Membrane properties in chronic inflammatory demyelinating polyneuropathy

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Summary

Threshold tracking was used to compare excitability properties (stimulus–response curves, strength–duration properties, recovery cycle and threshold electrotonus) of the median nerve in 11 patients with chronic inflammatory demyelinating polyneuropathy (CIDP) and 25 healthy controls. Stimulus–response curves were significantly different: threshold was much higher, the slope of the curves reduced and the spread of the thresholds greater in the CIDP group. The strength–duration time constant (τ_{SD}) was significantly shorter and the rheobase higher in the CIDP group. In the recovery cycle, the CIDP group had less refractoriness, supernormality and late subnormality than healthy controls, but the duration of the relatively refractory period was normal. These changes in τ_{SD} and the recovery cycle were not those previously predicted. There were no consistent changes in threshold electrotonus, suggesting that, for the studied axons, there are no consistent changes in accommodation properties that depend on internodal conductances. It is difficult to explain these changes in excitability on the basis of a change in membrane potential, or solely as the result of demyelination, and it is possible that other morphological factors such as variable remyelination and inflammatory oedema affected axonal excitability in the patients.

Keywords: chronic inflammatory demyelinating polyneuropathy; axonal excitability; recovery cycle; strength–duration properties; threshold electrotonus

Abbreviations: CIDP = chronic inflammatory demyelinating polyneuropathy; CMAP = compound muscle action potential; τ_{SD} = strength–duration time constant

Introduction

A number of indices of axonal excitability can be measured using the technique of threshold tracking (Bostock and Baker, 1988; Bostock *et al.*, 1998; Kiernan *et al.*, 2000). These indices depend on Na^+ and K^+ channels, membrane potential and the properties of the axonal membrane or myelin sheath. There are no prior reports of differences in these indices between patients with chronic inflammatory demyelinating polyneuropathy and healthy subjects but, on theoretical grounds, it has been predicted that the strength–duration time constant (τ_{SD}) would increase (Bostock, 1983; Bostock *et al.*, 1983, 1998; Mogyoros *et al.*, 2000), and it is likely that refractoriness would also increase.

Demyelination exposes the paranodal and internodal axonal membrane, altering the density of Na^+ and K^+ channels. In chronically demyelinated lesions, there may be changes in ion channel density in the involved axonal membrane (Shrager, 1989; Schwarz *et al.*, 1991; Waxman *et al.*, 1994, 1999; England *et al.*, 1996, 1998). This, in turn, may alter passive and active membrane properties. Accordingly the

findings in chronic disease states could differ from those due to the disruption of the myelin sheath in otherwise normal axons. It is important to know how these mechanisms are altered in disease because in chronic demyelinating diseases, many symptoms depend on the ability of critically affected axons to conduct impulses (McDonald, 1977; Waxman, 1988; Cappelen-Smith *et al.*, 2000; Kaji *et al.*, 2000).

The present study used threshold tracking to assess different aspects of nerve excitability in chronically demyelinated human motor axons. Some indices underwent changes that were not expected.

Methods

Experiments were performed on 11 patients with chronic inflammatory demyelinating polyneuropathy (CIDP) and 25 healthy adult subjects (five female, 20 male, aged 23–55 years), all of whom gave informed consent to experimental procedures, which had the approval of the Research Ethics

Table 1 *Clinical characterisatics of CIPD patients*

Clinical characteristics											
Age (years)	57	49	47	79	44	34	48	65	42	42	72
Gender	F	F	M	M	M	M	M	M	M	M	F
Hughes grade	2	1	2	2	2	2	2	2	1	1	1
APB strength (MRC scale)	4	4+	4–	4	4+	4	4–	4	5	5	5
Clinical state (symptomatic)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Muscle fatiguability	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Heat sensitivity	No	Yes	Yes	No	Yes	No	Yes	Yes	No	No	No
Duration of illness (years)	2	2	20	0.25	4	0.5	5	1	4	12	8
Sural nerve biopsy	DM	DM	ND	DM	DM	ND	DM	ND	ND	DM	DM
Conduction velocity (median)*	48 m/s	20 m/s	10 m/s	29 m/s	41 m/s	43 m/s	27 m/s	48 m/s	54 m/s	44 m/s	27 m/s

APB = abductor pollicis brevis; ND = not done; DM = demyelinating; M = male; F = female. *Conduction velocity in median motor axons in elbow–wrist segment.

Committee of the South Eastern Sydney Area Health Service (Eastern Division). A number of the healthy control subjects have been used in other previously published studies (Kiernan *et al.*, 2000; Kuwabara *et al.*, 2000), and seven of the patients were subjects in a previous study (Cappelen-Smith *et al.*, 2000).

Patients

Patient data are summarized in Table 1. The 11 patients (three female, eight male, aged 34–78 years) fulfilled the diagnostic criteria for CIDP recommended by the American Academy of Neurology (Cornblath *et al.*, 1991). Their disabilities were graded on the Hughes functional grading scale (grade 4, bed bound; grade 3, able to walk 5 m with aids; grade 2, ambulates independently; grade 1, minimal signs and symptoms, able to run; Hughes *et al.*, 1978). Eight patients were symptomatic and three were in clinical remission. Seven had biopsy-proven demyelinating neuropathy.

Neurophysiology

A computerized threshold tracking procedure (QTRAC version 4.3, written by Professor H. Bostock, Institute of Neurology, London, with multiple excitability protocol TRONDHM; see Kiernan *et al.*, 2000) was used to follow the excitability of motor axons in the median nerve at the wrist innervating the abductor pollicis brevis. The stimulus currents were delivered from a computer-controlled current source, through non-polarizable electrodes, with the cathode over the median nerve at the wrist and the anode ~10 cm proximal over the muscle. The amplitude of the compound muscle action potential (CMAP) was recorded from the abductor pollicis brevis with the active electrode at the motor point and the reference on the proximal phalanx.

Test current pulses of 0.2 or 1 ms duration were delivered regularly at 0.8 s intervals, combined with suprathreshold conditioning stimuli or subthreshold polarizing currents. The amplitude of the CMAP was measured from baseline to
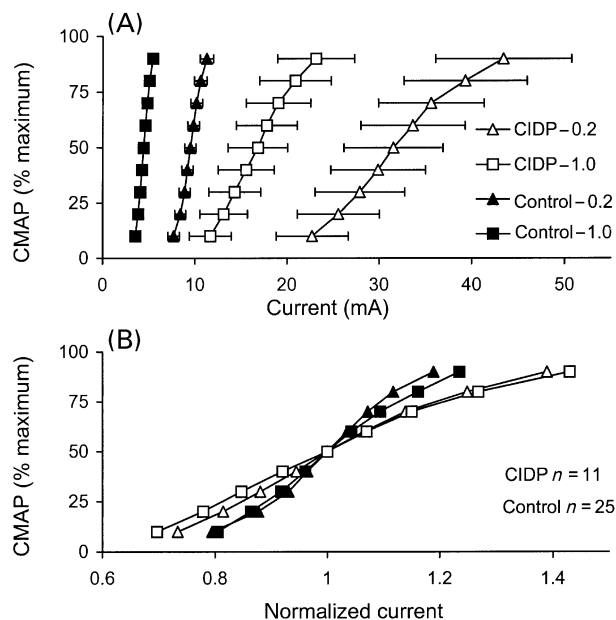

Fig. 1 Stimulus–response curves. (**A**) Absolute data (mean $\pm$ standard error of the mean) and (**B**) normalized curves (mean) of median motor axons at the wrist in 25 healthy controls and 11 CIDP patients for two stimulus durations (0.2 and 1.0 ms). In **B**, the data were normalized so that the threshold current for the 50% CMAP was 1.0. The threshold currents are much higher, the slope of the curves reduced and the spread of thresholds greater in the patients (**B**).

negative peak. For all tracking studies, the target CMAP was set to be 40% of maximum, on the fast rising phase of the stimulus–response curve (Fig. 1A). Skin temperature was measured near the stimulating site, and was maintained above 32°C using blankets and a heater when necessary.

The test protocol has been described in detail elsewhere (Kiernan and Bostock, 2000; Kiernan *et al.*, 2000). The stimulus–response curve was used with the tracking error (i.e. the difference between the actual and target responses) to optimize the threshold tracking. The measurement of the stimulus–response curves was done separately for test stimuli

of durations 0.2 and 1.0 ms (Fig. 1). From the stimulus–response curves, the currents required to produce CMAPs of 10–90% of the maximal response were measured, and used to calculate strength–duration properties for CMAPs of different size. The τ_{SD} is a nodal property and reflects the rate of decrease of threshold current as the duration of the test stimulus increases. The τ_{SD} was calculated using the following formula (Weiss, 1901; Bostock and Bergmans, 1994; Mogyoros *et al.*, 1996):

$$\tau_{SD} = 0.2\ (I_{0.2} - I_{1.0})/(I_{1.0} - 0.2\ I_{0.2})$$

where τ_{SD} is the strength–duration time constant, and $I_{0.2}$ and $I_{1.0}$ are the threshold currents using test stimuli of 0.2 and 1.0 ms duration, respectively.

Rheobase is the threshold current if the test stimulus could be infinitely long, and was calculated from the same data, using the formula:

$$I_{rh} = t \times I/(t + \tau_{SD})$$

where I_{rh} is rheobasic current, t is the stimulus duration, τ_{SD} is the strength–duration time constant, and I is stimulus current of duration τ.

To assess the time course of recovery of axonal excitability following a single supramaximal stimulus (the 'recovery cycle'), test stimuli of 1.0 ms duration were delivered at conditioning–test intervals of 2–200 ms after a supramaximal conditioning stimulus of 1.0 ms duration. The test response was measured after on-line subtraction of the CMAP produced by the conditioning stimulus.

In studies investigating the effects of changing membrane potential on the threshold for the test CMAP, membrane potential was altered using subthreshold depolarizing and hyperpolarizing currents lasting 100 ms. These currents were set at 40% of the unconditioned threshold, and the resulting changes in threshold represent 'threshold electrotonus'. In a further test with subthreshold conditioning, the test stimulus was delivered at the end of a polarizing current pulse lasting 200 ms. The strength of the current pulse was changed systematically from 50% depolarizing to 100% hyper-polarizing in 10% steps. This produced a current–threshold relationship analogous to the conventional current–voltage (I–V) relationship.

For each parameter, differences between the CIDP patients and the healthy controls were tested with Student's *t*-test. Because of the number of comparisons, significance was defined as $P < 0.01$. Data are given as mean $\pm$ standard error of the mean with, where appropriate, the extremes of the range.

Results
Resting CMAP amplitude, latency and conduction velocity

The amplitude of the negative peak of the maximal CMAP was 9.8 $\pm$ 0.6 mV (mean $\pm$ standard error of the mean) in the 25 healthy controls and 5.6 $\pm$ 1.1 mV in the 11 CIDP
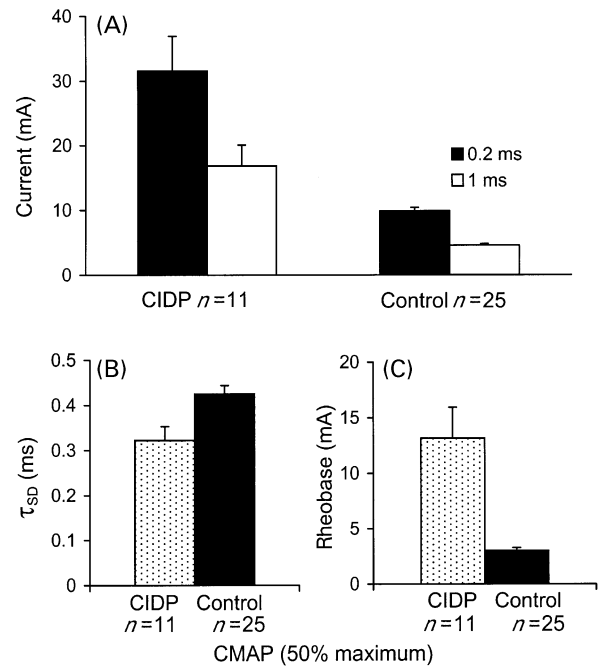

Fig. 2 Stimulus–response and strength–duration data for CMAPs 50% of maximum (mean $\pm$ standard error of the mean). (**A**) The absolute current required to produce the 50% CMAP for the 0.2 and 1.0 ms test stimulus in the 11 CIDP patients was ~3 times that in the 25 healthy controls. (**B**) The mean τ_{SD} for the 50% CMAP was ~100 μs longer in 25 healthy controls than in the 11 patients ($P < 0.001$). (**C**) Mean rheobase for the 50% CMAP was much lower for the 25 healthy controls than the 11 patients ($P = 0.004$).

patients ($P < 0.005$). The latency to half peak of the maximal CMAP was 4.3 $\pm$ 0.1 ms (range 3.2–5.4 ms) in the healthy control group and 9.0 $\pm$ 1.4 ms (range 5.0–21.7 ms) in the CIDP patients ($P = 0.007$). The median motor conduction velocity in the wrist–elbow segment was 35.5 $\pm$ 4.1 m/s (range 10–54 m/s) in the CIDP patients, compared with 56.7 $\pm$ 3.8 m/s (mean $\pm$ standard deviation), the accepted normal for this laboratory.

Stimulus–response curves and strength–duration properties

In the stimulus–response curves, threshold currents were significantly higher in the 11 CIDP patients than in the 25 healthy controls (Fig. 1A). The current required to produce a minimal (10%) CMAP in the patients was double that required to produce a maximal CMAP in healthy controls (Fig. 1A).

To produce a CMAP 50% of maximum, the mean absolute current for the 0.2 ms test stimulus was 31.6 $\pm$ 5.4 mA in the CIDP patients and 9.5 $\pm$ 0.6 mA in healthy controls. For the 1.0 ms test stimulus, the mean absolute current was 16.8 $\pm$ 3.2 mA in the patients and 4.4 $\pm$ 0.3 mA in the healthy controls. Hence the CIDP patients required more than threefold the current required by the healthy controls to
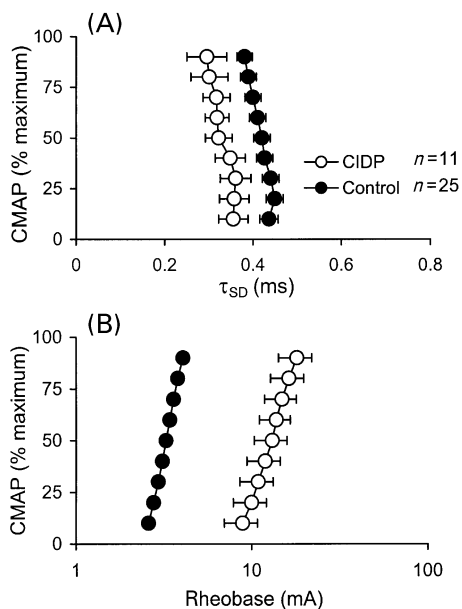
Fig. 3 Strength–duration properties. (**A**) τ_{SD}, and (**B**) rheobase for test CMAPs of different size (10–90% of maximum) in 25 healthy controls and 11 patients (mean $\pm$ standard error of the mean; in **B**, the error bars are smaller than the symbols for the control data). The τ_{SD} was significantly shorter by ~100 µs in the patients regardless of CMAP size. Rheobase was significantly higher in the patients regardless of CMAP size. Note the logarithmic scale for the rheobasic data in **B**.

produce a CMAP 50% of maximum whether the test stimulus was 0.2 or 1.0 ms duration ($P < 0.001$, Fig. 2A). Despite the stronger stimuli, the testing procedure was well tolerated by the patients, presumably because the threshold for discomfort was also increased.

Normalized stimulus–response curves were significantly different, the slope of the curves reduced and the spread of the thresholds greater in the CIDP patients (Fig. 1B). The greater spread of the CIDP curves was noted particularly at the highest thresholds. Using a 1.0 ms stimulus, the necessary current to produce a 10% CMAP was 69% (range 54–79%) of that required for a 50% CMAP, while in healthy controls it was 80% (Fig. 1B). This is relevant to the responses to prolonged depolarizing currents used in threshold electrotonus studies (see below).

The τ_{SD} for a 50% CMAP in the CIDP patients was 322 ± 30 µs (range 177–484 µs), significantly shorter than that for the healthy controls (424 ± 26 µs, range 283–629 µs; $P < 0.001$, Fig. 2B). As in healthy controls, the τ_{SD} changed little for test CMAPs of different sizes (Fig. 3A), despite the prominent range of thresholds from 10 to 90% (Fig. 1A and B). The τ_{SD} was significantly shorter by ~100 µs in the CIDP patients for CMAPs from 10 to 90% of maximum (Fig. 3A).

The patients had a higher rheobase threshold (13.2 ± 2.8 mA as against 3.3 ± 0.2 mA for healthy controls) for a CMAP 50% of maximum (Figs 2C and 3B). There was an inverse relationship between the τ_{SD} and rheobase in the
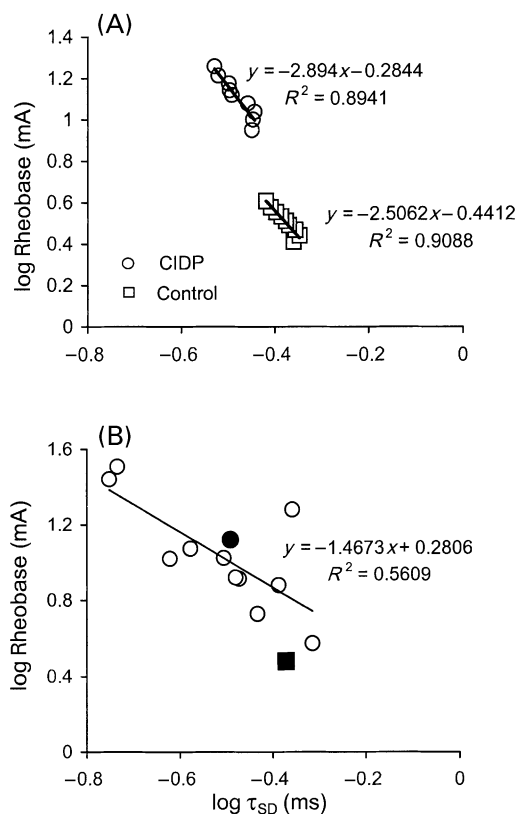

Fig. 4 Relationship between the logarithm of rheobase and the logarithm of τ_{SD}. (**A**) Mean data for the 10–90% CMAPs for the 11 CIDP patients and 25 controls. The relationship in each group is comparable, indicating maintenance of the usual reciprocal relationship seen between τ_{SD} and rheobase in normal axons. (**B**) Data for the 50% CMAP in each of the 11 patients ($R^2 = 0.56$). The mean data for 11 patients and 25 controls are shown as a filled circle and a filled square, respectively. The mean values for the patients are shifted upwards and to the left, relative to those for control subjects.

CIDP patients, whether plotted using the mean data for CMAPs 10–90% of maximum (Fig. 4A) or the 50% CMAP data for individual patients (Fig. 4B). As in healthy controls (Mogyoros *et al.*, 1996, 2000), these relationships became linear when plotted as the logarithm of rheobase against the logarithm of τ_{SD}. The data for the patients were shifted upwards and to the left (Fig. 4A and filled symbols in Fig. 4B).

Recovery cycle of axonal excitability
The pattern of the recovery cycles for the 11 CIDP patients and 25 healthy controls was similar, with relative refractoriness lasting <4 ms, supernormality maximal at the 7 ms conditioning–test interval, and late subnormality maximal at ~40 ms (Fig. 5). There was a significantly lower threshold change during the refractory and supernormal periods ($P = 0.001$ and 0.004, at the 2 and 7 ms intervals, respectively) in the CIDP patients compared with the healthy controls. There was also a tendency for lesser threshold changes in the late subnormal period in the CIDP patients.
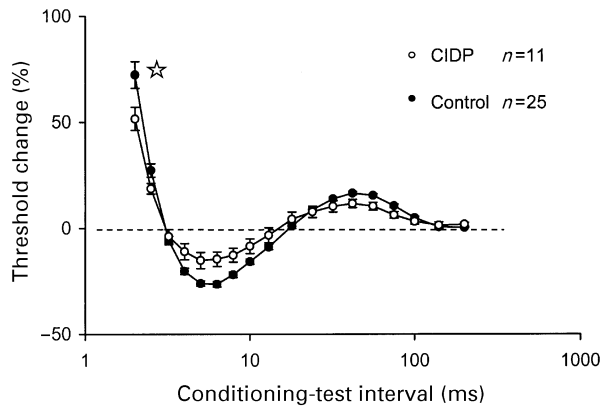
Fig. 5 The recovery cycle of axonal excitability following a single conditioning stimulus (mean ± standard error of the mean) for patients (open circles) and control subjects (filled circles). Note that the relatively refractory period had the same duration in both groups but the threshold changes were smaller in the patients. In this figure, an increase in threshold (i.e. a decrease in excitability) is plotted upwards. For the controls, the 2 ms data point (star) is based on 23 subjects ($P = 0.001$ at 2 ms; 0.03 at 2.5 ms).

However, while the extent of refractoriness was less in the patients, the duration of the relatively refractory period did not differ (Fig. 5).

Threshold electrotonus and current–voltage relationships

The mean threshold changes produced by subthreshold depolarizing or hyperpolarizing currents that lasted 100 ms are shown for 25 healthy controls and 10 CIDP patients in Fig. 6A, the data for the eleventh patient being marred by artefact. The data for the healthy controls (mean ± 95% confidence intervals) are shown in Fig. 6B and the equivalent data for the CIDP patients in Fig. 6C. As is conventional for threshold electrotonus (Bostock and Baker, 1988; Bostock *et al.*, 1998), an increase in excitability (a 'threshold reduction') is plotted as an upwards deflection, in contrast to the recovery cycle where greater excitability is plotted as a decrease in threshold, downwards. While the mean responses were similar, the waveforms were more variable in the CIDP patients (compare B and C in Fig. 6).

In response to depolarizing conditioning stimuli, the initial fast threshold change was identical for the healthy control and CIDP patients. The maximal threshold change was slightly greater in the hyperpolarizing but not the depolarizing direction. In the healthy controls, the maximal threshold reduction produced by the depolarizing current reached ~65% at ~25 ms. This would have remained subthreshold for normal subjects (see Fig. 1B, filled squares), for whom the threshold change would have to reach ~80% before low threshold axons were stimulated. In the patients, 65% of the 1.0 ms threshold would have stimulated some axons contributing to the first 10% of the CMAP (Fig. 1B, open squares). Activation
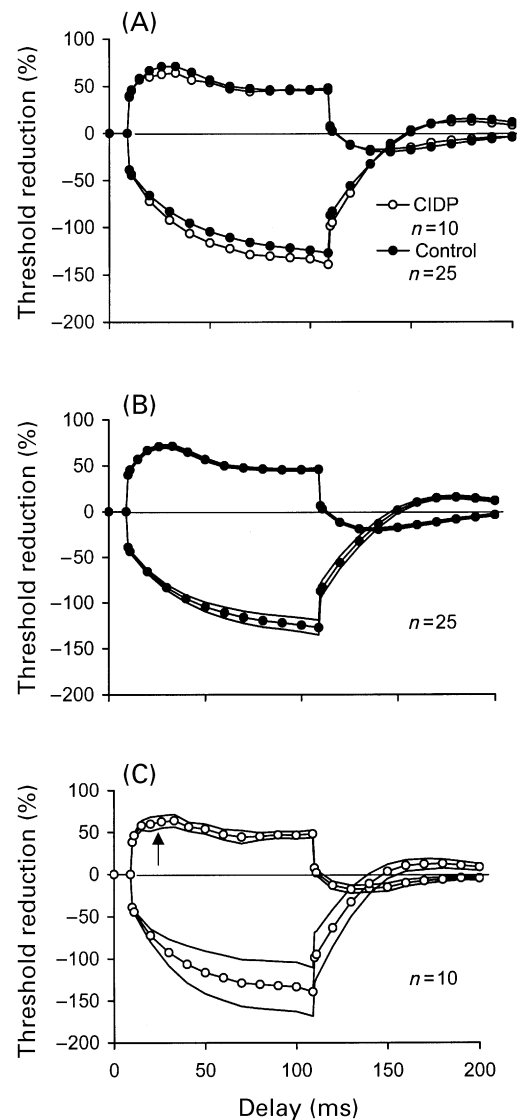

Fig. 6 Threshold electrotonus with 100 ms conditioning currents (±40% of unconditioned threshold). (**A**) The mean threshold changes produced by subthreshold depolarizing or hyperpolarizing currents for 25 healthy controls and 10 CIDP patients. (**B**) The data for the 25 healthy controls (mean ± 95% confidence intervals). (**C**) The data for the 10 patients (mean ± 95% confidence intervals). In **C**, the arrow indicates irregularity on the plot because, in three patients, the 40% depolarizing current exceeded threshold for the most excitable axons. In this figure, a reduction in threshold (i.e. an increase in excitability) is plotted upwards.

of some low threshold axons occurred with the depolarizing current in three patients and produced an irregularity in the plot of threshold electrotonus (indicated by the arrow on the averaged trace for the 10 CIDP patients in Fig. 6C). In five of the patients and 13 healthy controls, repeat studies were therefore performed using weaker conditioning currents (20% of the unconditioned threshold). The threshold electrotonus curves using 20% subthreshold currents were not significantly different in the two groups.
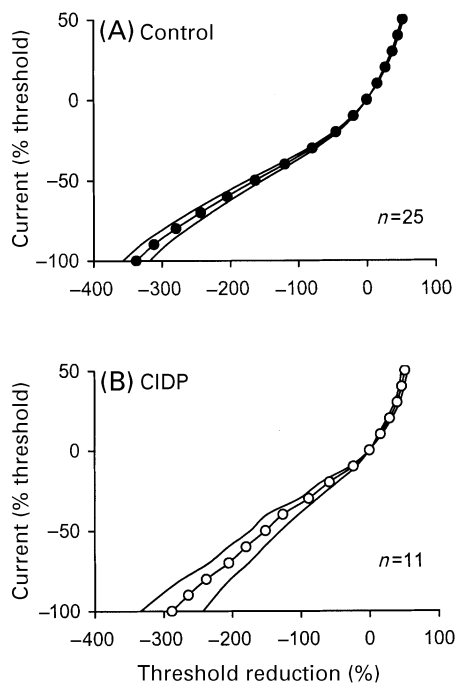
Fig. 7 Current–threshold relationships using conditioning currents lasting 200 ms for healthy controls (**A**) and patients (**B**). The intensity of the conditioning current was changed systematically from 50% (depolarizing) to –100% (hyperpolarizing) in 10% steps (mean ± 95% confidence intervals). These plots reveal no differences in accommodation to depolarizing currents (the upper data, to the right of the curves) or to hyperpolarizing currents (the lower data, to the left of the curves).

It should be noted that the threshold changes produced by 40% depolarizing currents were similar at the end of the 100 ms current pulse in patients and controls. In addition, there was no significant difference in the threshold change at the end of the 100 ms hyperpolarizing current. The threshold undershoot after the end of the 100 ms depolarizing current was also similar, as was the threshold overshoot following the hyperpolarizing current. These findings suggest that, on average, accommodation to depolarizing and hyperpolarizing currents in the patients did not differ significantly from that in healthy controls. This conclusion is supported by the current–threshold relationships which were virtually identical for the 25 healthy controls and 11 CIDP patients in the depolarizing direction. They diverged slightly in the hyperpolarizing direction (Fig. 7), but the differences were not significant. As with other indices, there was greater variability in the responses of the patients.

The variability of the threshold electrotonus curves were largely due to the responses of two patients. One had greater threshold changes to depolarizing and, particularly, hyperpolarizing currents, together with slightly less refractoriness, longer lasting supernormality and a greater threshold reduction at 200 ms in the current–voltage relationship. Such changes would be consistent with hyper-polarization of the tested axons at rest. The second patient had much smaller threshold changes to depolarizing and

hyperpolarizing currents, less supernormality and a lesser threshold reduction at 200 ms, changes which could indicate these axons were depolarized at rest. In both patients, the τ_{SD} was 184 and 265 µs, respectively, i.e. shorter than the mean for the patients (322 µs), indicating that changes in membrane potential cannot explain the shorter τ_{SD} in CIDP.

Correlation with clinical status and activity

Three of the patients were in clinical remission. There were no statistically significant differences between the eight symptomatic patients and the three in remission for any of the parameters tested. There were weak trends in the expected directions, namely a slightly longer τ_{SD} (331 ± 80 µs compared with 322 ± 30 µs) and lower threshold currents in the stimulus–response curves for the patients in remission. For the 0.2 ms stimulus, the mean absolute current was 32.5 mA to produce a CMAP 50% of maximum in the symptomatic CIDP patients compared with 28.9 mA for those in remission. Conduction velocity over the wrist–elbow segment of the median nerve was slightly greater for the patients in remission than those with symptomatic disease (41.7 ± 7.9 m/s, compared with 33.3 ± 4.9 m/s), and the distal motor latency slightly shorter (6.5 ± 1.5 ms, compared with 9.0 ± 1.4 ms), but neither difference was significant. There was no correlation between threshold or τ_{SD} and conduction velocity or distal motor latency.

Discussion

The present study has documented a number of differences in excitability between healthy and chronically demyelinated human axons *in vivo*. An expected finding was the significantly different stimulus–response curves, threshold being higher, the slope of the curves reduced and the spread of the thresholds greater in the CIDP patients (Meulstee *et al.*, 1997). Unexpectedly, however, the strength–duration time constant was significantly shorter in the CIDP patients, and they had less refractoriness, supernormality and late subnormality than healthy controls. Accommodative responses to long-lasting subthreshold currents were similar.

Differences in stimulus–response curves

In a normal peripheral nerve, the thresholds of individual motor axons are similar, and this is reflected in the steep slope of the stimulus–response curve in healthy controls (Fig. 1A). There was a greater spread of the CIDP curves, particularly at the highest thresholds. In normal subjects, it would be expected that, in general, the higher the threshold the smaller the axon. Intuitively, one would expect that in CIDP the higher threshold axons were those with the more severe demyelinating pathology. However, other factors could have contributed to the higher thresholds in the patients, including the effects of subperineural oedema, which could cause short-circuiting of applied current. Either way, these

findings confirm and extend the stimulus–response curve abnormalities described by Meulstee and colleagues (Meulstee *et al.*, 1997) in patients with demyelinating polyneuropathies.

Resting membrane potential and its effects on axonal excitability

The indices of axonal excitability studied here are voltage dependent and are therefore influenced by resting membrane potential. However, some findings in the CIDP patients, such as a decrease in both refractoriness and supernormality, cannot be explained adequately by a change in membrane potential. In addition, a number of independent findings suggest that membrane potential was normal in the patients. The duration of the relative refractory period was identical in the patients and the controls, and there was no significant difference in either the overall threshold electrotonus waveforms or the current–threshold relationships (see Kiernan and Bostock, 2000). Some of the data for the two extreme patients were compatible with opposite changes in membrane potential, but there were no such trends in the group data, and it is therefore necessary to consider mechanisms other than membrane potential to explain the group differences noted above.

Axonal excitability in CIDP

In CIDP, remyelination is active, even if often defective, and the effects of neural oedema could distort thresholds, as noted earlier. It is therefore possible that morphological factors other than demyelination could have contributed to the recorded changes in axonal excitability. However, one would expect oedema to be more prominent during the acute phases of Guillain–Barré syndrome and in acute motor axonal neuropathy but, in a limited number of recordings made to date in Sydney ($n = 4$) and Chiba ($n = 14$), there has been no evidence of shortening of the τ_{SD}.

Different pathologies (e.g. injury or chronic demyelination) can lead to changes in expression of ion channels in involved axons and dorsal root ganglia (Shrager, 1989; Schwarz *et al.*, 1991; Waxman *et al.*, 1994, 1999; England *et al.*, 1996, 1998), but whether this occurs in CIDP is unknown. It is therefore instructive to see if the changes in excitability indices in CIDP can be explained without invoking these plastic changes.

Strength–duration time constant

τ_{SD} mean values were significantly shorter in the CIDP patients than in healthy controls. This was unexpected (Bostock, 1983; Bostock *et al.*, 1983, 1998; Mogyoros *et al.*, 2000) and, as discussed above, cannot be explained by a hyperpolarizing shift in resting membrane potential. For example, if the slightly greater mean threshold electrotonus

response to hyperpolarizing current were due to a change in membrane potential, the data of Kiernan and Bostock (Kiernan and Bostock, 2000) suggest that this would have reduced τ_{SD} by only 10–15 μs, not by 100 μs. Demyelination may expose additional membrane at the node of Ranvier, increase nodal capacitance and increase the passive membrane time constant (Brismar, 1981; Bostock, 1983). However, this should increase the τ_{SD} and, indeed, it has been shown to do so in subacutely demyelinated rat motor axons (8 days after experimental demyelination with diphtheria toxin, Bostock *et al.*, 1983).

The τ_{SD} also depends on a threshold conductance, probably due to persistent Na^+ channels (Bostock and Rothwell, 1997). Na^+ channels have low density at the internode, and it is possible that an effective increase in nodal area by the inclusion of previously paranodal membrane would lower Na^+ channel density sufficiently to counteract the effects of the increased nodal capacitance. Alternatively, the shorter τ_{SD} could be the result primarily of remyelination effectively limiting the increase in nodal capacitance but without correcting a reduced Na^+ channel density. Other possible factors include short-circuiting of the applied current through areas of subperineural oedema and associated alterations in stimulus geometry. Future studies using the technique of latent addition (Bostock and Rothwell, 1997) might elucidate the contribution of active and passive membrane properties to τ_{SD} in CIDP.

Rheobase

Rheobase is defined as the threshold current required to excite an axon when the stimulus duration is infinitely long. According to Weiss' law (Weiss, 1901), there is a reciprocal relationship between the τ_{SD} and rheobasic current in healthy axons, i.e. when the τ_{SD} increases, rheobase decreases and vice versa, and this has been confirmed for human axons (Mogyoros *et al.*, 1996, 2000). The higher rheobase in the CIDP patients can be attributed, in part, to the higher threshold of demyelinated axons, as seen in the stimulus–response curves.

Threshold electrotonus

The threshold electrotonus allows accommodative responses to hyperpolarizing and depolarizing currents to be documented (Bostock *et al.*, 1998). The responsible conductances are located on the internodal membrane. There were no significant differences in the accommodative responses to prolonged subthreshold depolarizing and hyperpolarizing currents, a finding consistent with a previous (unrefereed) report (Kaji and Kojima, 1997). This suggests that, for the axons studied (i.e. conducting axons contributing to the first 40% of the CMAP), the behaviour of internodal accommodative conductances is largely unaffected by the demyelinating pathology. The normality of the accommodative responses is intriguing because this implies

that the access of current to the internodal membrane was relatively normal, and not enhanced as one might expect with paranodal demyelination and, particularly, with the stronger polarizing currents. This finding suggests that not all of the applied current reaches the axon, perhaps due to short-circuiting associated with inflammatory oedema, and that, as a result, the increases in threshold seen in CIDP are not solely due to demyelination, *per se*.

The recovery cycle

The recovery cycle findings cannot be explained by differences in membrane potential (see earlier) or temperature (because the duration of relative refractoriness was unaltered; see Kiernan and Bostock, 2001), but they can be explained by a smaller action potential. One would expect a greater driving current at demyelinated nodes in the patients (Bostock and Grafe, 1985; Kaji, 1997; Inglis *et al*., 1998; Kaji *et al*., 2000), but most of this current goes towards exciting the next node and the expected potential changes are actually smaller. A smaller action potential could also result from limitations on the driving current imposed by a reduced Na^+ channel density and the exposure of paranodal K^+ channels (Bowe *et al*., 1985; Eng *et al*., 1988). In addition to its effects on the recovery cycle, a smaller driving current would contribute to the reduction in conduction velocity in the patients.

Clinical implications

Threshold tracking measures the properties of the axonal membrane at the point of stimulation, not conduction over a length of axon. Hence the abnormalities described here result from neural pathology at the site of stimulation. Defects in conduction associated with these abnormalities have been the subject of a previous communication (Cappelen-Smith *et al*., 2000).

The extent of demyelination and its effects on axonal properties will vary for different axons in the same patient and for different patients. It is therefore not surprising that the variability of each measure was higher in the patients than in the controls. It is also possible that, by focusing on the significant differences in the mean response, variant behaviours may have gone undetected in the present study. Nevertheless, there were statistically significant differences between controls and patients and none between symptomatic patients and those in remission. This implies that excitability abnormalities persist despite restoration of secure conduction in some axons. Further implications are, first, that the present results cannot be extrapolated to acutely demyelinated axons and, secondly, that the measured properties are not accurate indices of disease activity.

In critically conducting human axons, it is probable that the driving current can last longer than 1 ms (Inglis *et al*., 1998), and the 1 ms threshold is therefore a more clinically relevant measure of threshold than the 0.2 ms threshold. In CIDP, thresholds were 3–4 times those of control subjects. Even accepting that part of this threshold increase was due to current short-circuiting, it is likely that greater current is required to keep an impaired axon conducting. It follows that motor axons in CIDP are much more likely to undergo conduction failure than healthy axons, much as has been demonstrated to occur when natural activity results in hyperpolarization of the active axons (Cappelen-Smith *et al*., 2000; see also Kaji *et al*., 2000).

References
Bostock H. The strength–duration relationship for excitation of myelinated nerve: computed dependence on membrane parameters. J Physiol (Lond) 1983; 341: 59–74.

Bostock H, Baker M. Evidence for two types of potassium channel in human motor axons in vivo. Brain Res 1988; 462: 354–58.

Bostock H, Bergmans J. Post-tetanic excitability changes and ectopic discharges in a human motor axon. Brain 1994; 117: 913–28.

Bostock H, Grafe P. Activity-dependent excitability changes in normal and demyelinated rat spinal root axons. J Physiol (Lond) 1985; 365: 239–57.

Bostock H, Rothwell JC. Latent addition in motor and sensory fibres of human peripheral nerve. J Physiol (Lond) 1997; 498: 277–94.

Bostock H, Sears TA, Sherratt RM. The spatial distribution of excitability and membrane current in normal and demyelinated mammalian nerve fibres. J Physiol (Lond) 1983; 341: 41–58.

Bostock H, Cikurel K, Burke D. Threshold tracking techniques in the study of human peripheral nerve. [Review]. Muscle Nerve 1998; 21: 137–58.

Bowe CM, Kocsis JD, Waxman SG. Differences between mammalian ventral and dorsal spinal roots in response to blockade of potassium channels during maturation. Proc R Soc Lond B Biol Sci 1985; 224: 355–66.

Brismar T. Electrical properties of isolated demyelinated rat nerve fibres. Acta Physiol Scand 1981; 113: 161–6.

Cappelen-Smith C, Kuwabara S, Lin CS-Y, Mogyoros I, Burke D. Activity-dependent hyperpolarization and conduction block in chronic inflammatory demyelinating polyneuropathy. Ann Neurol 2000; 48: 826–32.

Cornblath DR, Asbury AK, Albers JW, Feasby TE, Hahn AF, McLeod JG, et al. Research criteria for diagnosis of chronic inflammatory demyelinating polyneuropathy (CIDP). Neurology 1991; 41: 617–18.

Eng DL, Gordon TR, Kocsis JD, Waxman SG. Development of 4-AP and TEA sensitivities in mammalian myelinated nerve fibers. J Neurophysiol 1988; 60: 2168–79.

England JD, Levinson SR, Shrager P. Immunocytochemical investigations of sodium channels along nodal and internodal portions of demyelinated axons. [Review]. Microsc Res Tech 1996; 34: 445–51.

England JD, Happel LT, Liu ZP, Thouron CL, Kline DG. Abnormal distributions of potassium channels in human neuromas. Neurosci Lett 1998; 255: 37–40.

Hughes RA, Newsom-Davis JM, Perkin GD, Pierce JM. Controlled trial of prednisolone in acute polyneuropathy. Lancet 1978; 2: 750–3.

Inglis JT, Leeper JB, Wilson LR, Gandevia SC, Burke D. The development of conduction block in single human axons following a focal nerve injury. J Physiol (Lond) 1998; 513: 127–33.

Kaji R. Physiological and technical basis of peripheral nerve and motoneuron testing. In: Kimura J, Kaji R, editors. Physiology of ALS and related diseases. Amsterdam: Elsevier; 1997. p. 85–98.

Kaji R, Kojima Y. Pathophysiology and clinical variants of multifocal motor neuropathy. In: Kimura J, Kaji R, editors. Physiology of ALS and related diseases. Amsterdam: Elsevier; 1997. p. 85–98.

Kaji R, Bostock H, Kohara N, Murase N, Kimura J. Activity-dependent conduction block in multifocal motor neuropathy. Brain 2000; 123: 1602–11.

Kiernan MC, Bostock H. Effects of membrane polarization and ischaemia on the excitability properties of human motor axons. Brain 2000; 123: 2542–51.

Kiernan MC, Burke D, Andersen KV, Bostock H. Multiple measures of axonal excitability: a new approach in clinical testing. Muscle Nerve 2000; 23: 399–409.

Kiernan MC, Cikurel K, Bostock H. Effects of temperature on the excitability properties of human motor axons. Brain 2001; 124: 816–25.

Kuwabara S, Cappelen-Smith C, Lin CS-Y, Mogyoros I, Bostock H, Burke D. Excitability properties of median and peroneal motor axons. Muscle Nerve 2000; 23: 1365–73.

McDonald WI. Pathophysiology of conduction in central nerve fibres. In: Desmedt JE, editor. Visual evoked potentials in man: new developments. Oxford: Clarendon Press; 1977. p. 427–37.

Meulstee J, Darbas A, van Doorn PA, van Briemen L, van der Meché FG. Decreased electrical excitability of peripheral nerves in demyelinating polyneuropathies. J Neurol Neurosurg Psychiatry 1997; 62: 398–400.

Mogyoros I, Kiernan MC, Burke D. Strength–duration properties of human peripheral nerve. Brain 1996; 119: 439–47.

Mogyoros I, Lin CS-Y, Kuwabara S, Cappelen-Smith C, Burke D. Strength–duration properties and their voltage dependence as measures of a threshold conductance at the node of Ranvier of single motor axons. Muscle Nerve 2000; 23: 1719–26.

Schwarz JR, Corrette BJ, Mann K, Wiethölter H. Changes of ionic channel distribution in myelinated nerve fibres from rats with experimental allergic neuritis. Neurosci Lett 1991; 122: 205–9.

Shrager P. Sodium channels in single demyelinated mammalian axons. Brain Res 1989; 483: 149–54.

Waxman SG. Clinical course and electrophysiology of multiple sclerosis. In: Waxman SG, editor. Functional recovery in neurological disease. Adv Neurol, Vol. 47. New York: Raven Press; 1988. p. 157–84.

Waxman SG, Kocsis JD, Black JA. Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is re-expressed following axotomy. J Neurophysiol 1994; 72: 466–70.

Waxman SG, Cummins TR, Dib-Hajj S, Fjell J, Black JA. Sodium channels, excitability of primary sensory neurons, and the molecular basis of pain. [Review]. Muscle Nerve 1999; 22: 1177–87.

Weiss C. Sur la possibilité de rendre comparables entre eux les appareils à servant l'excitation électrique. Arch Ital Biol 1901; 35: 418–46.