Increase of sodium channels in demyelinated lesions of multiple sclerosis

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Redistribution of sodium channels along demyelinated pathways in multiple sclerosis (MS) could be an important event in restoring conduction prior to other reparative mechanisms such as remyelination. Sodium channels in human multiple sclerosis lesions were identified by quantitative light microscopic autoradiography using tritiated saxitoxin (STX), a highly specific sodium channel ligand. Demyelinated areas in various central nervous system regions containing denuded but vital axons exhibited a high increase of STX-binding sites by up to a factor of 4 as compared to normal human white matter. This important finding could explain aspects of fast clinical remissions and 'silent' MS lesions on functional and morphological properties. Demyelinated axons may functionally reorganize their membranes and adapt properties similar to those of slow conducting unmyelinated nerve fibres which have a higher amount and a more diffuse distribution of STX binding sites. This report is the first description of an altered distribution of voltage-sensitive sodium channels in human multiple sclerosis lesions.

Multiple sclerosis (MS) is a relapsing chronic demyelinating disease of the central nervous system (CNS) which often leads to conduction block of affected myelinated nerve fiber tracts and consequently to paresis during attacks. It is a common observation that clinical remission from these attacks does not depend solely on facultative remyelination. Therefore, more subtle and faster structural adaptations might be responsible for regaining conductivity, a hypothesis that is contradictory in the literature²⁴. Conduction of nerve fibers is generated and propagated by activation of sodium channels during the rising phase of the action potential. In myelinated fibers these Na⁺ channels are located mainly in nodes of Ranvier enabling fast saltatory conduction. Conversely, in unmyelinated nerve fibers, the distribution of Na⁺ channels is more homogeneous along the axonal membrane, conduction is slower and continuous. Early morphological evidence of a rearrangement and continuous distribution of sodium channels in experimentally demyelinated axons came from Foster et al. using ferric ion-ferrocyanide (FeFCN) staining and electron microscopy⁹. However, the FeFCN binding mechanism to the sodium channel protein is not thoroughly understood and sodium channel redistribution has not been confirmed in a human demyelinating disease so far.

The neurotoxin saxitoxin (STX) has been widely used to analyze structure and function of Na⁺ channels¹²,¹³. STX specifically blocks the Na⁺ channel by binding at a site associated with the selectivity filter. In a previous study the distribution of Na⁺ channels has been characterized in rat and normal human brain structures¹⁸–²⁰. Generally high densities were found in neural layers of neocortex, hippocampal formation, cerebellum and substantia nigra. Other grey areas presented intermediate levels. Unaltered white matter was essentially devoid of STX binding sites. To evaluate a possible effect of demyelination on the appearance of detectable Na⁺ channels in plaques of multiple sclerosis, we used tritiated saxitoxin ([³H]STX) as a specific ligand in a quantitative autoradiographic investigation.

Postmortem tissue specimens from numerous brain and spinal cord regions containing demyelinated plaques and normal white matter were obtained at autopsy from 4 multiple sclerosis patients (aged 48, 66, 69 and 78 years). Postmortem delay varied between 20 and 48 h. Observed brain regions were optic tract, forebrain white matter and cerebellum as well as cervical, thoracic and lumbar spinal cord in longitudinal and horizontal sections. The tissue was quick-frozen in contact with blocks of dry ice, cut in 15-μm sections on a cryostat-microtome, mounted on chrome-gelatine coated glass slides and stored at −80 °C until use for autoradiography as previously described¹⁸–²⁰.

In equilibrium binding studies, the brain tissue sections
were incubated at 4 °C with 3.6 nM [3H]STX dissolved in 20 mM Tris-HCl buffer at pH 7.4 containing (in mM): N-methyl-glucosamine 140, KCl 5.4, CaCl₂ 2.8 and MgSO₄ 1.3. The non-specific binding component was determined in the presence of 1 μM unlabeled STX. After 60 min of incubation, tissue sections were rinsed twice for 5 sec in the same buffer and twice for 5 sec in distilled water. Then the slices were dried and exposed to tritium-sensitive film (³H Hyperfilm) with tritium labeled standards (Amersham) and exposed for 4 months. Autoradiograms were then analyzed and quantified using a computerized image-analysis system (Numelec). The...

Fig. 1. a–c: recent demyelinated lesion in periventricular forebrain white matter (bright zone), characteristically located around a small vein that shows little perivascular inflammation (a; right from center; Luxol stain; 15×). Corresponding strong [³H]STX-binding signal in adjacent section (b; autoradiograph; 15×) that leaves vein blank (right from center). Intact axons can be found in this signal enhancing lesion at higher magnification (c; arrow; silver impregnation; 185×). d–f: old scarred demyelinated lesion of the medulla oblongata. Ventral pyramidal tract (2 arrowheads) is affected (a; Luxol stain; 11×). Undetectable corresponding [³H]STX-binding signal (e; autoradiograph; 11×) due to severe astrocytic scar and lack of surviving axons at higher resolution from within the lesion (f; silver impregnation; 185×).
specific binding value was determined as the difference between the total binding for a given area and the non-specific binding. The latter was homogeneous and weak (5% of total binding) over all the examined regions. The mean value of specific binding in a given area was calculated from 6 to 8 measurements. Adjacent tissue, that has been fixed in 7% neutral formaldehyde, was sectioned and stained for myelin and axons for conventional light microscopy using standard Luxol dye/silver impregnation procedures. Additionally, original tissue sections used for \(^3\)H-autoradiography were stained the same way with good results after 4 months of exposure to tritium-sensitive film.

Binding experiments in MS human CNS tissue sections with \(^3\)H-STX, which is highly specific for the voltage-sensitive Na\(^+\) channel, have revealed high densities of binding sites in neocortical and spinal cord grey matter whereas in unchanged white matter \(^3\)H-STX binding sites were undetectable. This localization is in agreement with the STX binding site distribution in normal human brain and spinal cord\(^2\). Fig. 1a–c shows a plaque of complete demyelination in subcortical white matter demonstrated by a loss of Luxol myelin stain. A strong local increase of STX binding sites is found in the corresponding autoradiogram (Table I). Lesions of the optic nerve and of spinal white matter presented a lower, but still increased labeling (Table I and Figs. 2 and 3). These plaques revealed only partial demyelination.

An increase of \(^3\)H-STX binding site densities could correspond to an adaptive augmentation of detectable Na\(^+\) channels or only reflect a decrease of quenching. The presence of myelin lipids significantly quenches the autoradiographic signal by autoabsorption of tritium B emission\(^10\). Therefore, we corrected the measured densities of STX binding sites using published quenching coefficients for different brain regions\(^10\). The mean quenching coefficient corresponds to a 35.6% and a 110.7% increase of the measured density of sites in grey
TABLE I

Distribution of STX binding sites (fmol/mg grey matter) and quenching-corrected values in human multiple sclerosis lesions

S.E.M. = Standard error of the mean.

<table>
<thead>
<tr>
<th>Localization</th>
<th>Specific binding (fmol/mg grey matter)</th>
<th>Specific binding corrected for quenching</th>
<th>Density ratio demyelinated/myelinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forebrain grey matter (cortex)</td>
<td>139.5 ± 16.2</td>
<td>188.3</td>
<td>4.02</td>
</tr>
<tr>
<td>Forebrain white matter demyelinated plaque</td>
<td>7.4 ± 0.5</td>
<td>15.5</td>
<td>2.06</td>
</tr>
<tr>
<td>Spinal cord grey matter</td>
<td>25.8 ± 0.9</td>
<td>34.9</td>
<td>1.35</td>
</tr>
<tr>
<td>Spinal cord white matter demyelinated plaque</td>
<td>3.8 ± 0.6</td>
<td>8.0</td>
<td>2.06</td>
</tr>
<tr>
<td>Optic nerve white matter demyelinated plaque</td>
<td>12.2 ± 0.2</td>
<td>16.5</td>
<td>1.35</td>
</tr>
<tr>
<td>Optic nerve white matter demyelinated plaque</td>
<td>5.3 ± 0.9</td>
<td>7.2</td>
<td>1.35</td>
</tr>
</tbody>
</table>

matter and white matter regions respectively. Because demyelinated MS plaques lack myelin lipids, we used the correcting factor for grey matter.

The analysis of the corrected values (Table I) indicates a large to moderate increase of [3H]STX binding sites in demyelinated plaques of subcortical white matter (a factor of 4), optic nerve (a factor of 2) and spinal cord (a factor of 2). It should be stressed that the calculated values presented in Table I are minimum values, since, if demyelination was only partial, the quenching coefficient that was used was too low and corrected values would be higher. The difference between normal white matter and demyelinated plaques would then be even more pronounced.

The demonstrated increase of Na⁺ channels in demyelinated lesions could be of glial or axonal origin. Astrocytic proliferation is a common finding in MS plaques and astrocytes are able to generate sodium currents which are inhibited by tetrodotoxin. However, the maximal density of STX binding sites in rat astrocytes is 10 times lower compared to neuronal elements. In our study, the presence of intact axons inside demyelinated plaques seems to be required for a higher level of STX signal (Fig. 1b,c). Heavily scarred plaques with a complete absence of axons were devoid of detectable STX binding sites (Fig. 1e,f), favoring a neuronal origin.

The pattern of Na⁺ channel distribution changes drastically with myelination during ontogenesis of the rat brain. In premyelinated fiber tracts STX binding sites are present at an intermediate density but the specific binding signal becomes weaker and eventually undetectable as myelination proceeds. The process of demyelination seems to induce an adaptive reverse phenomenon leading to the reappearance of Na⁺ channels in white matter areas, comparable to premyelination stages. This interpretation would be in agreement with clinical electrophysiological data from MS patients, presenting complete conduction block in acute demyelinated pathways. During clinical remission after a few days — an interval too short for remyelination to occur — evoked potentials with slowed conduction velocities reappear, as could be nicely demonstrated in the optic system. Again, an increase of Na⁺ channel density along demyelinated segments might cause changes of conduction properties which then resemble properties of unmyelinated or premyelinated nerve fibers.

An increase of Na⁺ channel density could be the result of a de novo synthesis of the sodium channel protein or/and of changes of axonal transport or of a transfer from astrocytes. An adaptation of sodium channel density similar to that described in the present paper for multiple sclerosis plaques has been seen in demyelinated axons of the sciatic nerve in the med/med mouse and in...
experimentally demyelinated axons using doxorubicin to kill Schwann cells. These studies suggest that an upregulation of sodium channel protein in experimentally demyelinated axons can occur without glial participation and is basically of neuronal origin. Our data indirectly support this and contrast the conclusion reached in a recent paper by Ritchie et al. studying sodium channels in Schwann cells: we could not detect STX binding sites in axon-free astrocytic scars of MS patients. However, recent data from cultured type-1 astrocytes and from the rat optic nerve after experimental enucleation, indicate that astrocytes lose their ability to carry sodium current when deprived of axonal contact. These findings point to an interdependently regulated axo-glial sodium channel expression which could, at least in part, also be responsible for undetectable STX-binding sites in axon-free astrocytic scars of MS patients. The reverse is not true: it has not been shown so far, that neurons and their processes depend on astrocytes in expressing the sodium channel protein.

In a preliminary immunohistochemical experiment we used anti-neurofilament antibodies to show a significant increase of neurofilament components (triplet) in axons passing demyelinated areas (data not shown). Whether this increase reflects a general upregulation of neuronal protein synthesis or is a sign of a disturbed axonal transport leading to protein accumulation cannot be decided so far.

If demyelinated segments in nerve fibers could adopt conduction properties of unmyelinated nerve fibers by an increase and/or a redistribution of sodium channels along their axonal membrane, this would be an interesting example of neuroaxonal plasticity. Bostock and Sears demonstrated continuous conduction along short segments of axons after demyelination by diptheria toxin. Clinically, in certain types of chronic demyelinating peripheral neuropathies, such as the hereditary neuropathy with liability to pressure palsies, a slowed conduction velocity is generally well tolerated by affected family members. Often they are free of any symptoms. In analogy, it may be that clinically asymptomatic or 'silent' demyelinated MS lesions, as have been described in the spinal cord, do not result in apparent paresis because of a functional adaptation of axonal membranes without necessary remyelination. The latter mechanism might inherit a significant change of sodium ion channel distribution, as shown here. Our observation could also help to explain a reasonable visual function in conditions of complete demyelination, as described in the optic nerve.

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11 Ghatak, N.R., Hirano, A., Lijtmaer, H. and Zimmerman, H.M., Asymptomatic demyelinated plaque in the spinal cord,


