demonstration that TTX, a potent and specific sodium channel blocker, decreases the phagocytic function of activated rat microglia by 40%. This study also demonstrated a substantial reduction of phagocytic capacity in macrophages/microglia from mutant *med* mice, which lack Na_v1.6 sodium channels.⁴⁶ These observations implicated Na_v1.6 in the function of murine macrophages and microglia, and provided evidence that sodium channels have a role in activation and phagocytic activity of microglia and macrophages in EAE. Hinting at a role for sodium channels in macrophages/microglia in human neuroinflammatory disease, the studies of Craner *et al.*⁴⁶ also revealed that Na_v1.6 channels are present in macrophages/microglia within acute MS lesions, and that the expression of these channels is upregulated on activation of these cells.

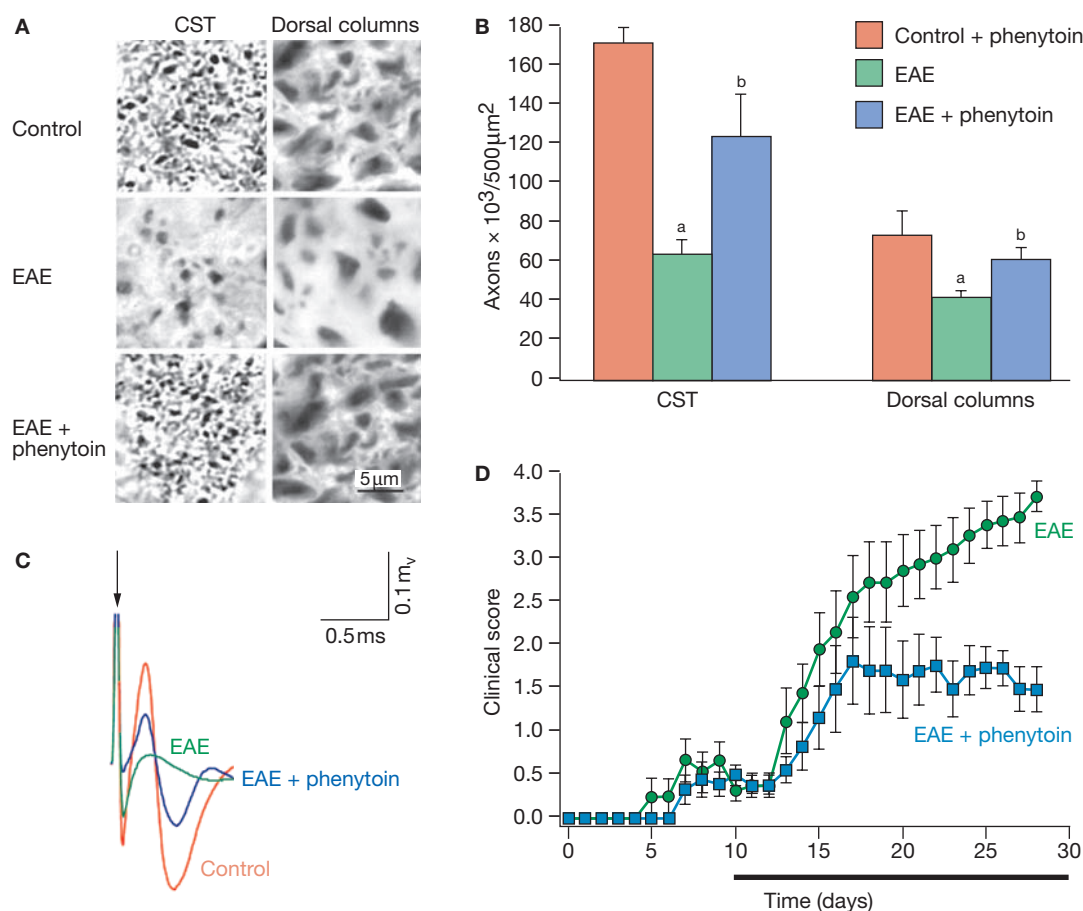


Figure 2 Treatment with phenytoin is protective in a mouse model of multiple sclerosis. **(A)** Representative sections showing the protective effect of treatment with phenytoin in the CST and dorsal columns (cuneate fasciculus) compared with untreated EAE. Control mice did not have EAE. Note the sparing of axons during treatment with phenytoin. **(B)** Quantitation of axon densities in dorsal CST and dorsal columns (cuneate fasciculus) from phenytoin-treated control mice, untreated EAE mice, and phenytoin-treated EAE mice. There was a substantial reduction in axon counts in untreated EAE^a compared with phenytoin-treated controls ($P < 0.05$) and untreated controls ($P < 0.05$, not shown). Phenytoin treatment of EAE^b resulted in a significant increase in axon density within both tracts compared with untreated EAE ($P < 0.05$). Bars, means \pm SE. **(C)** Supramaximal dorsal column compound action potentials are attenuated in untreated EAE, but robust compound action potentials with a normal configuration are preserved during phenytoin treatment. **(D)** Phenytoin treatment improves neurological status in EAE. Clinical scores (\pm SE) are shown for untreated EAE and for phenytoin-treated EAE. Oral administration of phenytoin, as indicated by the horizontal bar below the x-axis, was started on day 10 and continued through to the termination of the experiment on day 30. Abbreviations: CST, corticospinal tract; EAE, experimental autoimmune encephalomyelitis. Permission obtained from the American Physiological Society © Lo A *et al.* (2003) *J Neurophysiol* **90**: 3566–3572.

These studies indicated that sodium channel blockers might have an immunomodulatory action, and that a sodium-channel-blocker-induced attenuation of inflammation might contribute to the improved outcome seen during administration of these drugs in rodents with EAE. Importantly, however, these observations did not lessen the evidence for a direct protective effect on axons. Indeed, the protective effect of

sodium channel blockers on axons subjected to anoxia *in vitro*^{9,18,20} and immediately after exposure to elevated levels of NO *in vivo*²⁶—situations in which inflammatory activity is minimal or nonexistent—demonstrated that the mechanism of action of these agents involves, at least in part, a direct effect on axons. One interpretation of these studies was that sodium channel blockers might act via a dual

mechanism that involves both a direct action on axons and an immunomodulatory action on inflammatory cells.

Neuroprotection with sodium channel blockers: clinical studies

Building on the results in animal models, two clinical studies were planned to determine whether sodium channel blockers provide neuroprotection in MS: a trial of neuroprotection with lamotrigine in secondary progressive MS at the University College London Hospitals, London, UK (ClinicalTrials.gov identifier: NCT 00257855), and a trial of neuroprotection with phenytoin in primary progressive MS at Yale University, New Haven, CT, USA. Kapoor⁴⁷ has discussed some of the considerations involved in the design of this type of clinical study. A combination trial to determine whether topiramate, administered in conjunction with interferon β -1a, has a neuroprotective effect in relapsing–remitting MS, was also launched at the Multiple Sclerosis Institute, Philadelphia, PA, USA (ClinicalTrials.gov identifier: NCT 00217295). With use of different protocols, each of these trials was designed to measure the effect of a sodium channel blocker on the progression of brain atrophy and disease progression, with the University College London trial also examining new T1 low-intensity-signal lesion volume and new T2 high-intensity lesion volume, and the ratio of new T1 to new T2 lesions on MRI. The inclusion of a different group of patients (secondary progressive MS, primary progressive MS, and relapsing–remitting MS) in each of the three trials was, in part, a result of discussion between some of the clinical investigators about the derivation of information on different forms of the disease (a multicenter study was, at the time of inception of the studies, not possible for a number of reasons). It also, however, reflected differing views regarding which type of MS, if any, would be most likely to show a robust clinical response to sodium channel blockers.

Effects of withdrawal of sodium channel blockers

In a series of studies beginning in 2006, Black *et al.*⁴⁸ asked whether a protective effect would persist after withdrawal of phenytoin in the C57Bl mouse model of MOG-induced EAE. They wanted to test the hypothesis that there would be some degree of clinical progression after phenytoin treatment was stopped and to

study the rate of this progression. As in the original EAE studies,^{41,42} phenytoin was administered beginning on day 10 after the initial MOG injection and was withdrawn on day 28. Surprisingly, the sudden withdrawal of phenytoin resulted in acute exacerbation, accompanied by a markedly increased inflammatory infiltrate (consisting largely of macrophages and microglia, together with T lymphocytes) within the CNS. Death occurred in more than 50% of EAE mice following withdrawal of phenytoin (Figure 3). It was also noted that phenytoin withdrawal increased the vascular permeability of the brain and other organs.⁴⁸ These adverse effects were not seen when phenytoin was given to healthy mice (no EAE) for a similar period and then abruptly withdrawn.

Clinical worsening was also seen after withdrawal of carbamazepine from C57Bl mice with MOG-induced EAE.⁴⁸ The mice were treated orally with this sodium channel blocker at doses that achieved plasma levels within the human therapeutic range, following the same protocol as the study by Black *et al.* (i.e. treatment with carbamazepine beginning on day 10, with withdrawal at day 28). These experiments showed that carbamazepine is protective for as long as it is administered, producing significantly improved clinical scores in EAE mice compared with untreated EAE mice. Within 24 h of carbamazepine withdrawal, however, there was substantial worsening of clinical scores, and, by 7 days after withdrawal, the clinical scores were similar to those in mice with untreated EAE. As with phenytoin, withdrawal of carbamazepine was associated with a markedly increased inflammatory infiltrate within the CNS. Although the worsening appeared to be less severe than after withdrawal of phenytoin, 7.7% of EAE mice died during the 7-day period following withdrawal of carbamazepine.⁴⁸ The adverse effects did not occur after carbamazepine was given to healthy mice and subsequently withdrawn.

Despite the fact that phenytoin has been used successfully in the past to treat positive symptoms in patients with MS, the investigators at Yale University elected to put their phenytoin clinical study on hold in light of the observations on the effects of phenytoin withdrawal in EAE.⁴⁸ Their decision to postpone the trial in order to permit further preclinical studies was made before any patients were enrolled. The University College London and Multiple Sclerosis Institute,

Philadelphia trials of lamotrigine and topiramate, respectively, commenced before the effects of phenytoin and carbamazepine withdrawal were known and are still ongoing.

Prompted by the observations on withdrawal of sodium channel blockers, Carrithers *et al.*⁴⁹ examined the role of sodium channels in macrophage function in primary human monocyte-derived macrophages and a human monocytic cell line, THP-1. This study showed that Na_v1.5 sodium channels were present in late endosomes (cytoplasmic membrane-bound vesicles containing phagocytosed material) in human macrophages (Figure 4A,B), and that these sodium channels have an important role in phagocytosis (Figure 4D). Activation of the endosomal sodium channels in these macrophages provides a route for sodium efflux that offsets proton influx during endosomal acidification, an important step that aids the digestion of foreign material late in the phagocytotic process. On the basis of these observations, Carrithers *et al.*⁴⁹ speculated that sodium-channel-amplified acidification of endosomes within macrophages might enhance the destruction of infectious agents, and might, therefore, be an adaptation that is protective during acute infections. This mechanism might, however, amplify tissue injury in some pathological states that are characterized by inflammation.

Carrithers *et al.*⁴⁹ also confirmed the presence of the sodium channel Na_v1.6 in human macrophages. Na_v1.6 was associated with cytoskeletal structures such as actin stress fibers and the intermediate filament vimentin (Figure 4C), suggestive of an additional role for Na_v1.6 sodium channels in the regulation of macrophage cell shape and motility. Recent evidence indicates that sodium channels also modulate the motility of T lymphocytes.⁵⁰

CONCLUSIONS AND FUTURE PROSPECTS

At present, it is not known whether the unexpected observations of worsening following withdrawal of phenytoin and carbamazepine in a mouse model of MS can be extrapolated to other models of MS, to humans with MS, or to other sodium channel blockers. It is possible that there are species differences in the expression or roles of various types of sodium channels in immune cells, and it would not be surprising to find at least subtle differences in the actions of different sodium channel blockers. Nonetheless, it seems noteworthy that

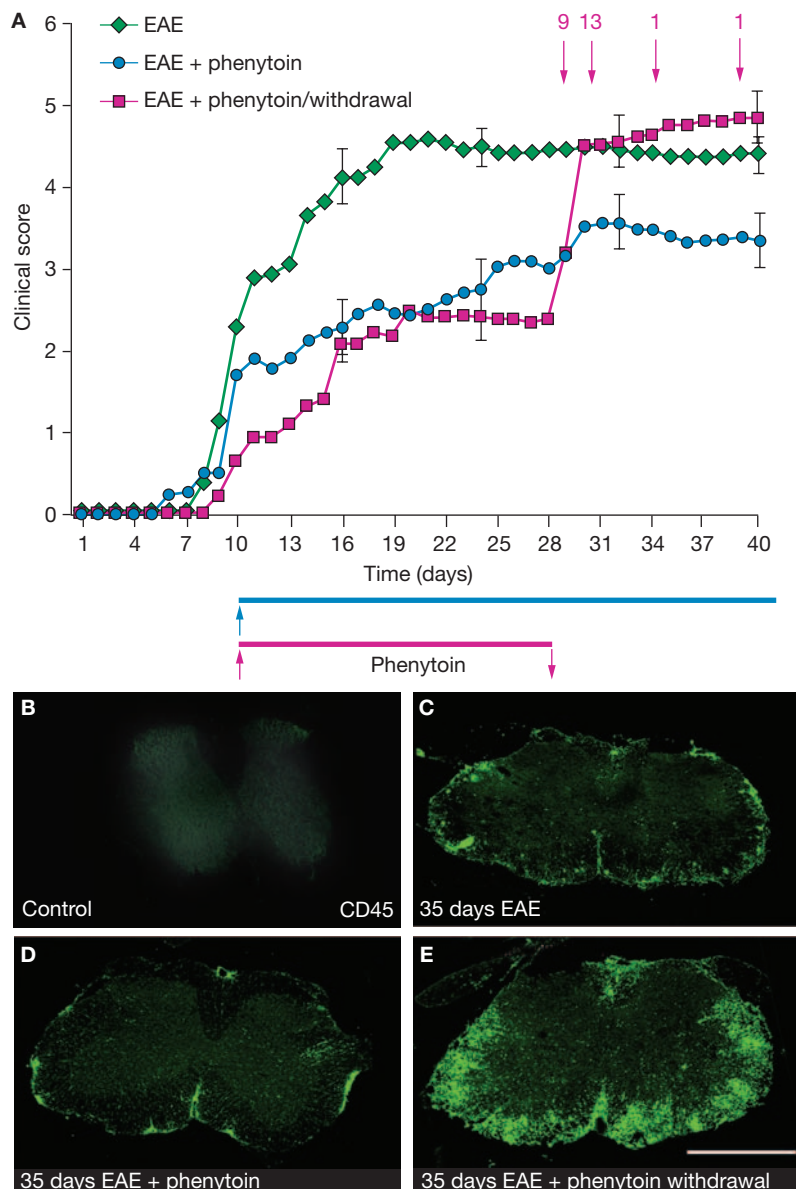


Figure 3 Withdrawal of phenytoin is followed by exacerbation in mice with experimental autoimmune encephalomyelitis.⁴⁸ (A) Mean clinical scores are shown for C57Bl mice with untreated EAE, EAE treated with phenytoin, and EAE initially treated with phenytoin, which was subsequently withdrawn on day 28 after induction of EAE. Phenytoin treatment is indicated by blue (continuous treatment) and magenta (withdrawal) bars. Treatment with phenytoin resulted in improved clinical scores, compared with untreated mice, on all days after day 12. Withdrawal of phenytoin at day 28 resulted in rapid worsening of clinical scores. The numbers above the magenta arrows indicate the number of deaths (within a group of 46 EAE mice from which phenytoin was withdrawn) at each postwithdrawal time point. No deaths occurred in mice with untreated EAE, or in mice with EAE in which treatment continued to day 40. For clarity, standard error bars are shown for days 16, 24, 32, and 40 only, but these are representative of all time points. (B) Cross section through the lumbar spinal cord of a C57Bl control mouse, stained with an anti-CD45 antibody to show absence of inflammatory cell infiltrate. (C) Section through the lumbar spinal cord of a C57Bl mouse after 35 days of EAE, stained with an anti-CD45 antibody to show inflammatory cell infiltrate. (D) Inflammatory infiltrate is reduced in phenytoin-treated EAE. (E) Inflammatory infiltrate is increased 7 days after withdrawal of phenytoin. Abbreviation: EAE, experimental autoimmune encephalomyelitis.

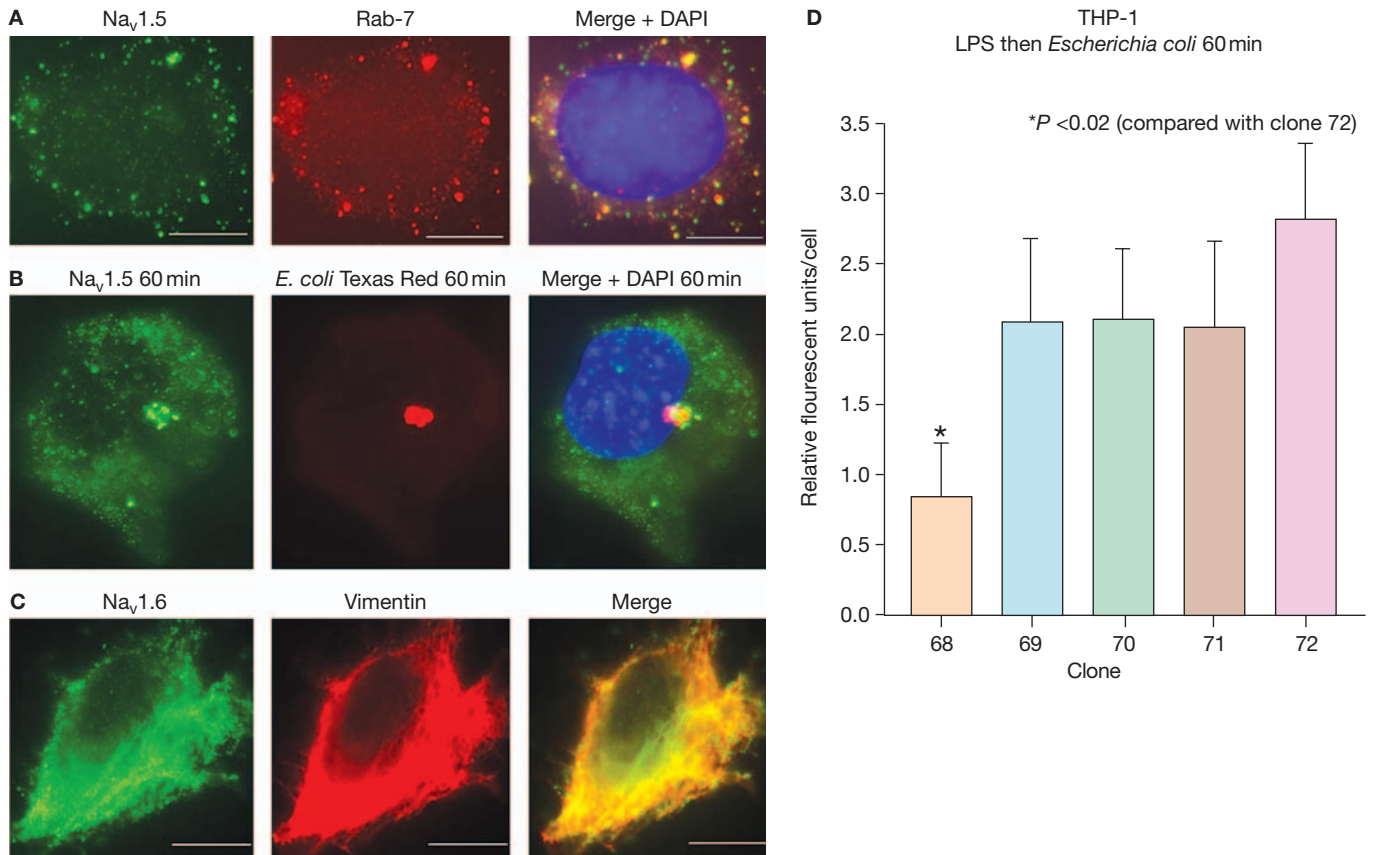


Figure 4 Sodium channels are expressed in, and regulate the function of, human macrophages. **(A)** Na_v1.5 sodium channels are present within human macrophages and the human monocytic cell line THP-1 (green, left panel), and are colocalized with Rab-7, a marker of late endosomes (red, middle panel). Right panel shows merged images (coexpression indicated in yellow; cell nucleus stained with DAPI [blue]). Scale bar 5 μm. **(B)** In human macrophages, Na_v1.5 sodium channels are situated in close proximity to phagocytosed particles in phagosomes. Texas Red[®]-labeled *Escherichia coli* (Molecular Probes, Eugene, OR) were incubated with differentiated, primed THP-1 cells. After 60 min, labeled *E. coli* (red, middle panel) were observed within intracellular vesicles, colocalized with Na_v1.5 (green, left panel). Right panel shows colocalization (yellow; cell nucleus stained with DAPI [blue]). **(C)** Na_v1.6 sodium channels are observed in association with cytoskeletal filaments in human macrophages. Note the colocalization (yellow, right panel) of Na_v1.6 immunoreactivity (green, left panel) with vimentin (red, middle panel). Scale bar 5 μm. **(D)** Na_v1.5 gene knockdown, mediated by short hairpin RNA (shRNA), decreases the level of phagocytosis in human macrophages. Phagocytosis in THP-1 cells was monitored by immunofluorescence. Phagocytosis was reduced by 70% in cells infected with shRNA clone 68 (which resulted in Na_v1.5 knockdown of ~88%) compared with phagocytosis in wild-type cells or cells infected with clone 72 (which produced no appreciable Na_v1.5 knockdown). Abbreviations: DAPI, 4',6'-diamidino-2-phenylindole; LPS, lipopolysaccharide. Permission obtained from the American Association of Immunologists, Inc. © Carrithers *et al.* (2007) *J Immunol* **178**: 7822–7832.

other chemically unrelated sodium channel blockers, such as TTX,^{46,49,50} and knockdown of sodium channels by use of short hairpin RNA (shRNA),⁴⁹ have profound effects on immune cells. Taken together with the observations on the effects of sodium channel blocker withdrawal in EAE,⁴⁸ these studies raise important questions regarding the functional roles of sodium channels in immune cells, and possibly in autoimmune and inflammatory disorders.

As outlined above, on the basis of laboratory studies beginning *in vitro* and then carried out

in rodent models of MS, three clinical studies were initiated to determine whether sodium channel blockers provide neuroprotection in MS (Table 1). The London study on lamotrigine and the Philadelphia study on topiramate are ongoing, but the Yale study on phenytoin was postponed following the observation of clinical worsening and increased inflammatory activity after withdrawal of sodium channel blockers in a mouse model of MS. It is important to consider these recent events in the context of a long history of use of sodium channel

Table 1 Sodium channel blockers under study as potential neuroprotectants in multiple sclerosis.

Drug	Primary clinical use	Protective effect during administration in animal models?	Effects of withdrawal in EAE	Clinical study
Phenytoin	Treatment of epilepsy	Protects axons, reduces inflammation and improves clinical status during administration to mice with MOG-induced EAE	Clinical worsening, increased CNS inflammation, death	Trial planned on primary progressive MS (Yale University, New Haven, CT, USA); study placed on hold
Flecainide	Antiarrhythmic agent	Protects axons, reduces inflammation and improves clinical outcome during administration in DA rats with relapsing–remitting EAE	Not known	None underway
Lamotrigine	Treatment of epilepsy	Protects axons, reduces inflammation and improves clinical status during administration to rats with spinal-cord-homogenate-induced EAE	Not known	Study has enrolled patients with secondary progressive MS (University College London Hospitals, London, UK [NCT 00257855])
Topiramate	Treatment of epilepsy	Not known	Not known	Combination study (topiramate and interferon β -1a) is enrolling patients with relapsing–remitting MS (Multiple Sclerosis Institute, Philadelphia, PA, USA [NCT 00217295])
Carbamazepine	Treatment of epilepsy; treatment of positive symptoms in MS	Protects axons, reduces inflammation, and improves clinical outcome during administration to mice with MOG-induced EAE	Clinical worsening, increased CNS inflammation	None underway

Abbreviations: EAE, experimental autoimmune encephalomyelitis; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis.

blockers, particularly carbamazepine, for the treatment of positive clinical phenomena in MS. There are no reports in the literature of acute worsening of MS following withdrawal of sodium channel blockers. Moreover, preliminary experiments suggest that gradual, tapering withdrawal of phenytoin over a 7-day period does not cause worsening of EAE (JA Black and SG Waxman, unpublished observations). On the other hand, the mechanisms that underlie the inflammatory rebound after abrupt withdrawal of phenytoin from mice with EAE are not understood, and the possibility of more-subtle, long-term effects in humans following withdrawal, resulting from a reprogramming of immune cells, has not been studied. As clinical studies progress, it will be important, therefore, to monitor patients closely not only in terms of neurological function and measures of axonal loss, but also via imaging for

new inflammatory events, and via serological and cerebrospinal fluid analysis to assess inflammatory and immune status. It is hoped that the ongoing trials will provide useful information within the next few years.

Currently, the jury is still out with respect to the safety and efficacy of sodium channel blockers as neuroprotective agents in patients with MS. As regards current neurological practice, given the long history of clinical use of carbamazepine and related agents in patients with MS who have trigeminal neuralgia and related disturbances, it seems appropriate to continue symptomatic treatment of appropriately selected patients. Nevertheless, it seems prudent to recommend careful selection of patients for treatment with sodium channel blockers, and to caution against abrupt drug withdrawal. If withdrawal does prove necessary, these medications should be withdrawn via a gradual taper.

KEY POINTS

- Voltage-gated sodium channels can contribute to axonal injury in multiple sclerosis (MS) by providing a pathway for sustained sodium influx that drives the Na⁺/Ca²⁺ exchanger to import calcium into axons
- Sodium channel blockers protect axons from degeneration in several *in vitro* models of axonal injury, and they prevent axon degeneration, maintain impulse conduction, and improve clinical status in experimental autoimmune encephalomyelitis, a mouse model of MS
- Sodium channels regulate the function of macrophages and microglia, so, in addition to a direct protective effect on axons, sodium channel blockers might have an immunomodulatory action
- Sudden withdrawal of the sodium channel blockers phenytoin and carbamazepine from mice with experimental autoimmune encephalomyelitis results in acute clinical exacerbation, accompanied by increased inflammatory infiltrate within the CNS
- Until more is known about the effects of sodium channel blocker withdrawal in humans with MS, clinical studies should monitor patients closely both in terms of neurological function and axonal loss and with respect to immune and inflammatory status
- If withdrawal of the sodium channel blocker is necessary in patients with MS treated with carbamazepine or phenytoin for trigeminal neuralgia or other positive disturbances, these medications should be discontinued via a gradual taper

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Competing interests

The author declared no competing interests.