Up-regulation of slow K⁺ channels in peripheral motor axons: a transcriptional channelopathy in multiple sclerosis

Karl Ng, James Howells, John D. Pollard and David Burke

Institute of Clinical Neurosciences, Royal Prince Alfred Hospital and The University of Sydney, Sydney, NSW 2006, Australia
Correspondence to: Karl Ng, MRCP, FRACP, Office of Research & Development, Medical Foundation Building – K25, University of Sydney, NSW 2006, Australia.
E-mail: kng@med.usyd.edu.au

Spinal lesions produce plastic changes in motoneuron properties. We have documented the excitability of motor axons in the median nerve of 12 patients with multiple sclerosis and 50 normal subjects, hypothesizing that plastic changes in the properties of spinal motoneurons might be reflected in the properties of peripheral motor axons and be demonstrable in vivo. In the patients, there were changes in physiological measures of axonal excitability attributable to increased slow K⁺ channel activity. Other measures were within control limits. These changes could be modelled by an 11% increase in slow K⁺ current, with compensatory changes in membrane potential, suggesting increased expression of the responsible channels. The changes cannot be explained solely by changes in membrane potential and are not those expected if peripheral nerve axons were involved in the inflammatory process of multiple sclerosis. They probably represent a transcriptional channelopathy, due to up-regulation of channel expression. The abnormalities do not imply that peripheral nerve function has been significantly compromised, but they do suggest that the properties of the parent motoneurons have changed. This study thus provides evidence for plasticity in motoneuronal properties at a molecular level, the first such evidence for intact human subjects.

Keywords: slow K channel; multiple sclerosis; motoneuron; channelopathy; plasticity

Abbreviations: CMAP = compound muscle action potential; SRC = stimulus–response curve; TE = threshold electrotonus; CTR = current–threshold relationship; RC = recovery cycle

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Introduction

The properties of motoneurons may undergo plastic changes following lesions that alter inputs to the motoneuron or, in intact animals, as a result of activity. Following spinal lesions, the excitability of motoneurons increases (Button et al., 2008), and this is due, in part, to the development of persistent inward currents (PICs), which contribute to the development of spasticity (Li et al., 2004; Heckmann et al., 2005, 2008). In the intact organism, the biophysical properties of α-motoneurons adapt to changes in activity levels (Gardiner et al., 2006; Hultborn, 2006). However, in addition, plasticity of spinal reflexes can have both primary and secondary effects on motoneuron properties, due to changes in inputs, both normal or disturbed, whether the spinal cord is intact or lesioned (Hultborn and Malmsten, 1983; Wolpaw and Tennissen, 2001; Hultborn, 2006).

The excitability of motor axons is determined by ion channels and pumps that are ultimately derived from the parent motoneuron. It would be intuitively reasonable for there to be subtle changes in axonal excitability when there are changes in motoneuron properties. While some channels expressed on the motoneuron are not active on normal axons, it is a premise of this study that changes in axonal properties may still indicate that there have been changes at motoneuron level. Accordingly, studies of axonal properties have reported marked changes in the excitability of motor axons in spinal cord injured patients (Lin et al., 2007) and studies from this unit have found changes in the hyperpolarization-activated conductance (Iₜₒ) following a cortical stroke that interrupted corticospinal inputs to spinal motoneurons (Jankelowitz et al., 2007a).

In this study, we present evidence for an increased slow K⁺ current in motor axons in the median nerve of patients...
with stable chronic multiple sclerosis. The changes cannot be explained by a demyelinating process, and we suggest that they represent a transcriptional channelopathy (i.e. a change in the expression of a non-mutated channel gene; Waxman, 2001), resulting from adaptive (plastic) changes in spinal motoneurons due to interruption of spinal circuits that influence motoneuron excitability.

**Methods**

**Subjects**

We studied 12 patients (aged 40–60, mean 48 years; 10 females) with multiple sclerosis, defined using revised McDonald criteria (Polman et al., 2005). Multiple sclerosis was chosen over spinal cord injury as a model of diffuse spinal cord disease because of (i) greater likelihood of diffuse rather than relatively discrete spinal pathology; (ii) greater availability of suitable patients and (iii) the greater mobility of multiple sclerosis patients than of comparably disabled spinal injured patients. The patient data were compared with data for 50 healthy subjects (aged 30–67, mean 44 years; 20 females). The patients and control were matched for age but not gender. Axonal excitability varies with the former but not the latter (Kiernan et al., 2000; Jandelowitz et al., 2007b). The studies were approved by the University of Sydney Human Research Ethics Committee, and informed consent was obtained from participants.

Patients were assessed for the following: multiple sclerosis subtype at the time of testing, disease duration, motor symptoms or signs, Kurtzke disability scales (Kurtzke, 1983) and radiological evidence of demyelination affecting the cervical cord at or above the C8/T1 level. The patients had stable long-standing deficits (Table 1). None were prescribed Na⁺ channel blocking agents (such as phenytoin, carbamazepine, valproate, lamotrigine and mexilitane), or suffered any peripheral nerve disease. All underwent routine nerve conduction studies to detect peripheral nerve disease (such as polyneuropathy and carpal tunnel syndrome) and those with identified abnormalities, whether symptomatic or not, were excluded. Late responses were recorded for the thenar muscles: minimal F-wave latency, persistence and chronodispersion from 30 stimuli at rest, and the H-reflex of the thenar muscles during weak voluntary abduction (Burke et al., 1989). The neurophysiological findings are summarized in Table 2.

**Axonal excitability**

Motor axons in the median nerve were stimulated at the wrist, and the compound muscle action potential (CMAP) was recorded from the thenar muscles. Stimuli were delivered using a constant-current linear bipolar source (Digitimer DS5, Welwyn Garden City, Herts) and using QTTracS software (© Prof. H. Bostock, Institute of Neurology, London). The duration of test stimuli was 1 ms (except when strength–duration properties were measured). The test stimuli were adjusted by computer to produce a CMAP ~40% of maximum (Kiernan et al., 2000), on the rapidly rising phase of the stimulus–response curve (see arrow in Fig. 1A).

A computerized threshold tracking technique was used to measure multiple indices of axonal excitability for motor axons of the median nerve proximal to the wrist, as described in full elsewhere (Bostock et al., 1998; Kiernan et al., 2000; Burke et al., 2001; Lin et al., 2006). Threshold tracking follows changes in the intensity of a test stimulus necessary to produce a constant submaximal test potential. The size of this test CMAP was determined from the stimulus–response curve. The required current is known as the ‘threshold’ for the test potential. Threshold was then altered by changing the duration of the test stimulus (to produce strength–duration curves), by changing membrane potential using subthreshold currents of long duration [to measure threshold electrotonus and the current–threshold relationship], or by a supramaximal stimulus that activated all axons in the nerve [to produce a recovery cycle]. Changes in threshold produced by conditioning stimuli are expressed as a percentage of the threshold for the unconditioned test CMAP. The biophysical properties measured in the test protocol have been validated in experiments performed in vitro and in vivo on cat and rat axons and pharmacological studies on these preparations (Bostock et al., 1998; Burke et al., 2001; Schwarz et al., 2006). The measures are all voltage-dependent, and there are age-related differences in them but no significant gender-related differences (Jandelowitz et al., 2007b). Coherent changes in the measures can give information about changes in resting membrane potential.

(i) Stimulus–response curves plot the increase in size of the unconditioned test potential as stimulus intensity is increased. These curves were used to set the size of the test potential (~40% of the maximal CMAP) for the subsequent measures (see arrow in Fig. 1A). Demyelination is characterized by a decrease in the slope of the stimulus–response curve (Meulstee et al., 1997; Cappelen-Smith et al., 2001; Sung et al., 2004), and such a finding would be expected in multiple sclerosis patients if abnormal excitability was due to peripheral nerve demyelination at the site of testing.

(ii) Strength–duration time constant (τSD; Fig. 1B) was determined from strength–duration curves which quantify the decrease in current required to produce a target potential as the duration of the stimulus is increased. The threshold for the test potential was measured using unconditioned stimuli of 0.2, 0.5, 0.8 and 1.0 ms duration, and the τSD was calculated using Weiss’ formula (Weiss, 1901), which relates stimulus change to stimulus duration. τSD depends on the resistive–capacitative properties of the nodal membrane and on conductances active at rest, predominantly (persistent) Na⁺ currents (Bostock and Rothwell, 1997). The voltage dependence of τSD depends on these conductances.

(iii) Threshold electrotonus (Fig. 2) measures the change in excitability during and after injection of subthreshold depolarizing and hyperpolarizing currents that last 100 ms. The strength of the subthreshold currents was ±40% of the threshold for the test potential. The changes in excitability reflect the changes in the membrane potential produced by the subthreshold currents. With rectangular depolarizing currents there is a rapid decrease in threshold followed by a continued slow threshold decrease (‘S1’), which reaches a maximum at ~20 ms. Axons then begin to accommodate to the depolarization, largely due to activation of slow K⁺ currents (GKs), and there is a gradual lessening of the depolarizing threshold change (termed ‘S2’). On termination of the 100 ms depolarization, there is a rapid decrease in threshold and then a slow undershoot, largely due to the slow deactivation of GKs. Hyperpolarizing currents produce a decrease in excitability, limited by the Ith, (though this
limitation does not produce reversal of the threshold change with 100 ms conditioning current). On termination of the 100-ms hyperpolarization, there is a threshold overshoot largely due to deactivation of \( I_H \).

(iv) Current–threshold relationship (Fig. 3) is a threshold analogue of a current–voltage (I/V) plot, and quantifies the rectifying properties of axons. The change in threshold for the test potential was measured 200 ms after the onset of a 220-ms conditioning current, and the strength of the conditioning current was changed in 10% steps from +50% in the depolarizing direction to 100% in the hyperpolarizing direction. The slope of the relationship represents input conductance, and an increased slope indicates greater rectification: outward rectification due to \( K^+ \) currents with depolarizing changes in membrane potential, and inward rectification due to \( I_H \) with hyperpolarizing changes.

Threshold electrotonus and current–threshold relationship are the only techniques available to provide insight into internodal channel function in vivo, and in particular, the accommodation of axons to the polarizing currents due to inwardly and outwardly rectifying currents.

(v) Recovery cycle (Fig. 1C) measures the fluctuations in axonal excitability following a conditioning discharge, as axons pass through the refractory, supernormal and late subnormal.

| Table 1 Clinical and radiological features in multiple sclerosis patients, in order of increasing S2 accommodation |
|---|---|---|---|---|---|---|---|
| Patient | Age | Sex | Duration multiple sclerosis (years) | Subtype | EDSS | Sensation | Tone | Power 1 | Cervical spine MRI | S2 accommodation (% change) |
| 1 | 54 | f | 16 | rr | 5 | objective | normal | 4.5 | Normal | 22.96 |
| 2 | 46 | m | 24 | sp | 9 | subjective | decr.”” | 2 | Not performed | 23.36 |
| 3 | 40 | m | 12 | sp | 8 | subjective | incr. | 5 | Acrophy cervical cord | 23.62 |
| 4 | 40 | f | 5 | rr | 3.5 | objective | normal | 4.5 | Left hemicord C2; | 24.28 |
| 5 | 47 | f | 8 | pr | 8 | normal | incr. | 5 | C2 lesion | 27.22 |
| 6 | 52 | f | 9 | sp | 8 | normal | incr. | 4.5 | Multiple lesions Cl-6 | 27.25 |
| 7 | 51 | f | 14 | rr | 2.5 | normal | normal | 5 | Central lesion C3 | 28.82 |
| 8 | 60 | f | 18 | rr | 6.5 | subjective | incr. | 3 | Not performed | 29.26 |
| 9 | 43 | f | 11 | rr | 6 | subjective | normal | 4.5 | Posteroentral C3; | 29.47 |
| 10 | 40 | f | 8 | pp | 8 | normal | normal | 4.5 | Not performed | 29.62 |
| 11 | 53 | f | 22 | rr | 2 | normal | normal | 5 | Normal | 30.44 |
| 12 | 49 | f | 20 | rr | 3.5 | normal | normal | 5 | Posterior C2 | 31.82 |

EDSS = expanded disability status scale; rr = relapsing-remitting; sp = secondary progressive; pr = progressive relapsing; pp = primary progressive.

UK Medical Research Council scale, **baclofen pump, ’wheelchair bound.

| Table 2 Nerve conduction studies in order of increasing S2 accommodation |
|---|---|---|---|---|---|---|
| Patient | Excitability (S2 accomm) (%) | Sensory CV (m/s) | Median motor (m/s) | Thenar F-waves (ms) | Thenar H-reflex latency (ms) |
| | Median digit II-wrist | Median forearm | Ulnar digit V-wrist | DML | CV | min latency | chrono latency | persistence (%) |
| 1 | 22.96 | 53 | 56 | 50 | 3.6 | 55 | 273 | 4.4 | 100 | 30.2 |
| 2 | 23.36 | 60 | – | 53 | 3.9 | 50 | 25.5 | 5 | 97 | absent” |
| 3 | 23.62 | 55 | 60 | 49 | 4.1 | 53 | 25.1 | 5 | 100 | 33.1 |
| 4 | 24.28 | 61 | 61 | 50 | 3.8 | 56 | 23.1 | 3 | 100 | 24.6 |
| 5 | 27.22 | 57 | 58 | 51 | 3.5 | 52 | 24.3 | 2.1 | 100 | 276 |
| 6 | 27.25 | 57 | 60 | 51 | 3.1 | 50 | 25.5 | 2.1 | 100 | 25.5 |
| 7 | 28.82 | 62 | 74 | 67 | 3.9 | 52 | 25.6 | 3.5 | 100 | 275 |
| 8 | 29.26 | 61 | 63 | 44 | 3.5 | 65 | 24 | 2.9 | 100 | 239 |
| 9 | 29.47 | 59 | 62 | 50 | 3.4 | 56 | 26.6 | 4.9 | 100 | 277 |
| 10 | 2962 | 59 | 67 | 51 | 3.5 | 50 | 275 | 2.9 | 100 | 277 |
| 11 | 30.44 | 58 | 63 | 45 | 4 | 59 | 28.7 | 2.3 | 100 | 31 |
| 12 | 31.82 | 54 | 55 | 50 | 3.5 | 50 | 275 | 2.9 | 100 | 277 |
| Mean | 27.34 | 58 | 61.7 | 50.9 | 3.7 | 55.5 | 22.6 | 3.3 | 98 | 279 |

CV = conduction velocity; DML = distal motor latency.

“on baclofen pump; ** dependent on limb length; Limit = laboratory normal values.
periods. Threshold for the test potential was measured following a supramaximal conditioning stimulus at conditioning-test intervals of 2–200 ms. The CMAP produced by the conditioning stimulus was subtracted online from the response to the conditioning-test pair so that the test CMAP could be defined accurately.

Skin temperature was maintained throughout the experiments, at 33.2°C ± 0.3°C for the patients, and at 32.7°C ± 0.1°C for controls (mean ± SEM; P = 0.648).

Mathematical modelling

An established mathematical model of the human myelinated motor axon (see Appendix A in Jankelowitz et al., 2007a), as used in previous studies (Bostock et al., 1991; Kiernan et al., 2005; Bostock, 2006; Kanai et al., 2006; Jankelowitz et al., 2007a, b), was used to determine whether changes in the measures of axonal excitability could be attributed to changes in the maximal slow K+ current (or to some other conductance) or to myelin disruption (myelin thinning; paranodal demyelination). Using the computer program MEMFIT (© Professor H. Bostock, Institute of Neurology, London; Bostock, 2006), the effects of changes in different parameters were determined on the goodness-of-fit of the model to the observed excitability data. The modelling involved iterative changes in up to three parameters at a time to reduce the total error in the physiological measures: strength–duration parameters, TE, the CTR and the RC.

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Fig. 1 Excitability of median motor axons at the wrist. All data are mean ± SEM for patients (filled symbols, n = 12) and normal subjects (open symbols, n = 50). (A) Stimulus–response curve. The curves for patients and normal subjects are normalized to a stimulus that elicits 50% of the maximal CMAP response. There is no significant difference in the curves for any given CMAP amplitude. In particular, the slopes of the relationships are identical. The arrow indicates that the test CMAPs (~40% of maximum) fell on the steepest phase of the relevant stimulus–response curve. (B) Strength–duration time constant is virtually identical for the two groups. (C) Recovery cycle. Threshold changes following a supramaximal conditioning stimulus. There is no difference in the duration of the relative refractory period or the threshold increase during this period. In the patients, there is a significant decrease in the excitability change during the supernormal period (*) and a significant increase in the threshold change during the late subnormal period (**), and these findings are quantified in Table 3.

Fig. 2 Threshold electrotonus. (A) Changes in threshold of motor axons in the median nerve produced by 100 ms long depolarizing currents (upwards) and hyperpolarizing currents (downwards) in the patients (filled symbols) and normal subjects (open symbols). All data are mean ± SEM. The polarizing currents were ± 40% of the threshold for the test CMAP. The response to depolarization peaks ∼20 ms after current onset, and then returns towards baseline in the 'S2' accommodation. (B) The response to depolarizing current on an expanded Y-axis. The peak depolarization ('S1') is identical but, in the patients, the accommodation to depolarization is greater, and there is greater undershoot in threshold when the current is turned off. Note that the data were highly reproducible in both the patients and the normal subjects, and that error bars are, in most instances, smaller than the symbols.
The parameters of the model were first adjusted to fit the control data for healthy subjects, allowing the total Na⁺ (and the percentages of transient and persistent current; at the node), K⁺ (slow and fast; at the node and internode), I_H and leak conductances (at the node and internode) to vary. The membrane potentials at the node and internode were clamped together by the separate adjustment of the pump currents at the node and internode. The patient data were then modelled using the ‘control’ model as a template and allowing parameters to vary, without clamping membrane potential.

**Results**

**Characteristics of the study population**

The average disease duration from the onset of symptoms was 13.9 years (range 5–24). Seven patients had relapsing–remitting, three secondary progressive, one primary progressive and one progressive relapsing forms of multiple sclerosis. Six were wheelchair-dependent. The mean Kurtzke EDSS score (Kurtzke, 1983) was 5.8 (range 2–9). In the tested hand, two patients had no symptoms or signs. Eight had weakness of thumb abduction of grade 0–4.5 on the Medical Research Council scale. Eight had weakness of thumb abduction of grade 0–4.5 on the Medical Research Council scale. Three of those with normal power had increased tone in the tested limb and two had tendon jerk hyperreflexia (Table 1). In other words, 11 of 12 patients had motor abnormalities in the tested limb. On MRI, all patients had typical white matter lesions, often extensive, in the cerebral hemispheres. Nine patients underwent cervical MRI, and abnormalities were present in seven (Table 1). All but one was using or had used immunomodulatory therapy, and eight were being treated at the time of the study. These agents are not known to affect axonal excitability.

Sensory and motor nerve conduction studies were within laboratory normal limits (Table 2) with no evidence for a widespread axonal or demyelinating process or of median mononeuropathy [amplitude of the compound sensory action potential (CSAP) 18.8 ± 6.2 μV; amplitude of the CMAP 7.8 ± 0.6 mV (mean ± SD)]. In one patient, the thenar H-reflex could not be recorded, probably due to continuous intrathecal baclofen administration, but F-wave studies were within normal limits (minimal latency 25.5 ms; chronodispersion 5 ms; persistence 97%).

**Excitability changes**

**Resting membrane potential**

The stimulus–response curves were similar for the patients and controls (Fig. 1A). For eight of the 16 measures in Table 3, the mean patient data were well within the 95% confidence limits for the normal subjects (Figs 1–3). These measures were: (i) strength–duration time constant; (ii) the duration of the relative refractory period and (iii) the threshold increase during the relative refractory period in the recovery cycle; (iv) the maximal decrease in threshold produced by depolarizing current (‘S1’), and (v) the maximal threshold increase produced by hyperpolarizing currents and (vi) the threshold overshoot following the termination of hyperpolarization in threshold electrotonus; (vii) the maximal threshold increase to hyperpolarizing currents; (viii) its slope in the current–threshold relationship. These eight measures are very sensitive to membrane potential (Bostock et al., 1998; Kiernan and Bostock, 2000; SEM, standard error of the mean control data. The probabilities given in Table 3 were derived from these Z scores.

**Data analysis**

Excitability data are presented in Table 3 for the two populations as mean ± SEM. The mean data for the measures of excitability (n = 16; Table 3) were compared to the 95% confidence limits for the controls, and Z scores derived for each index using:

\[ Z = \frac{(X - \mu)}{\sigma/\sqrt{n}} = \frac{(X - \mu)}{SEM} \]

where, X is the average patient data; μ control population mean; \( \sigma \) standard deviation of control mean; n, number of controls and
Kiernan et al., 2000), and their normality suggests that the changes in other parameters cannot be attributed to a change in membrane potential.

**Physiological evidence for an increased slow K\(^+\) current**

For eight of the 16 measures in Table 3, the mean patient data fell outside the 95% confidence limits for the normal subjects. The eight measures include conventional measures of slow K\(^+\) conductances (Schwarz et al., 2006), and other less-specific measures that would change if there were a change in slow K\(^+\) conductances (see below).

These eight abnormal measures were:

- **Threshold electrotonus** (Fig. 2): (i) lesser threshold change at the end of the 100 ms depolarizing current, (ii) greater accommodation to depolarizing current (‘S2 accommodation’) and (iii) greater threshold undershoot when the 100 ms depolarizing current was terminated;
- **Current-threshold relationship** (Fig. 3): (iv) lesser maximal threshold change at 200 ms produced by 50% depolarizing currents, (v) increased resting slope of the relationship and (vi) increased slope of the relationship with depolarizing currents; and
- **Recovery cycle** (Fig. 1C): (vii) increased late subnormality and (viii) decreased supernormality.

Measures (i) and (ii), measures (iv) and (vi) and measures (vii) and (viii) would be expected to co-vary, but the eight significant differences still sample five independent processes. Importantly, the changes in all eight measures would be expected with an increase in a slowly activated outwardly rectifying conductance in the patients, i.e. an enhanced slow K\(^+\) conductance (G\(_{Ks}\)) (Bostock et al., 1998; Kiernan and Bostock, 2000; Kiernan et al., 2000; Schwarz et al., 2006). Importantly, there were significant changes in all parameters that could be affected by a change in G\(_{Ks}\), but not in other parameters, and this provides internal validation of the findings.

Three of the measures [accommodation to depolarizing currents (Fig. 2B), the threshold undershoot after the 100 ms current ends (Fig. 2B) and late subnormality (Fig. 1C)] are commonly used physiological indicators of slow K\(^+\) conductances in experiments on rat and human axons (Bostock et al., 1998; Lin et al., 2006). The other changes are less specific but are expected consequences of increased G\(_{Ks}\). The lesser threshold change to depolarizing currents in the current-threshold relationship in Fig. 3B and the greater slope of the relationship imply greater outward rectification, presumably due to greater K\(^+\) currents (though these could be fast or slow). An increase in resting slope of the current-threshold relationship

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**Table 3 Changes in multiple measures of axonal excitability**

<table>
<thead>
<tr>
<th>Excitability Measure</th>
<th>Patients N = 12</th>
<th>controls N = 50</th>
<th>95% confidence intervals</th>
<th>Z-score</th>
<th>P-value</th>
<th>(\Delta) in measures</th>
<th>(\Delta) expected with incr G(_{Ks})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strength-duration time constant ((\mu)s)</td>
<td>423 ± 31.9 418 ± 14.5</td>
<td>3896 ± 446.4</td>
<td>0.3448 ± 0.6331</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Relative refractory period (ms)</td>
<td>3.01 ± 0.3 3.07 ± 0.05</td>
<td>2.97 ± 3.17</td>
<td>-1.2000 ± 0.8849</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Refractoriness at 2 ms (%)</td>
<td>115.8 ± 42.2 1399 ± 31.6</td>
<td>78 ± 201.8</td>
<td>-0.7627 ± 0.7764</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Supernormality (%)</td>
<td>-22.8 ± 11.4 -24.8 ± 0.88</td>
<td>-26.5 ± 23</td>
<td>2.2727 &lt;0.02</td>
<td>Decr. Decr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late sub-normality (%)</td>
<td>16.6 ± 11.5 14.4 ± 0.66</td>
<td>13.1 ± 15.7</td>
<td>3.3333 &lt;0.0005</td>
<td>Incr. Incr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threshold electrotonus to ± 40% currents</td>
<td>696 ± 0.71 693 ± 0.75</td>
<td>678 ± 70.8</td>
<td>0.0400 ± 0.6554</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maximal threshold change to depolarizing current (‘S1’)</td>
<td>273 ± 0.89 23.9 ± 0.54</td>
<td>22.9 ± 25</td>
<td>6.2963 &lt;0.0001</td>
<td>Incr. Incr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow accommodation to depolarizing current (‘S2’)</td>
<td>42.3 ± 0.99 45.4 ± 0.69</td>
<td>44.1 ± 46.8</td>
<td>-4.4928 &lt;0.0001</td>
<td>Decr. Decr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depolarizing threshold undershoot (%)</td>
<td>-22.1 ± 0.7 -18.9 ± 0.6</td>
<td>-20.1 ± 17.7</td>
<td>-5.3333 &lt;0.0001</td>
<td>Incr. incr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperpolarizing threshold at 90–100 ms (%)</td>
<td>-120.4 ± 4.95 -124 ± 2.68</td>
<td>-129.25 ± 118.75</td>
<td>1.3433 0.9099</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hyperpolarizing threshold undershoot (%)</td>
<td>16.1 ± 1.25 15.8 ± 0.6</td>
<td>14.6 ± 17</td>
<td>0.5000 ± 0.6915</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The only patient data outside the 95% confidence intervals for the normal subjects (second last column [\(\Delta\)]) are those indices dependent on G\(_{Ks}\) (last column). S1 refers to the initial slow threshold change produced by polarizing currents and, with depolarization, this reaches a maximum 10–20 ms after the onset of the polarizing current. S2 = the accommodation to 40% current lasting 100 ms, measured from peak depolarization at 20 ms to the end of the current (Fig. 2). TE parameters are expressed as a threshold change. For I/V parameters, threshold was measured 200 ms after the onset of long polarizing currents (see legend of Fig. 3). The slope parameters reflect the input conductance at resting membrane potential, during depolarization (greater steepness implies greater outward rectification) and during hyperpolarization (greater steepness implies greater inward rectification).
implies a greater input conductance at resting membrane potential, as would occur with greater \( G_{K_s} \) (but would also occur with appropriate changes in other conductances active at rest). A decrease in the threshold change during supernormality would be expected if the after-hyperpolarization responsible for late subnormality was increased due to greater \( G_{K_s} \).

**Mathematical modelling**

The changes in the physiological measures of axonal excitability (threshold electrotonus, current–threshold relationship, recovery cycle and strength–duration properties) were reproduced using a computer program, MEMFIT (Bostock, 2006), which implements the same model of the human motor axon (see Appendix A in Jankelowitz et al., 2007a) as used in previous studies (Bostock et al., 1991; Kiernan et al., 2005; Kanai et al., 2006; Jankelowitz et al., 2007a, b). The differences between the parameters for the ‘control’ model and the patient recordings were best explained by an increase in \( G_{K_s} \) by 11% at the node and internode, accompanied by compensatory changes in pump activity. This reduced the discrepancy between the patient and control recordings for the full data set (strength–duration properties; threshold electrotonus, current–threshold relationship and recovery cycle) by 62.8%. For individual measures, there was no change for the strength–duration properties, and the error for TE to depolarizing currents was reduced by 61.6%, the error for the current–voltage relationship to depolarizing currents by 88.5% and the error for the recovery cycle by 72.1%. Importantly, changes in model properties that would occur with exposure of fast K\(^+\) channels (due to paranodal demyelination) or loss of myelin lamellae were unable to reproduce the excitability data in this study.

**Correlations with clinical features**

None of the above parameters was correlated with multiple sclerosis subtype, disease duration, disability scales, the presence of motor involvement clinically or the amplitude of the CMAP. Further, there was no correlation with radiologically evident lesions in the cervical spine (although MRI of the cervical spine was not available in three patients).

**Discussion**

The present findings provide physiological evidence for an enhanced slow K\(^+\) current in motor axons of peripheral nerves in patients with clinical and/or radiological evidence of spinal lesions due to multiple sclerosis. As discussed below, the explanation is likely to be up-regulation of slow K\(^+\) channels on large myelinated axons, presumably due to plastic changes in the properties of the spinal motoneuron. However, the possibility of a primary change in peripheral nerve properties due to an inflammatory attack against the myelin or an immune attack against ion channel proteins needs to be considered.

There have been numerous accounts of the peripheral effects of a central injury. At a clinical level, muscle wasting can be seen in some parietal lobe injuries and, neurophysiologically, spontaneous (‘denervation’) activity has been documented in the acute period following cerebral stroke and other central nervous system lesions (Johnson et al., 1975; Spaans and Wilts, 1982). There was no evidence for compressive neuropathy, polyneuropathy or nutritional deficiency in our patients, factors that have been invoked in other studies (see below). On the other hand, in animal experiments, adaptive responses affecting the expression of ion channels have been documented centrally one, two and even three synapses remote from the primary lesion (Dib-Hajj et al., 1999; Hains et al., 2004; Zhao et al., 2006).

Recently, stroke patients were found to have altered inward rectification in peripheral nerve axons using the techniques of this study (Jankelowitz et al., 2007a) and, in patients with severe spinal injury, motor axons become profoundly hypoexcitable (Lin et al., 2007). We address differences from these studies below, argue that a disease-specific process is unlikely, and suggest that the change in slow K\(^+\) current results from a motoneuron adaptation, perhaps compensation for increased motoneuron excitability.

**Disturbance of peripheral nerve myelin**

Ultrastuctural changes have been noted in peripheral nerve in multiple sclerosis. In the sural nerves of 10 patients with multiple sclerosis, Pollock and colleagues reported a reduction in thickness of the myelin sheath by 50% for the size of the axon and a shortening of internodal length (Pollock et al., 1977). Prior to this, Hasson and colleagues described slight to severe demyelination in 12 of 20 cases of multiple sclerosis, but attributed this to nutritional deficiency or pressure palsies (Hasson et al., 1958). Another study found peripheral nerve involvement in only three of 54 cases (Miglietta and Lowenthal, 1961). There have also been individual reports of multiple sclerosis coexisting with demyelinating peripheral neuropathy, sometimes with hypertrophic nerves and onion bulbs, or polyradiculitis (Schoene et al., 1977; Lassmann et al., 1981; Ro et al., 1983).

There have been four reports and one letter describing abnormalities of the recovery cycle in multiple sclerosis. Four were on cutaneous axons and one on median motor axons. Three of the five studies reported an increased duration of the relative refractory period (Hopf and Eyholzt, 1978; Antonini et al., 1995; Boërio et al., 2007), and three reported less supernormality (Eisen et al., 1982; Shefner et al., 1992; Boërio et al., 2007). The findings were interpreted as evidence for subtle peripheral nerve involvement in the disease process. However, the techniques used to measure the relative refractory period and the degree of supernormality are less reliable than threshold-tracking
because they have high variability (Bostock et al., 1998) and they provide data for only one physiological process. This renders interpretation difficult. Greater refractoriness and decreased supernormality could occur if axons were depolarized or if axons were cooler than normal, possibilities excluded in the present study. Overlapping processes contribute to the changes in excitability measured during the three phases of the recovery cycle (the relative refractory, supernormal and late subnormal periods). Greater after hyperpolarization due to an increase in $G_K$ could reduce supernormality in addition to increasing late subnormality, and indeed we evoke this mechanism to explain the decreased threshold change during the supernormal period in the present study. Similarly, a decrease in supernormality without change in its time course could produce mild prolongation of the relative refractory period. In human studies, individual measures of axonal excitability can be difficult to interpret, and conclusions will have greater validity when based on multiple measures.

All tests of axonal excitability measure axonal properties at the site of stimulation. Demyelination is characterized by a decrease in the slope of the stimulus–response curve (Meulstee et al., 1997; Cappelen-Smith et al., 2001; Sung et al., 2004), and one would anticipate a lower slope of the curve in multiple sclerosis patients if there was peripheral nerve demyelination at the site of testing. The absence of such a change supports the results of the mathematical modelling that the findings seen in the present study cannot be explained by peripheral nerve involvement in the disease process.

**Immune attack on ion channel proteins**

It is conceivable that there may have been a direct antibody-mediated immune attack against voltage-gated Na⁺ or K⁺ channels in multiple sclerosis (Waxman, 1993), but it is unlikely that interference with channel function in this way would enhance channel function. In acquired neuromyotonia, peripheral nerve hyperexcitability is associated with antibodies directed against fast K⁺ channels (Hart et al., 2002). This is intellectually satisfying because blocking K⁺ channels could increase axonal excitability. However, with myelinated motor axons, blocking fast K⁺ channels using 4-aminopyridine (Judge and Bever, 2006) does not produce ectopic motor discharges. It widens the action potential of motor axons, and generates ectopic activity only with sensory axons (Kocsis et al., 1983, 1986). No abnormality of axonal excitability has been found in neuromyotonia using threshold tracking techniques (Kiernan et al., 2001). While it would be logical for a reduced channel current to contribute to the axonal hyperexcitability responsible for neuromyotonic activity, the site and mechanism of the ectopic motor activity in that disorder are still not fully explained.

Recently Mathey et al. (2007) have demonstrated that antibodies against neurofascin-155/186, which is located in the paranodal region of central and peripheral axons, can produce conduction block in experimental allergic encephalomyelitis. It is possible that these antibodies play an important pathogenic role in multiple sclerosis and, if so, the peripheral axon would not be immune. However, while such an action could ‘reveal’ fast K⁺ currents, it is difficult to see how it would boost nodal slow K⁺ currents.

**Plastic changes in motoneuron properties**

Waxman raised the possibility that, in multiple sclerosis, subtle peripheral nerve abnormalities could be an indirect result of central pathology, rather than a direct antibody-mediated action on ion channels (Waxman, 1993). We suggest that the peripheral nerve abnormalities were the result of central pathology, at least one synapse rostral to the motoneuron and its axon, and that the enhanced $G_K$ results from a change in expression of a non-mutated channel, i.e. a ‘transcriptional’ channelopathy (Waxman, 2001).

The adaptive neuronal changes to injury may be associated with changes in the expression of ion channels on neurons remote from the primary lesion. For example, peripheral nerve lesions produce changes in neuronal Na⁺ currents, primarily $Na\textsubscript{v}1.3$, in dorsal root ganglion and higher-order projections in rats, a number of synapses remote from the peripheral nerve lesion (Dib-Hajj et al., 1999; Hains et al., 2004; Zhao et al., 2006). Suprasegmental spinal lesions can lead to enhanced PICs in motoneurons (Li et al., 2004; Heckmann et al., 2005, 2008; Button et al., 2008), and are likely to produce changes in other conductances as well, thus opening up the potential for a change in the properties of motor axons. Our data suggesting increased $G_K$ are consistent with a change in motoneuron properties manifested by increased expression of KCNQ2/3 ($K\textsubscript{7.2/7.3}$) channels, the isoforms expressed on mammalian myelinated axons (Schwarz et al., 2006).

These downstream effects differ from changes seen in peripheral motor axons in stroke patients (Janelkowitz et al., 2007a), presumably because multiple sclerosis is associated with a more complex disturbance to the descending and segmental inputs to the motoneuron than occurs following the withdrawal of corticospinal inputs. In addition, a stroke produces an acute deficit, maximal at onset, often with ‘shock’, whereas in multiple sclerosis the lesions may initially be subclinical, the deficits are cumulative and there is no ‘shock’. The spinal disturbance in multiple sclerosis is less devastating than in spinal injury, where spinal shock may be severe, producing prolonged hyperexcitability of the motoneuron pool. It is therefore not surprising that the present findings differ from those reported by Lin et al. (2007) in 15 spinal cord injured patients. They found increased axonal thresholds, diminished CMAP amplitudes, reduced slope of the
stimulus–response curve, reduced strength–duration time constant and ‘fanning in’ of threshold electrotonus, and they concluded that multiple mechanisms were in play, including loss of motoneurons. The patients had deficits of variable severity (six clinically complete or near complete), at various levels (seven thoracic), at various times after the injury (from 1 month to 10 years). It is possible that the loss of motoneurons was due to profound hypo-excitability and/or segmental involvement. These findings contrast with the more subtle changes in axonal excitability seen in both stroke, where there is presumably more selective withdrawal of corticospinal drives, and multiple sclerosis, where the deficit is less acute and devastating. The differences in the axonal changes presumably reflect differences in the ability of the motoneuron pool to respond to different insults, and this implies that the adaptive response can vary with the nature of the insult.

The question then arises whether the change in $G_{Ks}$ is a primary event or secondary to other changes in the motoneuron, and whether the present findings are functionally important. We favour the view that the increased activity of $G_{Ks}$ represents an adaptation to offset the enhanced motoneuron excitability that would occur with greater PICs. This would be functionally important because it would help control the excitability of the quiescent motoneurons, without depressing their enhanced reactivity to afferent inputs, typical of spasticity. With regard to axonal function, the increase in $G_{Ks}$ was offset by compensatory changes in membrane potential, so that it is unlikely that impulse conduction in the axon would be jeopardized. The scientific importance of the present findings lies in that (i) they are further evidence of plasticity occurring at a molecular level, demonstrable in vivo; (ii) they provide some insight into the motoneuron plasticity that underlies the motor unit adaptations to spinal injury; and (iii) they provide a model for changes that presumably occur elsewhere within central pathways when there is a cerebral lesion. The clinical relevance lies in the fact that the different changes seen in multiple sclerosis, stroke and spinal cord injury confirm that the adaptive responses of neurons to different central nervous system (CNS) lesions can vary, i.e. that neuronal plasticity truly represents adaptation to the nature of the lesion.

We conclude that the spinal lesions of multiple sclerosis resulted in plastic changes in motoneurons remote from the primary pathology, and that the changes seen in the motor axon represent an adaptive response of the motoneuron pool designed to maintain motoneuron stability by offsetting increases in its excitability. This report presents evidence for human subjects in vivo for remote biophysical changes, and confirms that the excitability of downstream axons may be altered by remote lesions in the CNS. The limited repertoire of ion channels at the node of Ranvier of large myelinated axons can explain the restriction of the changes to a single ion channel.

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