Differences in accommodative properties of median and peroneal motor axons

S Kuwabara, C Cappelen-Smith, C S-Y Lin, I Mogyoros, D Burke

Abstract

Objectives—To investigate whether accommodation to depolarising and hyperpolarising currents differs for motor axons of human upper and lower limb nerves.

Methods—The threshold tracking technique was used to measure threshold electrotonus for median and peroneal motor axons. The threshold current that produced a compound muscle action potential 50% of maximum was measured, and membrane potential was altered using subthreshold polarising currents of 330 ms duration but of variable intensity, from +40% (depolarising) to -100% (hyperpolarising) of the unconditioned threshold.

Results—The maximal threshold changes (the peak of the S1 phase of threshold electrotonus) were significantly greater in median axons for both depolarising and hyperpolarising currents. The subsequent phases of accommodation to depolarising currents (S2) and to hyperpolarising currents (S3) were also significantly greater in median axons. These findings raised the possibility that greater accommodation (S2 and S3) in median axons resulted from greater changes in membrane potential. However, regression of S2 against S1 to depolarising currents disclosed significantly greater accommodation (27.8%) for median axons, suggesting that slow K⁺ conductances may be more prominent on median than peroneal axons. By contrast, the relation between S3 and S1 to hyperpolarising currents was similar for the two nerves, suggesting that the difference in inward rectification was merely because the conductance varies with the extent of hyperpolarisation.

Conclusions—Slow K⁺ conductances are more prominent for median motor axons than for peroneal axons. It would therefore be expected that axons innervating the lower limbs have less protection from depolarising stress and could develop ectopic activity more readily.

(J Neurol Neurosurg Psychiatry 2001;70:372-376)

Keywords: threshold electrotonus; threshold tracking; slow K⁺ channel; inward rectification

There are subtle differences in axonal properties in different human nerves, presumably reflecting adaptation to the discharge patterns normally carried by axons. For example, sensory axons have different strength-duration properties,^{1,2} presumably due to a more promi-

nent Na⁺ conductance which is active at resting membrane potential,³ and greater inward rectification than motor axons.⁴ In addition, it has recently been reported that there are significant differences in axonal properties between nerves in the upper and lower limbs.^{5,6}

Positive and negative symptoms in patients with polyneuropathy are more prominent in the lower limbs, and this may partly result from differences in axonal properties between nerves in the upper and lower limbs. It is not surprising that there may be biophysical differences in motor axons innervating muscles in the hand and foot, because their maximal discharge rates and discharge patterns probably differ.⁷⁻⁹ A previous study comparing median and peroneal motor axons suggested that accommodation to prolonged depolarising stimuli (presumably reflecting slow K⁺ channel activity) and accommodation to hyperpolarising stimuli (probably reflecting inward rectification) are more prominent in median axons.⁶ However, the study also found that median axons have greater threshold changes to both depolarising and hyperpolarising conditioning currents. Assuming that threshold varies with membrane potential, this raises the possibility that the more prominent accommodation on median axons was merely the result of a greater change in membrane potential. To demonstrate that there are differences in internodally located conductances responsible for accommodation to depolarising and hyperpolarising currents would require studies in which accommodation is measured in response to similar changes in membrane potential.

The present study was undertaken to investigate whether there are quantitative differences in accommodation between median and peroneal motor axons, by comparing the accommodative changes in threshold in response to currents of graded intensity. The data suggest that the two nerves respond differently to prolonged subthreshold currents and, accordingly, it might be expected that they have different behaviour under stress.

Methods

SUBJECTS

Fourteen experiments were performed on seven normal volunteers (aged 24 to 55 years, mean 36 years), who had no clinical or electrophysiological evidence of a peripheral nerve disorder. The subjects gave informed consent to the experimental procedures, which had been approved by the committee on experimental procedures involving human subjects of the University of New South Wales.

Department of Neurology, The Prince of Wales Hospital and Prince of Wales Medical Research Institute, University of South Wales, Barker Street, Randwick, NSW 2031, Sydney, Australia
S Kuwabara
C Cappelen-Smith
C S-Y Lin
I Mogyoros (deceased)
D Burke

Correspondence to:
Dr S Kuwabara
kwbr@mb.infoweb.ne.jp

Received 8 May 2000 and in revised form
8 November 2000
Accepted 22 November 2000

METHODS

In all studies, the current required to produce a compound muscle action potential (CMAP) that was 50% of maximum was determined using a computerised threshold-tracking program (QTRAC20, © Institute of Neurology, Queen Square, London, UK).^{10,11} The median CMAP was recorded from the abductor pollicis brevis, and the peroneal CMAP from the extensor digitorum brevis. The peroneal nerve was chosen in the lower limb because it innervates only one intrinsic muscle of the foot, such that the EMG recording would not be contaminated by activity from other muscles. For nerve stimulation, the active electrode was over the nerve at the wrist for the median nerve and at the ankle for the peroneal nerve, and the reference electrode was 10 to 20 cm proximal to the active electrode. Skin temperature was measured near the stimulus site, and was maintained at or above 30°C using blankets and a heater when necessary. Temperatures were 32.1 (0.8)°C at the wrist, and 31.4 (1.1)°C at the ankle (mean (SD)). It should be noted that threshold electrotonus is relatively insensitive to temperature, at least within the range 30°C–34°C.¹²

To investigate the effects of changing membrane potential, subthreshold depolarising or hyperpolarising DC of 300 ms duration was used to activate the accommodative conductances. Figure 1 shows the changes in threshold for the test CMAP produced by the polarising currents (threshold electrotonus) from one subject. Threshold currents required to produce an unconditioned CMAP of 50% maximum were measured using a test stimulus of

Figure 1 Threshold electrotonus of median (filled circles) and peroneal (open circles) motor axons from a normal subject. Threshold currents required to produce a compound muscle action potential of 50% maximum were measured using test stimuli of 1.0 ms duration. Polarising currents were of 300 ms duration, and their intensity was set +40%, -40%, and -80% of the unconditioned threshold. The F, S1, S2, and S3 phases are defined as in Vagg et al.²³ (see also text), for the 40% depolarising current and the 80% hyperpolarising current. In accordance with convention,¹⁰ threshold reduction is plotted on the y axis so that responses to depolarising and hyperpolarising currents are upwards and downwards, respectively. The interrupted vertical lines are at 25 ms and 100 ms—that is, at the peaks of S1_d and S1_h, respectively.

1.0 ms duration. The strength of the DC polarising current was +40% (depolarising), and -40% and -80% (hyperpolarising) of the unconditioned threshold. Threshold measurements were made from 10 ms before the onset of the polarising currents to 50 ms after their end. The peaks of the maximal threshold changes occurred at ~25 ms with depolarising currents and at ~100 ms with hyperpolarising currents (fig 1).

Based on the threshold electrotonus curves in fig 1, the changes in threshold for the test potential were measured 25 ms, 100 ms, and 300 ms after the onset of subthreshold polarising currents lasting 330 ms, using a 1.0 ms test stimulus. The strength of the polarising current was varied systematically from +40% (depolarising) to -100% (hyperpolarising) of the unconditioned threshold in 10% steps. The changes in threshold produced by the DC polarising current were normalised to the threshold for an unconditioned CMAP, and were defined as follows (fig 1):

F = the abrupt threshold change corresponding to the first electrotonic response to the polarising current.

S1 (depolarising) = first slow phase of threshold electrotonus to depolarising current = $T_{25d} - F$.

S1 (hyperpolarising) = first slow phase of threshold electrotonus to hyperpolarising current = $T_{100h} - F$.

S2 = accommodation to depolarising potential changes = $T_{25d} - T_{300d}$.

S3 = accommodation to hyperpolarising potential changes = $T_{100h} - T_{300h}$.

where T_{25} , T_{100} , and T_{300} represent normalised threshold values measured 25 ms, 100 ms, and 300 ms after the onset of depolarising (d) or hyperpolarising (h) currents, respectively.

Although the onsets of S2 and S3 occur at ~25 ms and ~100 ms respectively, this does not imply that the conductances responsible for the accommodative threshold changes are inactive until these intervals. However, the change in membrane potentials driving the conductance are probably maximal at or near these intervals.

Results

The threshold changes produced by DC lasting 300 ms are illustrated for one subject in fig 1. For equivalently strong currents, accommodation to both depolarising current (S2) and hyperpolarising current (S3) was greater for median axons than peroneal axons. However, the maximal threshold changes (S1_d and S1_h) were also greater for median axons.

POLARISING LEVEL AND S1

Figure 2 shows the relation between the strength of the polarising currents and the change in threshold produced by prolonged subthreshold depolarising and hyperpolarising currents. The maximal threshold changes measured 25 ms after the onset of depolarisation (equivalent to F + S1_d) were greater for median motor axons than for peroneal motor axons in all seven subjects ($p = 0.0008$ for the 40% depolarising current, paired t test; fig 2

Figure 2 Polarising level and maximal threshold change to (A) depolarising and (B) hyperpolarising currents. $F+S1_d$ is the threshold measured 25 ms after the onset of depolarising current. $S1_h$ is the threshold at 100 ms after the onset of hyperpolarising current minus the fast threshold change, F . * $p=0.0008$; ** $p=0.009$, paired t test.

A). The relations were roughly linear and the slopes of the regression lines for the two nerves differed significantly ($p<0.05$, t test for slope). For the response to depolarising currents, the peak threshold change ($F+S1_d$) was measured because the accommodative conductance (a slow K^+ conductance, G_{Ks}) is located on both the nodal and internodal membrane. The findings were similar whether $F+S1_d$ or only $S1_d$ was used to reflect the threshold change during S2.

With hyperpolarising currents, only the slow (S1) phase of threshold electrotonus was measured, excluding the initial rapid threshold change (F), because inward rectification is located in the internode. It should be noted that the findings were similar whether $F+S1_h$ or $S1_h$ was used to reflect the threshold change driving S3. The extent of S1 on hyperpolarisation was again greater for median axons for six of seven subjects and identical in the seventh, a difference that was significant ($p=0.009$ for the 100% hyperpolarising current, paired t test; fig 2 B). The differences between the nerves were greater the stronger the depolarising or hyperpolarising current. These findings showed that depolarising and hyperpolarising currents produce greater changes in threshold for median motor axons, presumably because they produce greater changes in membrane potential.

POLARISING LEVEL AND ACCOMMODATION

Figure 3 A presents the relation between the polarising level and the difference in the threshold changes measured 25 ms and 300 ms

Figure 3 Polarising level and accommodation. (A) S2 is the difference in threshold between 25 ms and 300 ms after the onset of the depolarising current. (B) S3 is the difference in threshold between 100 ms and 300 ms after the onset of hyperpolarising current.

after the onset of the prolonged subthreshold depolarising current. The S2 accommodative phase was estimated by subtracting the threshold change at 300 ms from the threshold change at 25 ms. S2 was greater in median axons.

To measure S3, the threshold change produced by a subthreshold hyperpolarising current at 300 ms was subtracted from the threshold change at 100 ms. For six of seven subjects both S2 and S3 accommodation were greater in response to the same polarisation level in median axons, and they were the same in the seventh subject (fig 3 A and B). These differences in the S2 and S3 phases of the threshold electrotonus raise the possibility that median motor axons have a greater slow K^+ conductance and greater inward rectification than peroneal motor axons. However, these differences might be explained by greater changes in membrane potential produced by the conditioning current. To determine whether this was the case, the relation between the accommodative responses and S1 was investigated.

RELATION BETWEEN ACCOMMODATION AND THE DRIVING THRESHOLD CHANGE

Figure 4 A shows the relation between the driving threshold change measured as $F+S1_d$, and the S2 accommodation. There was a linear relation for both median and peroneal motor axons. The slope was significantly steeper for median motor axons than for peroneal axons ($p=0.04$, t test for slope). With median axons, a

Figure 4 Relation between accommodation and driving threshold change. (A) “F+S1_d” is threshold measured 25 ms after the onset of depolarising current reflecting the change in membrane potential that drives S2. In response to a ~60% change in “F+S1_d”, median axons have significantly greater S2 than peroneal axons ($p=0.01$, paired t test). Moreover, the slope of the regression line was significantly steeper for median axons ($p=0.04$, t test for slope). (B) S1_h is the threshold 100 ms after the onset of hyperpolarising current minus F. The relation between S1_h and S3 is similar for the two nerves.

30% depolarising current produced a 60% decrease in threshold, whereas in peroneal axons a 40% depolarising current was required to produce the same decrease in threshold. For a 60% decrease in threshold, S2 was significantly greater in median axons ($p=0.01$, paired t test). The difference in the slopes suggest that median axons have 27.8% greater S2 accommodation in response to the same extent of S1.

Figure 4 B shows the relation of S3 to the driving threshold change, S1_h, during prolonged hyperpolarising currents. There was a similar relation for median motor axons and peroneal motor axons, apart from the greater maximal S1 threshold increase in median axons. This finding suggests that the difference in S3 accommodation in fig 3 B can be fully explained by the difference in S1_h in fig 2 B.

Discussion

This study has documented differences between motor axons in the median and peroneal nerves. Firstly, the applied currents produced greater changes in threshold (S1) and presumably greater changes in membrane potential for median axons. Secondly, in response to the same change in threshold (and presumably the same change in membrane potential), the S2

phase of threshold electrotonus was significantly greater in median motor axons than in peroneal axons. Thirdly, the S3 phase was similar between the two nerves when the driving threshold changes were matched. The S2 phase of threshold electrotonus mainly reflects nodal and internodal slow K⁺ conductances, whereas S3 is largely due to inward rectification, a conductance located in the internode.¹¹ The results therefore provide evidence that there is an internerve difference in the expression of slow K⁺ conductances but not in inward rectification.

The possibility that factors such as axonal size and skin resistance affected the results of threshold electrotonus cannot be excluded. However, a difference in the threshold changes during polarising currents is unlikely to be explicable by such differences. Refractoriness after a conditioning stimulus is inversely related to axonal size, but there is no significant difference in refractoriness of median axons at the wrist and of peroneal axons at the ankle.⁶ Skin resistance would have little or no effect on the results because the changes in threshold current were normalised to the unconditioned threshold.

S1 is thought to reflect changes in membrane potential of the internodal axolemma, caused by current passing through or under the myelin sheath.¹³ The magnitude of S1 depends on the access resistance to the internodal axolemma and also membrane potential. For example, when an axon is depolarised, the resistance of the internodal axolemma falls, due to activation of voltage dependent potassium channels, and S1 decreases significantly.^{11–14} The differences seen in the present study are unlikely to be due to differences in membrane potential because axonal excitability parameters sensitive to the membrane potential (refractoriness and strength-duration time constant)^{15–16} do not differ for median and peroneal motor axons.⁶ The differences in S1 could reflect the resistance of the internodal axolemma, although whether this is due to a difference in expression of ion channels or passive membrane properties is unknown at present.

Slow K⁺ channels are voltage dependent: ~30% are open at resting membrane potential¹⁷ and more are activated as the axon is depolarised¹¹ but, as mentioned above, a significant difference in membrane potential is unlikely. We therefore suggest that median motor axons have more functional slow K⁺ channels than peroneal motor axons. The difference in expression of nodal and internodal slow K⁺ conductances could be related to differences in the habitual discharge patterns of median and peroneal motor axons. Maximal firing rates in thenar motor units are about 30 Hz,^{7–8} though they may reach 100 Hz.¹⁸ Maximal firing rates of 25–30 Hz have been reported for motor axons of tibialis anterior,^{9–10} but there are limited data on the discharge patterns during common daily tasks. The thenar muscles are used more than the muscles in the foot for discrete movements, and habitual discharge patterns are likely to differ significantly, even if

motor axons innervating both muscles can be driven to similar rates.

In some human peripheral neuropathies such as in diabetes mellitus or vasculitic disorders, depolarising stresses are created by nerve ischaemia or paralysis of the $\text{Na}^+\text{-K}^+$ pump.²⁰ A lesser accommodation to depolarisation in motor axons innervating the lower limb muscles would provide less protection from depolarising stress. This could be a factor in the greater tendency for lower limb motor axons to become ectopically active, thus explaining why fasciculation, myokymia, and cramp are more prominent in lower limb muscles, both in normal subjects and in patients with peripheral neuropathy.

Our results showed that the extent of inward rectification was not significantly different for median and peroneal motor axons. Inward rectification is activated by prolonged hyperpolarisation, and produces a depolarising accommodation, limiting the extent of hyperpolarisation.^{4 11 21} Inward rectification could become significant when the axons conduct impulse trains. During prolonged impulse trains, human axons undergo substantial hyperpolarisation^{22 23} and this can lead to conduction block at the sites of impaired safety margin for impulse conduction.²⁴ Our results, however, do not support the hypothesis that a difference in the extent of inward rectification in motor axons of the different nerves contributes to more prominent weakness in the distal lower limbs of patients with polyneuropathy.

We conclude that slow K^+ conductances are not identical in different motor axons. It can therefore be expected that upper and lower limb axons will respond differently to depolarising stress, and this may contribute to the geographic differences in positive motor symptoms.

This study was supported by the National Health and Medical Research Council of Australia, the National Multiple Sclerosis Association of Australia, and the Uehara Memorial Foundation (Japan).

- 1 Panizza M, Nilsson J, Roth BJ, et al. The time constants of motor and sensory peripheral nerve fibers measured with the method of latent addition. *Electroencephalogr Clin Neurophysiol* 1994;93:147–54.
- 2 Mogyoros I, Kiernan MC, Burke D. Strength-duration properties of human peripheral nerve. *Brain* 1996;119:439–47.

- 3 Bostock H, Rothwell JC. Latent addition in motor and sensory fibers of human peripheral nerve. *J Physiol (Lond)* 1997;498:277–94.
- 4 Bostock H, Burke D, Hales JP. Differences in behaviour of sensory and motor axons following release of ischaemia. *Brain* 1994;117:225–34.
- 5 Lin C, Mogyoros I, Burke D. Differences in the properties of cutaneous afferents in median and sural nerves. *Muscle Nerve* 2000;23:763–70.
- 6 Kuwabara S, Cappelen-Smith C, Lin C, et al. Differences in excitability properties of median and peroneal motor axons. *Muscle Nerve* 2000;23:1365–73.
- 7 Bellemare F, Woods JJ, Johansson R, et al. Motor-unit discharge rates in maximal voluntary contraction of three human muscles. *J Neurophysiol* 1983;50:1380–92.
- 8 Bigland-Ritchie BR, Dawson NJ, Johansson RS, et al. Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. *J Physiol (Lond)* 1986;379:451–9.
- 9 Macefield VG, Gandevia SC, Bigland-Ritchie B, et al. The firing rates of human motoneurons voluntarily activated in the absence of muscle afferent feedback. *J Physiol (Lond)* 1993;471:429–43.
- 10 Bostock H, Baker M. Evidence for two types of potassium channel in human motor nerves in vivo. *Brain Res* 1988;462:354–8.
- 11 Bostock H, Cikurel K, Burke D. Threshold tracking techniques in the study of human peripheral nerve. *Muscle Nerve* 1998;21:137–58.
- 12 Kiernan M, Burke D, Andersen K, et al. Multiple measures of axonal excitability: a new approach in clinical testing. *Muscle Nerve* 2000;23:399–409.
- 13 Barrett EF, Barrett JN. Intracellular recording from vertebrate myelinated axons. *J Physiol (Lond)* 1982;323:117–44.
- 14 Baker M, Bostock H. Depolarization changes the mechanism of accommodation in rat and human motor axons. *J Physiol (Lond)* 1989;411:545–61.
- 15 Burke D, Mogyoros I, Vagg R, et al. Quantitative description of the voltage dependence of axonal excitability in human cutaneous afferents. *Brain* 1998;121:1975–83.
- 16 Burke D, Mogyoros I, Vagg R, et al. Temperature dependence of excitability indices of human cutaneous afferents. *Muscle Nerve* 1999;22:51–61.
- 17 Röper J, Schwarz JR. Heterogeneous distribution of fast and slow potassium channels in myelinated rat nerve fibres. *J Physiol (Lond)* 1989;416:93–110.
- 18 Marsden CD, Meadows JC, Merton PA. Isolated single motor units in human muscle and their rate of discharge during maximal voluntary effort. *J Physiol (Lond)* 1971;217:12–3.
- 19 Bigland-Ritchie BR, Furbush FH, Gandevia SC, et al. Voluntary discharge frequencies of human motoneurons at different muscle length. *Muscle Nerve* 1992;15:130–7.
- 20 Quasthoff S. The role of axonal ion conductances in diabetic neuropathy: a review. *Muscle Nerve* 1998;21:1246–55.
- 21 Pape HC. Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annu Rev Physiol* 1996;58:299–327.
- 22 Kiernan MC, Mogyoros I, Hales J P, et al. Excitability changes in human cutaneous afferents induced by prolonged repetitive axonal activity. *J Physiol (Lond)* 1997;500:255–64.
- 23 Vagg R, Mogyoros I, Kiernan M, et al. Activity-dependent hyperpolarization of human motor axons produced by natural activity. *J Physiol (Lond)* 1998;507:919–25.
- 24 Bostock H, Grafe P. Activity-dependent excitability changes in normal and demyelinated rat spinal root axons. *J Physiol (Lond)* 1985;365:229–57.