Evidence for axonal membrane hyperpolarization in multifocal motor neuropathy with conduction block

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Summary
Multiple nerve excitability measurements were used to investigate axonal membrane properties of patients diagnosed with multifocal motor neuropathy (MMN). Six patients were selected, all with evidence of distal focal motor conduction block involving the median nerve in the forearm. In all patients, the median nerve was stimulated at the wrist, just distal to the site of block, and the resulting compound muscle action potentials were recorded from abductor pollicis brevis. Stimulus–response behaviour, the strength–duration time constant, threshold electrotonus to 100 ms polarizing currents, a current–threshold relationship and the recovery of excitability following supramaximal activation were recorded using a protocol described recently. When compared with control values, patients demonstrated significantly greater superexcitability, a ‘fanning out’ of threshold electrotonus recordings, and a significant change in the slope of the current–threshold relationship. These abnormalities in axonal membrane excitability parameters closely resembled those in normal axons hyperpolarized following release from ischaemia. To test for axonal hyperpolarization, DC depolarizing currents were applied to the nerves of three patients, and all the excitability parameters were normalized by depolarization. Attempts to trace excitability measures proximally towards the site of block were unsuccessful, as the nerve became inexcitable in all cases. It is suggested that the distal hyperpolarization is probably linked to focal depolarization and that the clinical features of MMN are consistent with a depolarizing/hyperpolarizing lesion.

Keywords: multifocal motor neuropathy; axonal membrane; nerve excitability

Abbreviations: APB = abductor pollicis brevis; CMAP = compound muscle action potential; I/V = current–voltage; MMN = multifocal motor neuropathy; RRP = relative refractory period; TEd = depolarizing threshold electrotonus; TEh = hyperpolarizing threshold electrotonus

Introduction
Multifocal motor neuropathy (MMN) is a rare clinical entity, characterized by almost exclusive motor nerve involvement and producing a syndrome in affected patients of slowly progressive muscle atrophy and weakness (Roth et al., 1986; Parry and Clarke, 1988; Pestronk et al., 1988, 1990; Bouche et al., 1995; Taylor et al., 2000). A critical diagnostic feature is the demonstration of conduction block in multiple peripheral nerves on electrophysiological investigation (Sumner, 1997). Conduction block involves solely motor axons, with sensory conduction spared across the lesion (Kaji et al., 1993; Parry, 1997).

The presence of ‘positive’ symptoms and signs, such as cramp, myokymia and fasciculation, in the context of predominantly ‘negative’ features, such as depressed tendon jerks, muscle atrophy and weakness, remains unexplained in these patients. Nor has the mechanism of the conduction block itself been elucidated. Limited information is available from pathological studies, some of which provide evidence
favouring demyelination (Kaji et al., 1993; Parry, 1997). In an attempt to explore the basis of weakness and fatigue in patients with MMN, a recent study demonstrated that conduction block was exacerbated by activity-dependent hyperpolarization (Kaji et al., 2000).

New methods designed to measure axonal membrane excitability have recently been incorporated into the diagnostic assessment of patients with peripheral nerve disease (Kiernan and Bostock, 2000; Kiernan et al., 2000, 2001a, b). These techniques enable axonal membrane potential and ion channel function to be explored in vivo (Bostock et al., 1998). The present study was undertaken specifically to examine whether these new methods could provide further insight into the basis of conduction block in patients with MMN. Evidence is presented to indicate that the axonal membrane just distal to the site of conduction block is abnormal in patients with MMN. Measurements of excitability in these patients suggest that the axonal membrane is hyperpolarized at this site, behaving similarly to a post-ischaemic nerve. Further evidence for membrane hyperpolarization is provided by the normalization of excitability properties with the application of depolarizing currents.

**Methods**

Recordings were made on six patients with the clinical features and electrodiagnostic findings of MMN (aged 37–49 years; four male, two female). All six patients had evidence of focal conduction block of the median nerve in the forearm region (Table 1). All patients gave informed consent and the study was approved by the Joint Research Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology, London; for the patient studied in Japan, approval was granted by the Institutional Review Board of Kyoto University School of Medicine.

Studies were performed using a recently described protocol designed to measure a number of different nerve excitability parameters rapidly (Kiernan et al., 2000). Compound muscle action potentials (CMAPs) were recorded from thenar muscles using surface electrodes over the abductor pollicis brevis (APB), with the active electrode at the motor point and the reference on the proximal phalanx. The EMG signal was amplified (gain 500, bandwidth 1.6 Hz to 2 kHz) and digitized by computer (486 PC) with an A/D (anologue-to-digital) board (DT2812; Data Translation, Marlboro, Mass., USA) using a sampling rate of 10 kHz. Stimulus waveforms generated by the computer were converted to current with a purpose-built isolated linear bipolar constant current stimulator (maximum output (±50 mA). The stimulus currents were applied via non-polarizable electrodes (Red Dot; 3M Health Care, Borken, Germany), with the active electrode over the median nerve at the wrist and the reference electrode ~10 cm proximal over the muscle. Stimulation and recording were controlled by QTRAC software (version 5.2; copyright Institute of Neurology, London, with multiple excitability protocol TRONDXM).

Test current pulses of 0.2 or 1 ms were applied at 0.8 s intervals and combined with suprathreshold conditioning stimuli or subthreshold polarizing currents as required. The amplitude of the CMAP was measured from baseline to negative peak. Skin temperature was monitored close to the stimulation site.

The sequence of recordings followed that described previously (Kiernan et al., 2000). Stimulus–response curves were recorded separately for test stimuli of durations 0.2 and 1 ms (Fig. 1A). The stimuli were increased in 6% steps, with two responses averaged for each step, until three averages were considered maximal. The ratio between the 0.2 and 1 ms stimuli required to evoke the same response was used to estimate rheobase and the strength–duration time constant of axons of different threshold (Fig. 1D). A target response was then set at the steepest point on the stimulus–response curve between 30 and 50% of the maximum CMAP, and the 1.0 ms test stimuli was adjusted automatically by the computer to maintain this peak CMAP amplitude. For most recordings, ‘proportional tracking’ was used, whereby the change in stimulus amplitude from one trial to the next was made proportional to the ‘error’, i.e. the difference between the last response and the target response (Kiernan et al., 2000). The

**Table 1 Motor nerve conduction studies demonstrate focal conduction block of the median nerve in the forearm region for each of the six MMN patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years), gender</th>
<th>Sensory conduction</th>
<th>Median CMAP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wrist Left</td>
</tr>
<tr>
<td>2</td>
<td>47, F</td>
<td>Normal</td>
<td>4.8</td>
</tr>
<tr>
<td>1</td>
<td>44, M</td>
<td>Normal</td>
<td>3.6</td>
</tr>
<tr>
<td>3</td>
<td>37, M</td>
<td>Normal</td>
<td>7.5</td>
</tr>
<tr>
<td>4</td>
<td>45, M</td>
<td>Normal</td>
<td>9.5</td>
</tr>
<tr>
<td>5</td>
<td>51, F</td>
<td>Normal</td>
<td>3.7</td>
</tr>
<tr>
<td>6</td>
<td>49, M</td>
<td>Normal</td>
<td>6.7</td>
</tr>
</tbody>
</table>
Fig. 1 Six plots of the excitability data recorded in Patient 1 (continuous lines and circles) superimposed on the 95% confidence limits for 29 normal subjects (broken lines in B–F). The recordings from Patient 1 were made separately 10 weeks apart. (A) Absolute stimulus–response relationships for the test stimuli of duration 0.2 and 1.0 ms. The circle on the 1.0 ms response curve corresponds to the threshold for a CMAP 50% of maximum and the broken ellipse corresponds to the 95% confidence limits for this point. (B) Normalized stimulus–response relationships. (C) Current–threshold relationship reflecting the rectifying properties of the axon (both nodal and internodal axolemma). For each level of conditioning current, from +50% to −100% of the control threshold, the threshold change was measured. The plot is orientated such that depolarization occurs to the right and hyperpolarization to the left. (D) Distribution of strength–duration time constants. Nine populations, starting from axons contributing to the responses 5–15% of maximal, then 15–25% and further increasing in 10% steps to the maximum of 95%, are plotted against their corresponding CMAP responses. (E) Threshold electrotonus (continuous lines and circles representing ±40% of the resting threshold). The changes in threshold are plotted as threshold reductions with responses to depolarizing currents upwards, as is normal for electrotonus. (F) Recovery cycle, showing relative refractory period, superexcitability and late subexcitability.
slope of the stimulus–response curve was used to set the constant of proportionality and optimize the tracking efficiency. In some patients, however, only a few units were conducting, so that proportional tracking was inappropriate. The program detected this from the steepness of the stimulus–response curve and switched to fixed-step tracking, in which the stimulus was altered in 2% steps.

Prolonged subthreshold currents were used to alter the potential difference across the internodal and nodal axonal membranes. The changes in threshold associated with these electrotonic changes in membrane potential normally have a similar time course and are known as threshold electrotonus (Bostock et al., 1998). In the present protocol, test stimuli of 1 ms duration were used to produce the target CMAP (40% of maximal). Threshold tracking was used to record the changes in threshold induced by 100 ms polarizing currents, set to 40% (depolarizing) and −40% (hyperpolarizing) of the control threshold current. The three stimulus combinations were tested in turn: test stimulus alone (to measure the control threshold current), test stimulus + depolarizing conditioning current, and test stimulus + hyperpolarizing conditioning current. Threshold was tested at 26 time points (maximum separation 10 ms) before, during and after the 100 ms conditioning currents (Fig. 1E). Each stimulus combination was repeated until three valid threshold estimates were recorded, as judged by the response being within 15% of the target response or alternate responses being either side of the target.

The current–threshold relationship (Fig. 1C) was tested with 1 ms pulses at the end of 200 ms polarizing currents, which were altered in a ramp fashion from +50% (depolarizing) to −100% (hyperpolarizing) of the control threshold in 10% steps. As with the conventional threshold electrotonus protocol, stimuli with conditioning currents were alternated with test stimuli alone, and each stimulus combination was repeated until three valid threshold estimates had been obtained.

The following data values were abstracted from each recording for numerical analysis. Latency (ms) was measured from the onset of the 1 ms stimulus to peak amplitude, averaged over the whole recording period. Peak CMAP (mV) was the average of the three largest responses on each stimulus–response curve. Threshold (mA) was the 1 ms stimulus required to elicit a CMAP that was 50% of maximal. The strength–duration time constant (ms) was calculated, as described above, for a 40% maximal CMAP, while rheobase (mA) was the corresponding rheobase. The stimulus–response slope was estimated from the normalized stimulus–response relationship (Fig. 1B), as the stimulus evoking a 75% maximal response minus that evoking a 25% maximal response, divided by that evoking a 50% maximal response. The resting I/V slope was the slope of the current/threshold relationship in Fig. 1C, calculated from the polarizing currents between −10% and +10% of resting threshold, while the minimal I/V slope was calculated by fitting a straight line to each three adjacent points in turn. RRP (ms), the relative refractory period, was calculated from the recovery cycle data in Fig. 1F as the first intercept on the x axis. Superexcitability (%) was measured as the minimum mean of three adjacent points and late subexcitability (%) as the maximum mean of three adjacent points after 10 ms. The remaining four excitability parameters were derived from the threshold electrotonus data in Fig. 1E. TEh (10–20 ms) and TEd (90–100 ms) were the mean threshold reductions between the specified latencies after depolarizing current onset, and TEh (10–20 ms) and TEd (90–100 ms) were the corresponding responses to hyperpolarizing current. Mean values of these parameters in the six patients were compared with mean values in 29 normal control subjects (Kiernan et al., 2000) using two-tailed Student’s t-tests. The subjects providing the two sets of recordings did not differ signifi-
cantly in skin temperature [controls, 32.3 ± 0.2; MMN, 32.7 ± 0.3; mean ± SEM (standard error of the mean)], age (controls, 39.4 ± 1.9 years; MMN, 45.3 ± 1.9 years) or sex ratio (controls, 21 male, eight female; MMN, four male, two female).

Case histories
Patient 1
Patient 1 was a 45-year old male who initially presented 6 years prior to the present study with left wrist weakness and fasciculation and cramp involving the right forearm. Examination demonstrated weakness in elbow extension bilaterally, finger extension and thumb abduction on the right (MRC grade 3–4/5). Reflexes in the upper limbs were reduced. Electrophysiological examination demonstrated motor conduction block in the right median nerve in the forearm (Table 1). Weakness improved with a 3-monthly regimen of intravenous immunoglobulin therapy after earlier trials of steroids (inconsistent response) and azathioprine (pancytopenia) had been stopped.

Patient 2
This patient was a 47-year-old female with a 9-year history of patchy upper limb weakness bilaterally. Examination revealed wasting of the left shoulder muscles, particularly the deltoid, hypothenar and thenar eminences bilaterally. Widespread fasciculations were present in the left upper limb. Generalized weakness was present in the upper limbs (MRC grade 1–4/5) without a flicker of contraction in right abductor pollicis brevis. Right biceps tendon reflex was present with reinforcement, while the remaining upper limb reflexes were absent. Lower limb examination was normal. Electrophysiological examination demonstrated motor conduction block in the median nerve in both forearms and in the ulnar nerve in the left forearm. Weakness improved with regular intravenous immunoglobulin therapy.

Patient 3
Patient 3 was a 37-year-old male who presented initially with left leg weakness, which later became bilateral and involved both upper limbs. Examination revealed no wasting, but evidence of left upper limb weakness that was most marked distally and involved APB (MRC grade 4/5) and generalized bilateral lower limb weakness that was more marked on the left. Upper limb reflexes were normal and symmetrical. Lower limb reflexes were absent bilaterally. Electrophysiology demonstrated motor conduction block in the median and ulnar nerves in the right forearm and the distal motor response from the left common peroneal was markedly attenuated. Previous therapy with corticosteroids and cyclosporin had shown no benefit. Six days after initial therapy with intravenous gammaglobulin, the patient noted marked improvement in strength. This therapy was continued at intervals of 3–6 months.

Patient 4
This patient, a 45-year-old male, had a 9-year history of weakness that began in the right arm and spread over 2 years to involve the left arm and both lower limbs. He described twitching movements of all four limbs. On examination there was mild wasting of the muscles of the right hand, particularly APB. There was generalized upper limb weakness, the right limb being more affected than the left, and weakness was most marked distally (MRC grade 4/5). There was no wasting or weakness of the lower limbs. Upper limb reflexes were present with reinforcement. Lower-limb reflexes were normal and symmetrical. Electrophysiology demonstrated conduction block in the left median nerve in the forearm, the right median nerve at Erb’s point and in the right and left ulnar nerves at the axilla.

Patient 5
This 51-year-old woman presented with a 20-year history of asymmetrical upper limb weakness, initially involving the left arm and spreading 5 years later to involve the right arm. She reported cramps chiefly in the right arm and fasciculations occurring bilaterally. Examination revealed right biceps hypertrophy with distal wasting of both upper limbs (MRC grade 4/5). Upper limb reflexes were absent. Electrophysiological examination demonstrated persistent motor conduction block in the median nerve in both forearms. Initial treatment consisted of corticosteroids, which gave minimal benefit, and she was subsequently treated with a course of intravenous immunoglobulin.

Patient 6
Patient 6 was a 49-year-old man who had been diagnosed as having MMN in 1991. Weakness was found in the territories of both median and both peroneal nerves and the left ulnar nerve, with no sensory deficits. The lesion in the left median nerve was localized to the distal forearm, sparing the anterior interosseous nerve. MRI showed focal nerve enlargement from 3 to 11 cm proximal to the wrist crease. Motor conduction studies revealed complete conduction block at a point between 4 and 6 cm proximal to the crease, with normal sensory conduction along the entire nerve.

In the following 4 years, he had received oral cyclophosphamide (50–100 mg/day for 10 months in total) and then three courses of intravenous immunoglobulin (400 mg/kg/day for 5 days per course). These treatments significantly improved the weakness of the left APB from 0/5 to 4+/5 on the MRC scale. The degree of conduction block also improved greatly (proximal distal ratio; from 0 to 0.3). On examination in December, 2000, there were occasional fasciculations and myokymia in the left thenar eminence.
Manual muscle testing revealed mild weakness in the left APB (MRC 4+/5). Detailed histories and clinical findings for this patient have been reported previously (Kaji et al., 1993, 2000)

Results
The full sequence of excitability measurements, as described in Methods, was recorded in each patient and the data from a patient (Patient 1) are plotted in a standard format in Fig. 1,
superimposed on the means and 95% confidence limits for normal control subjects (Kiernan et al., 2000). Two recordings from this patient, made 10 weeks apart, are superimposed and they show very similar deviations from the normal range. The six standard plots shown in Fig. 1 will be described in turn.

Stimulus–response curves for the test stimuli of duration 0.2 and 1 ms are plotted on log–log coordinates in Fig. 1A. The filled circle on the 1 ms response curve corresponds to the threshold for a CMAP 50% of maximum, and the broken ellipse corresponds to the 95% confidence limits for this point. Although CMAP amplitude falls just within the normal range, the normalized stimulus–response curve is abnormally steep (Fig. 1B) because the majority of the CMAP was generated by a single motor unit in this patient.

Figure 1C shows a plot of the normalized threshold changes at the end of 200 ms current pulses. For each level of conditioning current, from +50% to −100% of the control threshold, the threshold change was measured and the percentage threshold reduction is plotted and they show very similar deviations from the normal range. The six standard plots shown in Fig. 1 will be described in turn.

Stimulus–response curves for the test stimuli of duration 0.2 and 1 ms are plotted on log–log coordinates in Fig. 1A. The filled circle on the 1 ms response curve corresponds to the threshold for a CMAP 50% of maximum, and the broken ellipse corresponds to the 95% confidence limits for this point. Although CMAP amplitude falls just within the normal range, the normalized stimulus–response curve is abnormally steep (Fig. 1B) because the majority of the CMAP was generated by a single motor unit in this patient.

The changes in excitability occurring during and after long-duration (100 ms) subthreshold depolarizing and hyperpolarizing currents are illustrated for Patient 1 in Fig. 1E, and are collectively known as threshold electrotonus. The changes in threshold are plotted as threshold reductions, with responses to depolarizing currents upwards, as is normal for electrotonus. The fast changes in threshold that occur at delays of 0 and 100 ms are due to the rapid (<1 ms) changes in potential occurring at the nodes of Ranvier at the onset and offset of the polarizing currents. The slower excitability changes are caused by slower potential changes occurring passively on the internodal membrane, and by ion channels with slow kinetics, especially slow K⁺ channels at the nodes.

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>MMN patients</th>
<th>Controls</th>
<th>Significance (P)</th>
<th>Correct with depolarizing current</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SEM</td>
<td>Mean SEM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>7.29 0.48</td>
<td>6.62 0.12</td>
<td>&lt;0.05</td>
<td>+</td>
</tr>
<tr>
<td>Peak CMAP (mV)</td>
<td>4.45 1.10</td>
<td>9.02 0.56</td>
<td>&lt;0.01</td>
<td>++</td>
</tr>
<tr>
<td>Threshold (mA)</td>
<td>6.37 1.22</td>
<td>4.63 0.21</td>
<td>&lt;0.05</td>
<td>++</td>
</tr>
<tr>
<td>Rheobase (mA)</td>
<td>4.52 1.02</td>
<td>3.14 0.15</td>
<td>&lt;0.05</td>
<td>++</td>
</tr>
<tr>
<td>Strength–duration time constant (ms)</td>
<td>0.42 0.04</td>
<td>0.42 0.02</td>
<td>&gt;0.5</td>
<td></td>
</tr>
<tr>
<td>Stimulus–response slope</td>
<td>9.1 3.2</td>
<td>4.9 0.2</td>
<td>&lt;0.01</td>
<td>+</td>
</tr>
<tr>
<td>Resting I/V slope</td>
<td>0.43 0.03</td>
<td>0.62 0.02</td>
<td>&lt;0.0001</td>
<td>++</td>
</tr>
<tr>
<td>Minimum I/V slope</td>
<td>0.19 0.02</td>
<td>0.25 0.01</td>
<td>&lt;0.001</td>
<td>++</td>
</tr>
<tr>
<td>RRP (mS)</td>
<td>2.80 0.09</td>
<td>3.13 0.05</td>
<td>&lt;0.05</td>
<td>++</td>
</tr>
<tr>
<td>Subexcitability (%)</td>
<td>−39.85 3.94</td>
<td>−25.48 1.00</td>
<td>&lt;0.00005</td>
<td>+</td>
</tr>
<tr>
<td>TEd (10–20 ms) (%)</td>
<td>16.02 2.57</td>
<td>14.58 0.72</td>
<td>−0.5</td>
<td></td>
</tr>
<tr>
<td>TEd (90–100 ms) (%)</td>
<td>77.06 5.89</td>
<td>68.20 0.8</td>
<td>&lt;0.01</td>
<td>++</td>
</tr>
<tr>
<td>TEd (90–100 ms) (%)</td>
<td>47.04 1.55</td>
<td>42.08 0.71</td>
<td>&lt;0.01</td>
<td>++</td>
</tr>
<tr>
<td>TEd (90–100 ms) (%)</td>
<td>−83.78 6.18</td>
<td>−74.04 1.10</td>
<td>&lt;0.05</td>
<td>++</td>
</tr>
<tr>
<td>TEd (90–100 ms) (%)</td>
<td>−157.09 6.85</td>
<td>−117.52 2.75</td>
<td>&lt;0.00005</td>
<td>+</td>
</tr>
</tbody>
</table>

Student’s t was calculated for the difference in means, and P represents the probability of this occurring by chance alone (two-tailed test).

For the excitability parameters that were established to be abnormal, the right-hand column of the table shows those that became more normal (+) and those that came within the normal range (++) after the application of a depolarizing current. See text (Methods) for explanation of variables. SEM = standard error of the mean.

The changes in excitability parameters that were established to be abnormal, the right-hand column of the table shows those that became more normal (+) and those that came within the normal range (++) after the application of a depolarizing current. See text (Methods) for explanation of variables. SEM = standard error of the mean.
during the prolonged currents are increased, as occurs with membrane hyperpolarization (Kiernan and Bostock, 2000).

The final part of the program recorded the changes in excitability that occurred following a single supramaximal conditioning stimulus. Together, these changes (the absolute and relative refractory periods, the supernormal period and the late subnormal period) constitute the recovery cycle and have been well documented for human nerves. In Fig. 1F, the threshold changes are plotted with a logarithmic time scale to show more clearly the early events in the cycle while encompassing the time for complete recovery. As with threshold electrotonus, the differences in the recovery cycle recorded from Patient 1 compared with normal subjects were striking. The refractory period, determined by inactivation of Na⁺ channels, was within the normal range. However, the next phase of the recovery cycle, when axons enter a superexcitable period produced by the depolarizing afterpotential, was markedly lengthened. Superexcitability is voltage-dependent because it depends on the spread of depolarization to the internodal axolemma (Barrett and Barrett, 1982), which is limited by internodal K⁺ channels, especially fast K⁺ channels in the paranodal region (Baker et al., 1987; Waxman and Ritchie, 1993). The late subexcitable period, which is due to the activation of nodal voltage-dependent slow K⁺ channels (Baker et al., 1987; Bostock, 1995; Kiernan et al., 1996), although displaced in time, presumably due to the large superexcitable period, attained normal amplitude.

The recordings from Patients 5 and 6 showed abnormalities similar to those from Patient 1, while data from the three other patients were intermediate between those of Patient 1 and the mean for normal controls. Mean data from all six patients (± standard error of the mean) are presented in Fig. 2 in comparison with normal control data. It can be seen that the abnormalities exhibited by Patient 1 are a consistent feature of this patient group. Statistical data for each of the excitability measures described in Methods are presented in Table 2. In the patient group, the CMAPs were reduced and the stimulus–response slopes were greater, reflecting the presence of axonal degeneration and the large contribution to the CMAP of a single giant unit in two cases. The stimulus current required to evoke the target response was somewhat greater in the patient group, suggesting decreased excitability of the axonal membrane in the patients, but the relative

Fig. 3 Changes with polarization (H = 1 mA hyperpolarizing current; D = 1 mA depolarizing current) and ischaemia (I = 5 min ischaemia; PI = 5 min post-ischaemia) compared with mean data from three MMN patients (M = MMN patients) are shown for selected excitability parameters. (A) Minimum versus resting current/threshold slope. (B) Relative refractory period versus skin temperature. (C) Early-depolarizing versus late-hyperpolarizing threshold electrotonus. (D) Supereexcitability versus late subexcitability. Dotted lines indicate 95% confidence limits for normal controls values. The normalizing effect of depolarizing currents on values recorded from MMN patients is also illustrated (MD).
refractory period was reduced. The most significant ($P < 0.0001$) alterations in excitability properties in the MMN patients were: (i) the reduction in the minimum slope of the current–threshold ($I/V$) relationship (indicating a reduction in input conductance); (ii) TEh ($90–100 \text{ ms}$), the increase in threshold after $100 \text{ ms}$ of hyperpolarizing current; and (iii) the increase in superexcitability. All these excitability parameters, highly abnormal in the MMN patients, depend on the resting conductance of the paranodal and internodal axon membrane.

**Comparison with hyperpolarized normal nerves and effect of depolarization**

One way that the resting conductance of axonal membrane can be reduced is by membrane hyperpolarization. To explore the possibility that the nerve distal to conduction block in these patients was hyperpolarized, the results from the three patients with the most abnormal excitability properties were compared with those from previous recordings of polarized and ischaemic nerves in healthy subjects (Fig. 3) (Kiernan and Bostock, 2000). The magnitude and direction of the effects of polarizing currents and ischaemia on four pairs of excitability parameters were compared with the 95% confidence limits for these parameters in normal subjects. For simplicity, the data points plotted are restricted to the mean values for both control groups and for the effects of 1 mA depolarizing (D) and hyperpolarizing (H) currents, and for recordings started 5 min after applying a pressure cuff (I) and 5 min after its release (PI).

It can be seen that the patient data lie outside established control ranges in most parameters, behaving as if they were for a hyperpolarized and/or post-ischaemic nerve. One parameter affected differently by these two treatments was late subexcitability, probably because it depends on the extracellular potassium concentration as well as on membrane potential (Kiernan and Bostock, 2000). This parameter is plotted against superexcitability in Fig. 3D. Whereas both hyperpolarization and post-ischaemia increase superexcitability, late subexcitability is decreased by hyperpolarizing currents but remains unchanged when the nerve is post-ischaemic, and was unchanged or even increased in the patients despite the changes consistent with hyperpolarization in the other excitability parameters.

These comparisons suggest that the nerve just distal to the site of conduction block in these patients with MMN was behaving as if hyperpolarized, most closely resembling a post-ischaemic nerve. For a critical test of whether hyperpolarization was responsible for the abnormal membrane properties, a depolarizing current of 0.5 mA was applied to the nerves of the three patients, to test whether depolarization could reverse the abnormalities. It can be seen from Fig. 3 that this was indeed the case: all the excitability abnormalities were reduced by depolarization. The right-hand column of Table 2 shows, for the excitability parameters that were abnormal, those that became more normal (+) and those brought within the normal range (+++) by the depolarizing current. No excitability parameters were made abnormal or more abnormal by depolarization.

**Discussion**

**The nature of the distal membrane abnormality in multifocal motor neuropathy**

The present study examined the excitability properties of motor axons in patients with MMN in whom the conduction block was located distally. The excitability properties of the nerves tested just distal to the site of the lesion were in several cases strikingly abnormal (e.g. Fig. 1), with greatly increased superexcitability and marked fanning-out of threshold electrotonus, corresponding to reduced resting slope of the current–threshold relationship and increased apparent input impedance. These changes suggest a reduction in resting paranodal and internodal $K^+$ conductance, which could arise either by block of $K^+$ channels or by membrane hyperpolarization. Comparison with previous recordings from normal nerves hyperpolarized by applied current and by release of ischaemia showed very close correspondence between the excitability changes in MMN and those during post-ischaemic hyperpolarization.

A parameter that helps distinguish between membrane hyperpolarization and block of $K^+$ channels is refractoriness. This depends critically on Na$^+$ channel inactivation and therefore depends on membrane potential in a way that is independent of $K^+$ channels. Block of $K^+$ channels on its own would cause membrane depolarization (Grafe et al., 1994) and an increase in Na$^+$ channel inactivation, whereas a reduction in $K^+$ conductance due to membrane hyperpolarization would be accompanied by a fall in resting Na$^+$ inactivation and in refractoriness. The RRP was significantly reduced in the MMN patients (Table 2), as it is when normal axons are hyperpolarized by applied currents and in the post-ischaemic state (Kiernan and Bostock, 2000).

The most convincing evidence that the excitability changes in MMN were due primarily to membrane hyperpolarization was provided by the experiment in which the abnormalities were reversed by depolarizing current. It is extremely unlikely that normalization of the full range of excitability parameters, including those dependent principally on Na$^+$ channels as well as those dependent principally on K$^+$ channels, could be achieved by membrane depolarization if the abnormalities in MMN were caused by K$^+$ channel block or another channel abnormality rather than by membrane hyperpolarization.

One possible objection to this conclusion is that another potential-dependent excitability parameter, the strength–duration time constant, was not abnormal in the patients (Table 2). This apparent discrepancy probably reflects the combination of the low sensitivity of the strength–duration time constant to change in membrane potential (Kiernan and
Bostock, 2000) and the present degree of intra- and intersubject variability described previously for this parameter (Bostock et al., 1998; Kiernan et al., 2000). In contrast, the six excitability parameters found by Kiernan and Bostock (2000) to provide the best indices of a small change in membrane potential in motor axons [i.e. TEd (90–100 ms), resting I/V slope, superexcitability, relative refractory period, TEx (90–100 ms) and TExd (10–20 ms)] were all significantly abnormal for the MMN patients in the direction of membrane hyperpolarization (Table 2).

The nature of conduction block in multifocal motor neuropathy

Findings from the present study suggest that the nerve just distal to the site of conduction block in patients with MMN is hyperpolarized. Similar changes can be induced by overactivity of the electrogenic Na⁺/K⁺ pump, as occurs following ischaemia and tetanization (Ritchie and Straub, 1957; Bergmans, 1970; Raymond, 1979; Bergmans, 1982; Bostock and Grafe, 1985; Applegate and Burke, 1989; Gordon et al., 1990; Bostock and Bergmans, 1994; Kiernan et al., 1997a, b; Kiernan and Bostock, 2000). No other mechanism has been described which could account for substantial, long-lasting hyperpolarization of axons. But in patients the hyperpolarization is not merely long-lasting but represents a stable steady state. In Patient 1, two recordings taken 10 weeks apart showed almost identical excitability abnormalities (Fig. 1). Persistent overactivity of the Na⁺/K⁺ pump requires a persistent intra-axonal source of Na⁺ ions to drive the pump. These Na⁺ ions could not be entering the axon continuously at the recording site, otherwise they would (unless coupled with a compensatory ion flux) cause a net depolarization of the membrane.

Longitudinal diffusion or transport along the axon could provide an intra-axonal source of Na⁺ ions. Given that the lesion in these patients is focal, Na⁺/K⁺ pump activity could be blocked by oedema, for instance, preventing oxygen diffusion, or, given that MMN is immune-mediated, by antibodies preventing Na⁺/K⁺ pump function. In either case, prevention of Na⁺/K⁺ pump function would produce a depolarizing block with intracellular accumulation of Na⁺ at the site of the lesion. Otherwise, disruption of the blood–nerve barrier, as demonstrated in a previous study (Kaji et al., 1993), may increase the K⁺ concentration of the endoneurial fluid. This may further aggravate the depolarization block. If Na⁺ influx continued, a steady state would be achieved only by Na⁺ ions moving intracellularly along the axon to a site where the pump was still working, resulting in overactivity and membrane hyperpolarization. Therefore, depolarization at the site of the lesion would coexist with chronic hyperpolarization on one or both sides of this site. Such a lesion, with adjacent ischaemic and post-ischaemic lengths of nerve, would also be likely to generate ectopic activity, which is a hallmark of MMN, patients experiencing fasciculation or myokymia in the presence of conduction block.

Other clinical symptoms and studies provide further support for this hypothesis. Cold paralysis, describing a worsening in weakness with exposure to cold, and a term originally used for monomelic amyotrophy (Hirayama et al., 1963; Kiernan et al., 1999), also occurs in patients with MMN (Kaji and Kojima, 1997). The electrogenic Na⁺/K⁺ pump is temperature-sensitive, with slower kinetics at lower temperatures. On cooling, the reduced pump kinetics would exacerbate the effect of the already compromised Na⁺/K⁺ pump function to increase the depolarizing conduction block. This contrasts with the expected effect of cooling to alleviate conduction block due to demyelination (Waxman, 1978). Similarly, digitalis, a known blocker of Na⁺/K⁺ pump activity, paradoxically exacerbated the fanning-out changes seen in threshold electrotonus for a patient with MMN (Kaji and Kojima, 1997).

If the membrane hyperpolarization in MMN were due to longitudinal, intracellular diffusion of Na⁺ ions, as proposed, then it would be expected that the intracellular Na⁺ excess and electrogenic hyperpolarization should fall off with distance from the lesion. It was not possible to verify this with the patients in the present study, because of the location of the lesions close to the wrist. In patients with conduction block in the upper arm, however, it should be possible to test this prediction by comparing excitability measurements at the elbow and at the wrist. It was also not possible to verify that the axons became depolarized at the site of the lesion, by tracking excitability changes more proximally, because the nerves became inexcitable (cf. Yokota et al., 1996).

Patients may improve rapidly with intravenous immunoglobulin therapy, the treatment of choice for MMN (Hahn, 2000), suggesting an immune basis for the disease. It is possible that the focal Na⁺ influx inferred for MMN patients in the present study could occur through selective antibody interference with the Na⁺/K⁺ pump of motor axons. Alternatively, antibodies could provoke Na⁺ influx by a direct or indirect effect on Na⁺ channels. While no such antibody has yet been described in MMN patients, antibodies to GM1 ganglioside are known to be present in up to 50% of cases, although their role in disease pathophysiology has not been elucidated (Hirota et al., 1997; Kaji and Kojima, 1997; Benatar et al., 1999).

Axonal degeneration in multifocal motor neuropathy

A conspicuous feature of the motor nerves in the most affected patients in our group was the extent of axonal degeneration. This was shown by the number of giant motor units which presumably resulted from collateral sprouting and reinnervation by surviving axons. Evidence of axonal degeneration was greatest in the three patients (Patients 1, 5 and 6) who provided the most abnormal excitability record-
ings, and it might therefore be suggested that the apparent hyperpolarization was a consequence of tracking a single unit or of sprouting, or was somehow related to depletion of motor axons in the nerve. These possibilities are refuted by (i) the observation in Patient 1 that tracking a single unit, or a much larger compound response, produced similar, highly abnormal recordings, and (ii) the observations in previous studies of amyotrophic lateral sclerosis (K. Cikurel, N. M. F. Murray and H. Bostock, unpublished data) that, even with a single functional motor unit in a muscle, threshold electrotonus can be entirely normal. It seems more likely that the apparent correlation between axonal degeneration and distal hyperpolarization arises because both are caused by focal depolarization at the site of the lesion. The more extensive and profound the membrane depolarization, the more Na+ will accumulate at the site of the lesion. The more extensive and profound depolarization arises because both are caused by focal depolarization; the combination of depolarization and reduced transmembrane Na+ gradient will also lead to intracellular Ca2+ accumulation (since the Na+/Ca2+ exchange mechanism that normally removes intracellular Ca2+ can go into reverse) and consequent degeneration (Stys et al., 1991; Tatsumi and Katayama, 1995).

In conclusion, the use of excitability testing in patients with MMN provided evidence of membrane hyperpolarization distal to the site of conduction block and led to the hypothesis that the conduction block and ectopic discharges are caused primarily by juxtaposed lengths of depolarized and hyperpolarized axonal membrane, and that the block is accompanied by degeneration when the depolarization is severe.

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References


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