Sodium Channel Expression Within Chronic Multiple Sclerosis Plaques

Joel A. Black, PhD, Jia Newcombe, PhD, Bruce D. Trapp, PhD, and Stephen G. Waxman, MD, PhD

Abstract
Multiple sclerosis (MS) is characterized by focal destruction of myelin sheaths, gliotic scars, and axonal damage that contributes to the accumulation of nonremitting clinical deficits. Previous studies have demonstrated coexpression of sodium channel Na\textsubscript{v}1.6 and the sodium-calcium exchanger (NCX), together with β-amyloid precursor protein (β-APP), a marker of axonal damage, in degenerating axons within acute MS lesions. Axonal degeneration is less frequent within chronic MS lesions than in acute plaques, although current evidence suggests that axonal loss in chronic lesions (“slow burn”) is a major contributor to accumulating disability. It is not known, however, whether axonal degenerations in chronic and acute lesions share common mechanisms, despite radically differing extracellular milieu. In this study, the expression of sodium channels Na\textsubscript{v}1.2 and Na\textsubscript{v}1.6 and of NCX was examined in chronic MS plaques within the spinal cord. Na\textsubscript{v}1.2 immunostaining was not observed along demyelinated axons in chronic lesions but was expressed by scar and reactive astrocytes within the plaque. Na\textsubscript{v}1.6 immunoreactivity, which was intense at nodes of Ranvier in normal appearing white matter in the same sections, was present in approximately one-third of the demyelinated axons within these plaques in a patchy rather than continuous distribution. NCX was not detected in demyelinated axons within chronic lesions, although it was clearly present within the scar astrocytes surrounding the demyelinated axons. β-APP accumulation occurred in a small percentage of axons within chronic lesions within the spinal cord, but β-APP was not preferentially present in axons that expressed Na\textsubscript{v}1.6. These observations suggest that different mechanisms underlie axonal degeneration in acute and chronic MS lesions, with axonal injury occurring at sites of coexpression of Na\textsubscript{v}1.6 and NCX in acute lesions but independent of coexpression of these 2 molecules in chronic lesions.

Key Words: Axonal injury, Demyelination, Multiple sclerosis, Sodium channel, Spinal cord.

INTRODUCTION
Although multiple sclerosis (MS) has classically been characterized as a demyelinating disorder (1), the contribution of axonal pathology to clinical deficits has long been recognized and has recently become a focus of major interest (2–5). MS plaques are heterogeneous but have been broadly classified as active/acute or inactive/chronic, with subcategories defined by the presence or absence of specific cellular and/or molecular constituents (e.g., inflammatory infiltrates, cytokines, and myelin products [6, 7]). Whereas axonal damage is more prominent in acute plaques, a continuing loss of axons in chronic lesions (“slow burn”) has been suggested to play an important role in accumulating nonremitting neurologic deficits (3, 8). Indicators of axonal degeneration such as axonal accumulation of β-amyloid precursor protein (β-APP), an early and sensitive marker for axonal damage (9), are more prevalent in acute plaques than in chronic lesions (10–12). Although this variation could reflect the prior loss of axons in long-standing lesions, it also could be due to differences in the pathogenesis of axonal injury in acute and chronic plaques. Consistent with this loss, the degree of axonal accumulation of β-APP is correlated with macrophage infiltration (12, 13), which is greatest in active lesions.

The mechanisms responsible for axonal loss after demyelination are incompletely understood. A broad range of factors have been suggested to contribute to axonal degeneration after demyelination, including proteolytic enzymes, cytokines, nitric oxide, and persistent sodium influx, which can drive reverse Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange (for reviews, see References 2, 3, 14, 15). Involvement of voltage-gated sodium channels in the cascade leading to axonal damage is suggested by observations that sodium channel blockade can prevent axonal degeneration within white matter tracts in a number of disease models (16–20). Axonal sparing in experimental autoimmune encephalomyelitis, a model of MS, has been demonstrated during periods of administration of sodium channel blocking agents (21–24). Using triple-immunofluorescent labeling, Craner...
et al. (25, 26) have provided evidence that the coexpression of sodium channel Na_v1.6, which produces a substantial persistent sodium current (27), and the sodium-calcium exchanger (NCX) is associated with axonal injury within acute lesions in experimental autoimmune encephalomyelitis and MS tissue.

Chronic MS plaques contain demyelinated axons that are often surrounded by a dense meshwork of astrocyte processes, with relatively few immune cells present (28). Little is known about the molecular or physiologic characteristics of these chronically demyelinated axons, although decreased neurofilament staining (29) and the re-expression of PSA-NCAM (30) have been observed in these axons. An increase of binding of tritiated-saxitoxin, a sodium channel-specific ligand, has been reported in demyelinated MS lesions (31), although the clinical status of the plaques was not characterized, and this study could not distinguish between axonal and glial saxitoxin binding. In contrast to active MS lesions, where enhanced expression of sodium channels Na_v1.2 and Na_v1.6 has been demonstrated along demyelinated axons and where Na_v1.6 and NCX are associated with axonal injury (26), the expression of sodium channels and NCX within chronically demyelinated plaques has not been studied.

In the present study, we have examined the expression of Na_v1.2 and Na_v1.6 sodium channels and NCX, together with β-APP accumulation, in axons within chronic MS lesions, to determine whether expression of these sodium channels and/or NCX is associated with axonal injury. Our results demonstrate that Na_v1.6 is detectable in approximately one-third of demyelinated axons in chronic plaques, whereas the remaining axons do not express detectable levels of Na_v1.6 or Na_v1.2. We show that axonal β-APP accumulation occurs but is rare in these chronic lesions within the spinal cord and does not preferentially occur in axons that express Na_v1.6 or NCX. These observations suggest that differing underlying mechanisms may lead to axonal degeneration in acute and chronic MS plaques.

**MATERIALS AND METHODS**

**Multiple Sclerosis Tissue**

Postmortem cervical and thoracic spinal cord tissue acquired by means of a rapid protocol from patients with disabling secondary progressive MS (n = 5; age 59.4 ± 8.7 years; mean disease duration 24.9 ± 6.8 years) and from controls (n = 3; age 69.1 ± 3.8 years) was obtained from the UCL Institute of Neurology NeuroResource tissue bank (32) and the Cleveland Clinic Foundation (33). Tissue was rapidly frozen and stored at -80°C as previously described (11, 26); plaques were characterized as chronic on the basis of lack of myelin as judged by staining for myelin basic protein (MBP) and paucity of oil red O (34) or major histocompatibility complex class II-positive phagocytic macrophages (35).

Mean ± SE oil red O and perivenular inflammatory cuffing scores (scale 0–5, with 0 expected in normal control white matter [36]) for the chronic plaques were 0.2 ± 0.1 and 0.1 ± 0.0, respectively.

**FIGURE 1.** Myelin basic protein (MBP) and immune cells in a chronic multiple sclerosis (MS) lesion. (A) Low magnification montage of MBP immunostaining in a chronic MS plaque within the spinal cord. MBP immunoreactivity is nearly completely absent within the chronic plaque (white lines), but MBP staining is exhibited by an area of normal appearing white matter adjacent to the lesion. The tissue section is outlined by a dashed line. (B) CD14 (monocytes) and CD68 (monocytes/macrophages) immunolabeling in control and chronic and acute MS lesions within spinal cord. There is a paucity of CD14- and CD68-positive cells in control and chronic plaques; acute lesions exhibit substantial infiltration of CD14- and CD68-positive cells. Scale bars: = (A) 100 μm; (B) 50 μm.
Mean postmortem intervals were 14.6 hours (range 8–20 hours) for MS tissue and 15.0 hours (range 5–25 hours) for control tissue.

**Immunocytochemistry**

Tissue sections were processed as described previously (26). Briefly, 10-μm sections were initially fixed for 5 minutes in 4% paraformaldehyde in 0.14 M Sorenson’s phosphate buffer, pH 7.4, rinsed several times in PBS, and incubated in blocking solution (PBS with 5% normal goat serum, 1% bovine serum albumin, 0.1% Triton X-100, and 0.02% sodium azide) for 30 minutes at room temperature. Sections were then incubated simultaneously or in combination with primary antibodies (mouse anti-MBP IgG [1:4000, SMI 94; Sternberger Monoclonals, Lutherville, MD], mouse anti-phosphorylated and anti-nonphosphorylated neurofilament [each 1:20,000, SMI 31 and SMI 32; Sternberger Monoclonals], mouse anti-sodium-calcium...
exchanger [NCX] IgM [1:100; RDI, Flanders, NJ], mouse anti-Alzheimer precursor protein A4 [β-APP] IgG [1:100; Chemicon, Temecula, CA], rabbit anti-Na,1.2 IgG [1:100; Alomone Labs, Jerusalem, Israel], rabbit anti-Na,1.6 [PN4] IgG [1:100; Sigma-Aldrich, St. Louis, MO], rabbit anti-glial fibrillary acidic protein GFAP IgG [1:200; Chemicon], chicken anti-GFAP IgG [1:1000; Encor Biotechnology, Alachua, FL], and chicken anti-neurofilament [1:1500; Encor Biotechnology]) for 24 to 48 hours at 4°C. Sections were subsequently washed with PBS, incubated in appropriate secondary antibodies (goat anti-mouse IgG Alexa Fluor 488 or 568 [1:1000; Molecular Probes, Eugene, OR], goat anti-mouse IgM Alexa Fluor 488 [1:1000; Molecular Probes], goat anti-rabbit IgG Cy3 [1:2000; Amersham, Piscataway, NJ] or Alexa Fluor 633 [1:1000; Molecular Probes], or goat anti-chicken IgG Alexa Fluor 488 or 633 [1:1000; Molecular Probes]) for 12 to 24 hours at 4°C, washed with PBS, and coverslipped with Aqua Poly mount (Polysciences, Warrington, PA). Immunostaining of nodes of Ranvier in normal-appearing white matter for Na,1.6, and of astrocytes for Na,1.2 and NCX provided positive control for preserved antigenicity in the same sections.

**Tissue Analysis**

For analyses of chronic lesions within sections, multiple images were accrued with a Nikon PCM 2000 confocal microscope. Analyses were confined to regions of the sections that did not exhibit MBP staining and were at least 200 µm from the boundary of the chronic lesion. Images were composed and processed to enhance contrast in figures in Adobe Photoshop. For quantification, a target line extending across the width of the image and perpendicular to the axis of the axons was overlaid on 6 to 12 images (each 300 µm × 300 µm), the number of neurofilament (NF)-positive axonal profiles (NF immunofluorescent signal >2 times background) that intersected the target line were counted, and the expression of Na,1.2, Na,1.6, and/or β-APP immunostaining was assessed. This approach was used in preference to expressing data per unit area because it reduces the possibility of duplicating quantification for a given axon as it moves in and out of the tissue plane (26).

**FIGURE 4.** Na,1.2, Na,1.6, sodium-calcium exchanger (NCX), and β-amyloid precursor protein (β-APP) immunolabeling in control spinal cord. (A, B) Neurofilament (NF)-positive axons (blue) do not exhibit detectable β-APP (green) Na,1.2 or Na,1.6 (red) immunolabeling in presumably myelinated regions, although limited β-APP and Na,1.2 immunoreactivity is observed in surrounding presumptive astrocytes. (C, D) NF-positive axons (blue) do not exhibit detectable NCX (green) Na,1.2 or Na,1.6 (red) immunolabeling; NCX and Na,1.2 labeling is observed in surrounding presumptive astrocytes. (A, inset) Parallel fibers of control cerebellar molecular layer (mol) display strong Na,1.2 (red) immunolabeling (gr, granule cell layer). (B, top inset) Node of Ranvier in control spinal cord exhibits robust Na,1.6 (red) immunolabeling; nodal Na,1.6 cluster is bounded by Caspr-positive (green) paranodal staining. (B, bottom inset) Nodes of Ranvier in normal-appearing white matter adjacent to the chronic lesion exhibit robust Na,1.6 (red) immunolabeling. (C, insets) In an acute MS lesion, a linear profile exhibits Na,1.2 (red), NCX (green), and β-APP (blue) immunolabeling. (D, insets) A linear profile in an acute MS lesion exhibits Na,1.6 (red), NCX (green), and β-APP (blue) immunostaining. Scale bars = 10 µm.
total of 1,643 NF + profiles were examined in 7 chronic plaques from subjects with MS for the expression of Na,1.2, Na,1.6, and/or β-APP (NF/β-APP/Na,1.2, 508; NF/β-APP/Na,1.6: 448, NF/Na,1.2, 312; NF/Na,1.6, 228; and NF/β-APP, 147), with 21 to 73 NF + axons counted per plaque for each of the labeling paradigms undertaken. Statistical analysis was performed using the Student t-test.

**RESULTS**

Previous studies have demonstrated a significant increase in the number of axons exhibiting β-APP accumulation in acute (active) MS plaques compared with control tissue (10, 12, 26, 37). The accumulation of β-APP is thought to result from failure of axonal transport and has been widely used as an indicator of axonal damage (9, 13, 38). To examine degeneration of axons within chronic (inactive) MS plaques (“slow burn”) and the possible association of Na,1.2 and Na,1.6 sodium channels to this degenerative process, we used axonal β-APP immunoreactivity as a marker of axonal damage.

Chronic MS lesions within the spinal cord were judged to be inactive if they exhibited a paucity of macrophages and perivascular cuffing together with lack of MBP immunoreactivity (Fig. 1A). The chronic plaques exhibited limited numbers of CD14-positive monocytes and CD68-positive monocytes/macrophages compared with acute lesions (Fig. 1B). Only regions within the cores of chronic plaques ≥200 μm away from normal-appearing white matter were analyzed in the present study, ensuring that axonal characteristics in the regions of the chronic lesion that were examined were not confounded by possible active edges in these plaques. Immunostaining for NFs (using a combination of antibodies to phosphorylated and nonphosphorylated neurofilaments, to assure staining of both) provided a definitive marker for axons. Examination of the chronic lesions within the spinal cord demonstrated that NF-positive axons displaying β-APP accumulation were present but rare (Fig. 2), consistent with previous

**FIGURE 5.** Expression of Na,1.6 and β-amyloid precursor protein (β-APP) within axons in a chronic multiple sclerosis (MS) lesion. (A) Na,1.6-positive (red) demyelinated axons (blue) that exhibit β-APP immunolabeling (green) are rarely observed in chronic lesions (arrow). The Merged signal for neurofilament (NF), Na,1.6, and β-APP is white. Scale bar = 10 μm. (B) The percentages of Na,1.6-positive axons (y axis) of all NF + axons and of all β-APP + axons are shown; there is no significant difference in the percentage of all NF + axons that express Na,1.6 compared with the percentage of all β-APP + axons that express Na,1.6. (C) The percentages of β-APP-positive axons (y axis) of all NF + axons and of all Na,1.6 + axons are shown; there is no significant difference in the percentage of all NF + axons that express β-APP + compared with the percentage of all Na,1.6 axons that express β-APP +.
descriptions in brain (10, 12). Of the 1,103 NF-positive axons examined in sections stained for Aβ-APP, only 25 (2.3%) displayed Aβ-APP immunostaining. Based on a 10-Km-section thickness, this suggests that there were 2.47 x 10^2 Aβ-APP-positive axons/mm^3 of tissue, significantly fewer that the 7,500 Aβ-APP-positive axons/mm^3 (26) and the 11,000 transected axons/mm^3 (11) reported in acute MS lesions.

To examine the expression of Na_v1.2 and Na_v1.6 sodium channels in axons within chronic lesions, the tissue was reacted with antibodies to neurofilaments and Na_v1.2 or Na_v1.6. As shown in Figure 3, Na_v1.2 immunoreactivity was not detected in axons within these chronic lesions. Of 810 NF-positive axons from 5 chronic plaques studied after immunostaining for Na_v1.2, none displayed detectable Na_v1.2 immunoreactivity. Notably, however, some GFAP-positive astrocyte processes forming the dense meshwork surrounding the axons exhibited Na_v1.2 staining (Fig. 3, insets), consistent with previous reports of Na_v1.2 expression in astrocytes (39), demonstrating that channel protein was present in the sections and was detectable using this antibody. Parallel fibers within the molecular layer of control human cerebellum also exhibited robust Na_v1.2 immunoreactivity (Fig. 4A, inset), in agreement with previous descriptions (40), providing another positive control.

In contrast with the absence of Na_v1.2 immunostaining in axons within the chronic lesions, Na_v1.6 immunolabeling was present along some axons (Fig. 3). The Na_v1.6 labeling of axons was not continuous along the lengths of the axons but was heterogeneous and patchy. The intensity of the Na_v1.6 signal varied in different axons within each section, with some axons exhibiting robust Na_v1.6 labeling, whereas in others the signal was barely detectable. From a population of 676 NF-positive axons in tissue processed for both neurofilament and Na_v1.6 immunostaining, approximately one-third (231 of 676) displayed Na_v1.6 immunolabeling. The patchy distribution of Na_v1.6 was not preferentially localized along axons of a particular diameter but was present along axons of a range of diameters from small to large.

To determine whether there was an association between Aβ-APP accumulation and expression of Na_v1.6 in axons or between colocalization of NCX and Na_v1.6 in axons within chronic lesions, we used triple-immunocytochemical detection for NF, Na_v1.6, and β-APP or NCX. In control tissue obtained after similar postmortem intervals from subjects without neurologic disease (Fig. 4B, upper inset) and in normal-appearing white matter adjacent to chronic lesions (Fig. 4B, lower inset), robust Na_v1.6 immunofluorescence was present at nodes of Ranvier along myelinated axons, indicating that Na_v1.6 antigenicity had been maintained and was detectable immunocytochemically. Na_v1.2 and Na_v1.6 were detectable and were colocalized with NCX in axons from acute MS lesions (Fig. 4C, inset and Fig. 4D, inset, respectively), as previously reported (26), providing positive controls for the NCX as well as Na_v1.2 and Na_v1.6 antibodies. In addition, Na_v1.2 immunolabeling was displayed in presumptive astrocyte processes within the control tissue, which also exhibited weak β-APP (Fig. 4A, B) and NCX labeling (Fig. 4C, D).

As in double-immunolabeling experiments with NF and β-APP, triple-labeling analysis of chronic lesions (Fig. 5) also demonstrated that β-APP-positive axons were rare (13 of 448; 2.9%). Of the axons in these samples, 95 of

FIGURE 6. Expression of the sodium-calcium exchanger (NCX) in a chronic lesion. Neurofilament (NF)-positive (blue) axons do not exhibit NCX immunolabeling (green). However, processes that form a meshwork and surround the demyelinated axons display NCX immunoreactivity. At increased magnification (insets), some NCX-positive processes display an intimate association with the demyelinated axons. Scale bar = 10 μm; left inset = 5 μm; right inset = 1 μm.

FIGURE 7. Astrocyte processes express the sodium-calcium exchanger (NCX) in chronic multiple sclerosis lesions. Immunostaining for NCX (green) and glial fibrillary acidic protein (GFAP) (red) illustrates that many of the GFAP-positive astrocyte processes that form a dense meshwork surrounding the axons express NCX (arrows). The merged signal for NCX and GFAP is yellow. Scale bar = 10 μm.
448 (21.2%) exhibited Na\textsubscript{v}1.6 staining, and only 2 of 95 (2.1%) of the Na\textsubscript{v}1.6-positive axons displayed A\textsubscript{-APP} immunostaining. Of the 13 A\textsubscript{-APP}-positive axons, only 2 (15.4%) exhibited Na\textsubscript{v}1.6 immunolabeling. Thus, there was not a significant difference in the percentage of all NF + axons versus the percentage of all A\textsubscript{-APP} + axons displaying Na\textsubscript{v}1.6 immunoreactivity (Fig. 5B), nor was there a significant difference in the percentage of all NF + axons versus the percentage of Na\textsubscript{v}1.6+ axons displaying A\textsubscript{-APP} immunolabeling (Fig. 5C).

A recent study examining acute MS plaques demonstrated that Na\textsubscript{v}1.6 tends to be colocalized with NCX in A\textsubscript{-APP}-positive axons (26). In the present study, our examination of chronic plaques from 5 subjects with MS failed to reveal any NF-positive axons with detectable NCX immunoreactivity (Fig. 6). Although NCX immunolabeling was not displayed by NF-positive axons, a meshwork of NCX-positive processes surrounding the axons was observed; many of these processes ran around or across the axons. Double-immunolabeling with GFAP and NCX antibodies demonstrated that many of these NCX-positive profiles were astrocyte processes (Fig. 7). In addition, intense NCX immunoreactivity, which was substantially greater than that exhibited by the scar astrocyte processes forming the dense meshwork, was observed along slender processes that extended parallel to the demyelinated axons for at least 100 \(\mu\)m within chronic lesions; colocalization experiments demonstrated that these processes, which have a morphology consistent with previous descriptions of reactive astrocytes in MS plaques (36), were GFAP-positive (Fig. 8). Moreover, the astrocytes with elongated processes, which were rarely encountered in the chronic lesions, exhibited Na\textsubscript{v}1.2 immunofluorescence (Fig. 8), consistent with prior reports of Na\textsubscript{v}1.2 in astrocytes (39), although Na\textsubscript{v}1.6 staining was not detectable within these cells (not shown).

**DISCUSSION**

Accumulating axonal loss has been identified as a major contributor to nonremitting neurologic deficits in MS (8, 33). Although it is known that acute MS lesions exhibit \(>10\)-fold more damaged axons than chronic plaques (10–12), the question of whether this is due to prior loss of axons in chronic lesions, so that fewer axons remain at risk to be injured or, alternatively, whether different mechanisms drive axonal degeneration in acute versus chronic lesions is not fully understood. Active lesions are characterized by T-cell and macrophage-dominated infiltrates, demyelination, and production of cytotoxic cytokines and reactive oxygen and nitrogen species (1, 7, 40, 41). In contrast, chronic lesions are relatively devoid of immune cells and myelin debris and are composed primarily of demyelinated axons within a dense meshwork of astrocyte processes (28). In this study we show that, unlike acute MS plaques (26), only one-third of the demyelinated axons within chronic lesions in spinal cord tissue obtained at autopsy from patients with MS displayed detectable Na\textsubscript{v}1.6 sodium channel immunolabeling, although adjacent normal-appearing white matter exhibited intense Na\textsubscript{v}1.6 immunoreactivity at nodes of Ranvier. In addition, there was no apparent association between the colocalization of Na\textsubscript{v}1.6 and \(\beta\)-APP, an early marker of axonal damage (9).
In chronic lesions within spinal cord, demyelinated axons were surrounded by a dense meshwork of GFAP-positive cell processes. These astrocytic processes exhibited NCX and Na\textsubscript{1.2} immunofluorescence. Na\textsubscript{1.2} immunoreactivity has been previously observed in spinal cord astrocytes in situ (39), and functional sodium channels are present in spinal cord astrocytes in vitro (42). Likewise, NCX expression in astrocytes in vitro (43, 44) and in vivo (45) has been reported. NCX can contribute to the regulation of intracellular calcium ([Ca\textsuperscript{2+}]) levels via forward (Ca\textsuperscript{2+} extrusion) or reverse (Ca\textsuperscript{2+} influx) operation (46). Although the functional significance of NCX and Na\textsubscript{1.2} in these astrocytes is not clear at this time, the presence of NCX in these cells implies that these molecules may be involved in [Ca\textsuperscript{2+}] flux into or out of glial cells within chronic lesions.

The substantially differing milieu surrounding demyelinated axons in acute versus chronic plaques raises the question of whether the pathogenesis of axonal degeneration is similar in acute and chronic lesions. Craner et al (25, 26) provided evidence implicating the expression of a specific sodium channel isoform, Na\textsubscript{1.6}, along demyelinated axons in acute MS lesions in the cascade leading to axonal degeneration. They reported the preferential coexpression of Na\textsubscript{1.6}, which is known to produce a persistent sodium current (27, 47), and NCX along axons accumulating β-APP within acute lesions, supporting the hypothesis that persistent sodium current produced by Na\textsubscript{1.6} can drive reverse operation of NCX, resulting in an influx of Ca\textsuperscript{2+} that promotes activation of calcium-dependent injurious enzymes within axons in these acute lesions (16). Coman et al (48) also reported diffuse sodium channel immunoreactivity along axons within demyelinated MS plaques, with some axons displaying broad sodium channel aggregates. In the present study, axons accumulating β-APP were observed, albeit rarely (~2.5% of all NF\textsuperscript{−} axons), in chronic lesions, but NCX immunoreactivity was not detected in these axons or in β-APP-negative axons. In addition, only 15.4% of the β-APP-positive axons exhibited detectable levels of Na\textsubscript{1.6}. We cannot rule out the possibility that the present results may underestimate the number of axons that express Na\textsubscript{1.6} or NCX or of injured axons (i.e. axons containing β-APP), because the densities of these molecules might have been below the threshold for immunocytochemical detection in some axons or because their antigenicity might have been lost owing to proteolysis in injured axons before death or postmortem (49). However, robust Na\textsubscript{1.6} immunolabeling was present at nodes of Ranvier within normal-appearing white matter adjacent to chronic lesions and along white matter axons obtained at similar postmortem intervals from patients without neurologic disease. We also cannot exclude the possibility that sodium channels along demyelinated axons might be more susceptible to proteolysis compared with sodium channels at nodes because of differences in cytoskeletal architecture or in the extracellular matrix (50). However, Na\textsubscript{1.2} and NCX were detectable in glial cells within these chronic lesions, and, although similar considerations about proteolysis and about the densities of Na\textsubscript{1.6} and NCX could apply to acute lesions, Na\textsubscript{1.6} and NCX were clearly present and were colocalized in a majority of β-APP-positive axons in acute lesions (26). The most parsimonious explanation of these observations is that the mechanisms underlying axonal degeneration in acute lesions differ from those in chronic plaques. In this regard, mitochondrial dysfunction leading to reduced ATP production has been suggested to contribute to the cascade of deleterious events culminating in axonal degeneration of chronically demyelinated axons in progressive MS (51). It is possible that energy failure may be exacerbated in chronic lesions or that in chronically demyelinated axons increases in [Ca\textsuperscript{2+}], from sources other than the sodium channel-NCX cascade may overwhelm homeostatic mechanisms. Irrespective of the underlying mechanisms, it is clear that there is a transition in the expression of Na\textsubscript{1.6} and NCX in demyelinated axons, from a high level in acute lesions to lower levels in chronic plaques. A similar loss of axonal Na/K ATPase has been reported in chronic MS lesions (52).

The present results raise the question of whether functional consequences invariably ensue as a result of axonal degeneration within chronic lesions in MS. It remains to be determined whether a functionally significant number of demyelinated axons within spinal cord chronic lesions are capable of impulse conduction. Sodium channel expression was detected in only about one-third of the axons in this study. Na\textsubscript{1.6} immunostaining was not continuously distributed along these demyelinated axons but was, in contrast, patchy. It has yet to be established whether the multifocal distribution of Na\textsubscript{1.6} channels along demyelinated axons in chronic spinal cord MS plaques or the density of sodium channels within these foci can support secure action potential conduction. If a subpopulation of demyelinated axons within these chronic lesions is incapable of secure impulse conduction, then degeneration of these axons would not be anticipated to add to clinical deficits. Alternatively, if some demyelinated axons within chronic lesions in the spinal cord have acquired the capacity to securely conduct impulses through demyelinated lesions, as appears to occur in the optic nerve where functional vision can be regained after optic neuritis even in the absence of remyelination (53), then loss of these axons would be expected to result in incremental clinical impairment. After demyelination, discrete foci of inward currents have been observed along some peripheral nervous system axons before remyelination, and it has been speculated that these “phi-nodes” may support discontinuous impulse conduction along these fibers (54). It is also known that a low density of sodium channels along some remyelinated axons, possibly as low as 2/μm\textsuperscript{2}, can support “microsaltatory” conduction in which the action potential propagates via sequential activation of channels that are spaced at intervals of nearly 1 μm along the axon (55). It is conceivable that conduction in chronically demyelinated axons could be supported by sodium channels other than Na\textsubscript{1.2} or Na\textsubscript{1.6}, although none of the other sodium channels expressed in the CNS (Na\textsubscript{1.1} and Na\textsubscript{1.3}) have been reported to be localized along axons. Irrespective of whether chronically demyelinated axons conduct or not, the present results, in conjunction with the observations of Craner et al (26), demonstrate that the expression of sodium channels in demyelinated axons within chronic versus acute...
MS plaques is substantially different and support the suggestion that mechanisms leading to axonal degeneration differ in these 2 types of lesions.

REFERENCES

46. Hryshko LV, Philipson KD. Sodium-calcium exchange: Recent advances. Basic Res Cardiol 1997;92(Suppl 1):45–51

Downloaded from http://jnen.oxfordjournals.org/ by guest on October 8, 2016