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Strength–duration properties of sensory and motor axons in amyotrophic lateral sclerosis

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Summary

In normal subjects, the strength–duration time constant is longer for cutaneous afferents than for motor axons, probably because the former express a greater non-inactivating (persistent) Na^+ conductance that is active at threshold. Using a threshold-tracking system the strength–duration properties of cutaneous afferents and motor axons were recorded from 23 patients with amyotrophic lateral sclerosis, and compared with those of 32 healthy subjects. In control subjects and patients, the strength–duration time constant of sensory fibres declined with age, and there was no difference between the two groups when age was taken into account. The motor time constant did not change with age when expressed as a percentage of the time constant for sensory fibres in the same nerve, but was significantly longer for the patients than control subjects. In addition, motor rheobase was significantly lower for the patients, when expressed as a percentage of sensory rheobase. There was an inverse relationship between the time constant and rheobase for sensory and motor axons, and this was the same for the patients and the control subjects, suggesting that the variations in time constant within and between the groups were related to the expression of a common factor. Measurements of refractoriness and supernormality provided no evidence for a difference in resting membrane potential between the patients and control subjects. These findings are consistent with the interpretation that motor axons of the patients with amyotrophic lateral sclerosis have a greater persistent Na^+ conductance than normal motor axons. This could contribute to the ectopic activity responsible for fasciculation.

Keywords: amyotrophic lateral sclerosis; fasciculation; strength–duration properties; Na^+ conductance; motor axon

Abbreviations: ALS = amyotrophic lateral sclerosis; CMAP = compound muscle action potential; CSAP = compound sensory action potential

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive fatal neurodegenerative disorder of unknown pathogenesis. Fasciculation is a prominent feature of ALS and is due to ectopic activity of motor axons. The ectopic activity usually arises from the axon, particularly its terminals (Wettstein, 1979; Conradi *et al.*, 1982; Roth, 1982, 1984; Layzer, 1994), but it is likely that some fasciculation arises more proximally in the axon (Layzer, 1994) and this would imply a widespread disturbance of axonal excitability. This disturbed axonal function presumably reflects disturbed neuronal function and, accordingly, studies on peripheral nerve axons might reveal abnormalities of pathogenetic importance.

Nerve excitability is dependent on many factors, but perhaps the most important are voltage-dependent Na^+ channels. The strength–duration time constant of a nerve is a nodal property that is longer for cutaneous afferents than for motor axons (Panizza *et al*., 1992, 1994; Mogyoros *et al*., 1996). This difference between sensory and motor axons appears to be due to a greater voltage-dependent Na^+ conductance that is active at resting membrane potential in cutaneous afferents (Bostock and Rothwell, 1997), probably a non-inactivating Na^+ conductance due to 'persistent' Na^+ channels (see Crill, 1996; Baker and Bostock, 1997). The longer strength–duration time constant and the greater tendency of sensory axons to become ectopically active, e.g. during ischaemia and hyperventilation, are probably related

to the greater representation of this Na^+ conductance on cutaneous afferents (Mogyoros *et al.*, 1997*b*). While a number of factors can affect the time constant, including the membrane potential (Bostock, 1983; Bostock and Rothwell, 1997), its measurement may provide an indirect indication of the persistent Na^+ conductance (Mogyoros *et al.*, 1997*b*).

The present study was undertaken to determine the strength–duration properties of cutaneous afferents and motor axons of patients with ALS. The findings raise the possibility that motor axons in ALS patients may have a greater persistent Na^+ conductance than those in age-matched control subjects, and that this difference is not due to a difference in resting membrane potential.

Methods

Experiments were performed on 23 patients who conformed to the El Escorial criteria (World Federation of Neurology Research Group on Neuromuscular Diseases, 1994) for clinically probable or definite ALS (eight female and 15 male; aged 40–74 years, mean ± SD 59.1 ± 10.7 years). All patients and control subjects gave informed consent to the experimental procedures, which had the approval of the Committee on Experimental Procedures Involving Human Subjects of the University of New South Wales. The results obtained from the patients were compared with those for 32 healthy control subjects (15 male, 17 female; aged 21–68 years, mean ± SD 40.4 ± 14.1 years). The control group was divided into a young subgroup (<40 years, range 21–39 years, mean ± SD 29.5 ± 5.4 years; $n = 17$) and an older subgroup (>40 years, range 40–68 years, mean ± SD 52.8 ± 10.0 years; $n = 15$), and data are presented as such in the figures and text. The younger control subjects were from a previous study (Mogyoros *et al.*, 1996) and the older subjects were mostly the carers for the patients, only one of whom was a blood relative of the patient. Recruitment of elderly control subjects was limited by the need to exclude subjects who suffered from a disease process likely to affect peripheral nerve function. There was no significant difference between the mean ages of the patients and of the older control subjects ($P = 0.24$, one-tailed Student's t test).

The strength–duration properties of cutaneous afferents and motor axons were determined using a computerized threshold tracking procedure as described in full elsewhere (Mogyoros *et al.*, 1996). The median nerve was stimulated at the wrist level using surface electrodes of 1-cm diameter, taped to the skin 4 cm apart. The stimulus was a square-wave pulse with rise and fall times of 10 μs for a 40–50 mA stimulus. The antidromic compound sensory action potential (CSAP) was recorded from the index finger using ring electrodes 3 cm apart around the proximal phalanx. The orthodromic compound muscle action potential (CMAP) was recorded using surface electrodes over the abductor pollicis brevis. Skin temperature for control subjects aged <40 years was 33.5 ± 0.19°C (mean ± SEM; $n = 17$), for control subjects aged >40 years 32.9 ± 0.17°C ($n = 15$) and for patients 32.8 ± 0.12°C ($n = 23$).

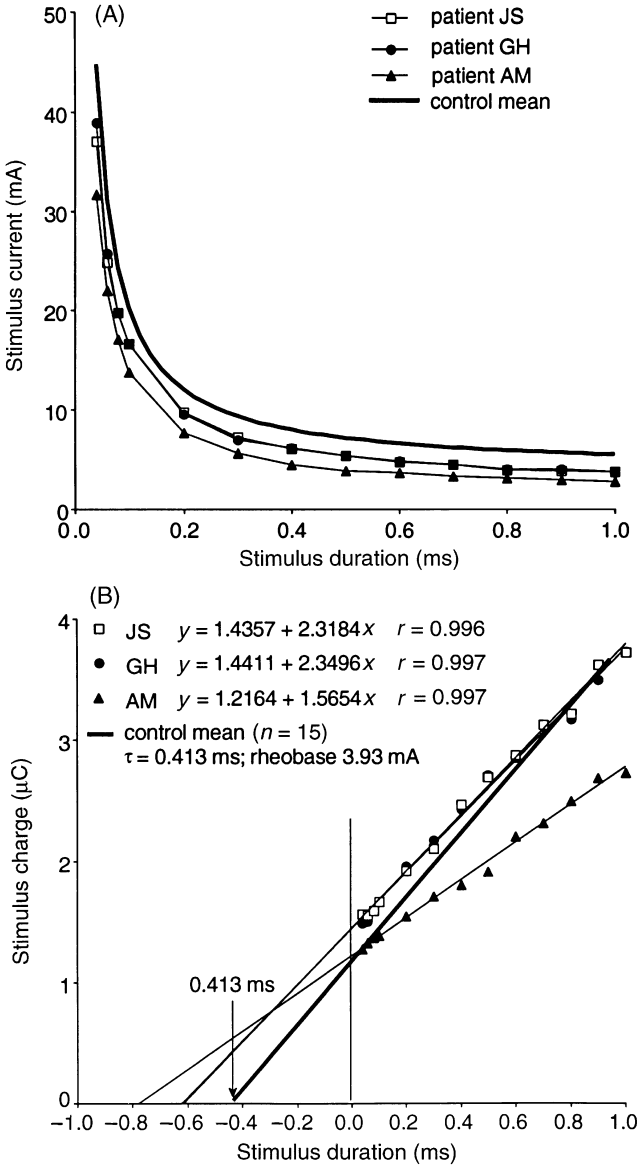

Fig. 1 (**A**) Strength–duration curves of motor axons obtained from three patients, and the mean curve for control subjects aged >40 years; the latter was recalculated from the mean time constant and mean rheobase. (**B**) Strength–duration time constant of the same three patients and the mean control determined from Weiss' formula using plots of threshold charge against stimulus duration. The time constant is given by the (negative) intercept of the linear regression line on the duration axis. The rheobase is given by the slope of the regression line.

The threshold currents required to produce a CSAP and a CMAP of 30–40% of maximum were determined by computer, using 5–12 stimulus durations between 40 μs and 1 ms (Fig. 1A). The resulting strength–duration curve is hyperbolic (Weiss, 1901; Bostock, 1983; Mogyoros *et al.*, 1996), and the strength–duration time constants for cutaneous afferent and motor axons were calculated using Weiss' formula (Weiss, 1901), according to which there is a linear

relationship between stimulus charge and stimulus duration. The time constant (or, more correctly, chronaxie) is given by the negative intercept of the regression line on the duration axis and the rheobase by the slope of the regression line (Fig. 1B). The time constant reflects the rate of decline of the current threshold with increasing stimulus duration. Rheobase is the threshold current if the stimulus could be infinitely long.

As an assessment of differences in resting membrane potential, the threshold decrease during the supernormal period and threshold increase produced by refractoriness for patients with ALS were compared with those for age-matched control subjects. These measures of nerve excitability are sensitive to changes in membrane potential and have been used as surrogate measures in previous studies (Kiernan *et al*., 1997; Mogyoros *et al*., 1997*b*). The degree of supernormality or refractoriness was measured as the change in threshold of a test potential when it was conditioned by a supramaximal stimulus delivered 7 ms or 2 ms earlier, respectively. The stimulus duration was 0.1 ms. The maximal potential produced by the supramaximal conditioning stimulus contaminated the conditioned potential at conditioning–test intervals of <10 ms. The conditioned potential was therefore measured after subtraction of the response to the conditioning stimulus, the subtraction being performed on line by computer (Kiernan *et al*., 1996).

As detailed in the Results section and figures, not all of the above parameters of nerve excitability could be measured in each patient. In particular, a maximal CMAP was not recorded in four patients; in two of these, the thenar muscles were so wasted that no motor studies could be performed. Data were first shown to conform to a normal distribution (Kolmogorov–Smirnov Test). Linear regression was used to look for age-related changes and an ANOVA (analysis of variance) was performed to determine whether there were significant differences in time constant and rheobase between the patients and control subjects when the effects of age were controlled. Supernormality and refractoriness of sensory and motor axons in the patients were compared with those in the older control subjects (see above) using Student's *t* test. Unless otherwise specified, data are given as mean ± SEM.

Results

There was no significant difference in the size of the CSAP recorded in the patients with ALS and that in the control subjects aged >40 years (17.3 ± 1.6 μV versus 19.6 ± 1.6 μV, $P = 0.427$). A CMAP could be recorded in 19 of the 23 patients with ALS, and was significantly smaller than for the control group (2.45 ± 0.28 mV versus 4.5 ± 0.449 mV, $P = 0.0009$).

Strength–duration time constant and rheobase in healthy subjects

There was a significant negative correlation for the entire population between age and the time constant for cutaneous afferents ($r = –0.38$; $P < 0.01$) but not for motor axons. As a result the sensory–motor difference in time constant also decreased with age ($r = –0.31$; $P < 0.05$).

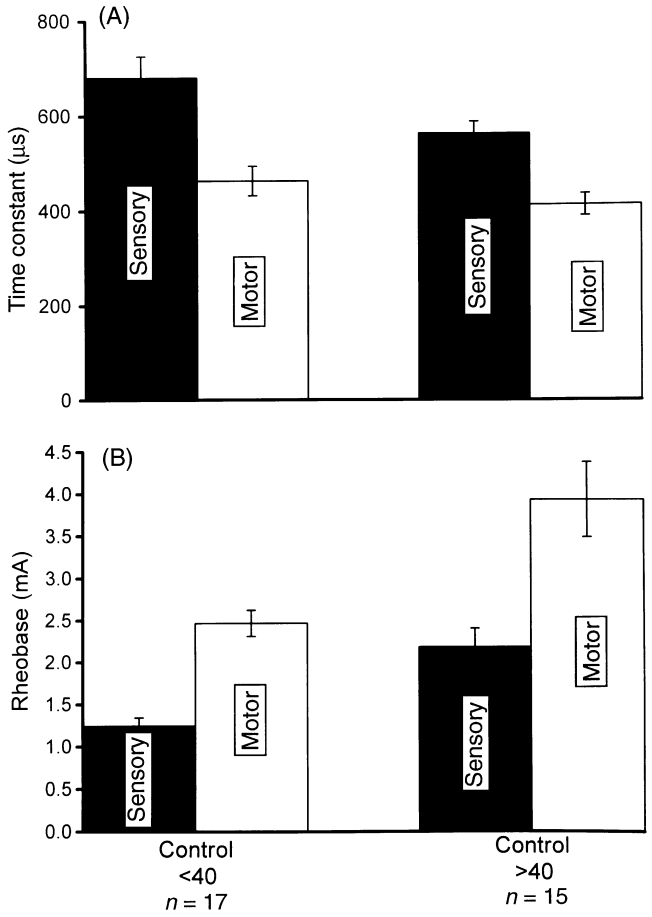

Fig. 2 Age-related changes in strength–duration time constant (**A**) and rheobase (**B**) for cutaneous afferents and motor axons in the control subjects (mean ± SEM).

The data for control subjects were therefore divided into two groups, younger (<40 years; $n = 17$) and older (>40 years; $n = 15$); this cut-off age was chosen because the youngest patient with ALS was aged 40 (Fig. 2). The mean strength–duration time constant of cutaneous afferents was longer in the younger control subjects (<40 years) than in the older control subjects (>40 years) (681.1 ± 45.3 μs versus 566.1 ± 26.3 μs, respectively; $P = 0.001$; Fig. 2). The mean strength–duration time constant for motor axons was slightly longer in the younger group but not significantly so (young, 463.2 ± 31.1 μs; older, 411 ± 23.9 μs; $P = 0.12$). The rheobase for cutaneous afferents and motor axons was significantly greater in the older control group (Fig. 2): for sensory axons 1.24 ± 0.1 mA for younger control subjects and 2.22 ± 0.226 mA for older control subjects ($P = 0.0009$), and for motor axons 2.46 ± 0.155 mA for younger control subjects and 3.97 ± 0.459 mA for older control subjects ($P = 0.0067$).

Together these findings are consistent with an age-related decrease in a conductance that is normally expressed more

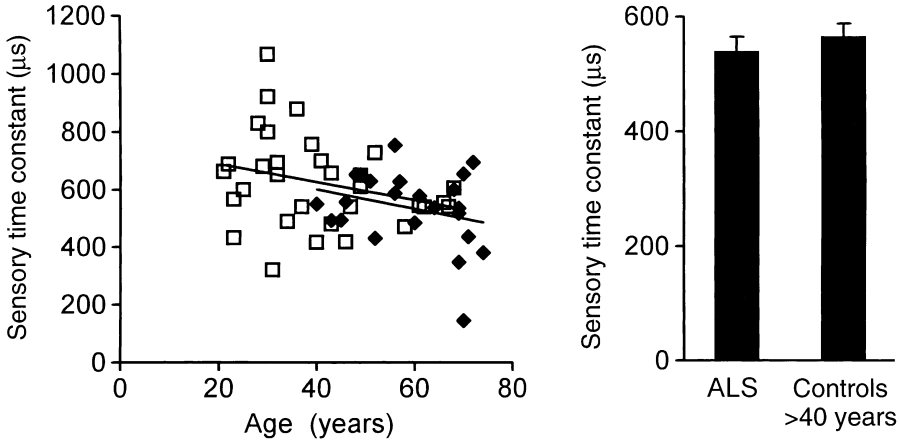

Fig. 3 The relationships between strength–duration time constant for sensory axons and age for patients (open symbols) and control subjects (filled symbols). The data form a homogeneous relationship. For sensory axons the regression lines for the patient data (open symbols) and the control data (filled symbols) are virtually identical. The right panel presents the strength–duration time constant (mean + SEM) for sensory axons for control subjects aged >40 years and patients.

on sensory axons, and which both increases the strength–duration time constant and reduces the rheobase.

Strength–duration time constant and rheobase in patients with ALS

There was a slight age difference between the patients and the older control subjects (59.1 ± 10.7 years, 52.8 ± 10 years, respectively; $P = 0.24$), a factor that was probably responsible for the slightly lower sensory time constant in patients than in the older control subjects (538.2 ± 27.5 μs versus 566.1 ± 26.3 μs; $P = 0.489$). Indeed, when sensory time constants were plotted against age, the patient and control data formed a homogeneous relationship and, when regressed separately, there was no difference in the slopes of the regression lines of the sensory time constant against age for the patients and the control subjects (Fig. 3).

The motor time constants of the patients (474 ± 31 μs) were greater than those of the older control subjects (411 ± 24 μs), but this difference was not significant ($P = 0.123$). To test whether a real difference was being masked by differences in age or other factors affecting both motor and sensory fibres, we also expressed each motor time constant as a percentage of the sensory time constant recorded from the same nerve (Fig. 4A). These motor : sensory ratios were not correlated with age in either control or patient population ($r = 0.086$, $P = 0.637$ and $r = –0.006$, $P = 0.98$, respectively). However, the motor : sensory ratios showed less scatter than the motor time constants themselves, and were significantly greater for the patient group (91.1 ± 4.9%) than either the younger (71.6 ± 5.5 %, $P = 0.0126$) or older (73.2 ± 2.6 %, $P = 0.003$) control groups (histograms in Fig. 4A). These results indicate that the normal difference in strength–duration relationships, between motor and sensory axons, is reduced or absent in the patients with ALS.

Further evidence for an abnormality of motor axon excitability in ALS was provided by the measurements of rheobase. Sensory rheobases were no different in the patients from those in age-matched control subjects ($P = 0.729$), whereas motor rheobases were lower, though not significantly so ($P = 0.21$). However, when expressed as percentages of the sensory rheobases in the same nerve, the motor rheobases were significantly lower in the patients (149.1 ± 11.6%, $n = 21$) than in either the younger (207 ± 12%, $n = 17$, $P = 0.0012$) or the older control subjects (183.1 ± 10%, $n = 15$, $P = 0.00162$; Fig. 4B). As with the time constants, expressing the motor rheobases as percentages of the sensory values reduced the variance in the values due to the relationship with age (and possibly to other variables concerned with access of the delivered current to the axons) and allowed an effect of the disease to become evident.

Motor axons therefore have both a lower rheobase and a longer strength–duration time constant in ALS than expected from the properties of the sensory axons in the same nerve, although both properties of the sensory axons appear indistinguishable from those in the age-matched control subjects. We conclude, therefore, that motor axons are abnormally excitable in ALS, with properties more like those of sensory axons.

Correlation between strength–duration time constant and rheobase

Considering all data (cutaneous afferent and motor) for the 32 control subjects, there was a reciprocal relationship between strength–duration time constant and rheobase, best demonstrated when the time constant was plotted against the logarithm of rheobase (Fig. 5). A relationship of this kind might be expected if both depend on a common factor, such as resting potential or a nodal ion conductance (Mogyoros *et al.*, 1996; Bostock and Rothwell, 1997). There was a virtually identical relationship for the patients, with no

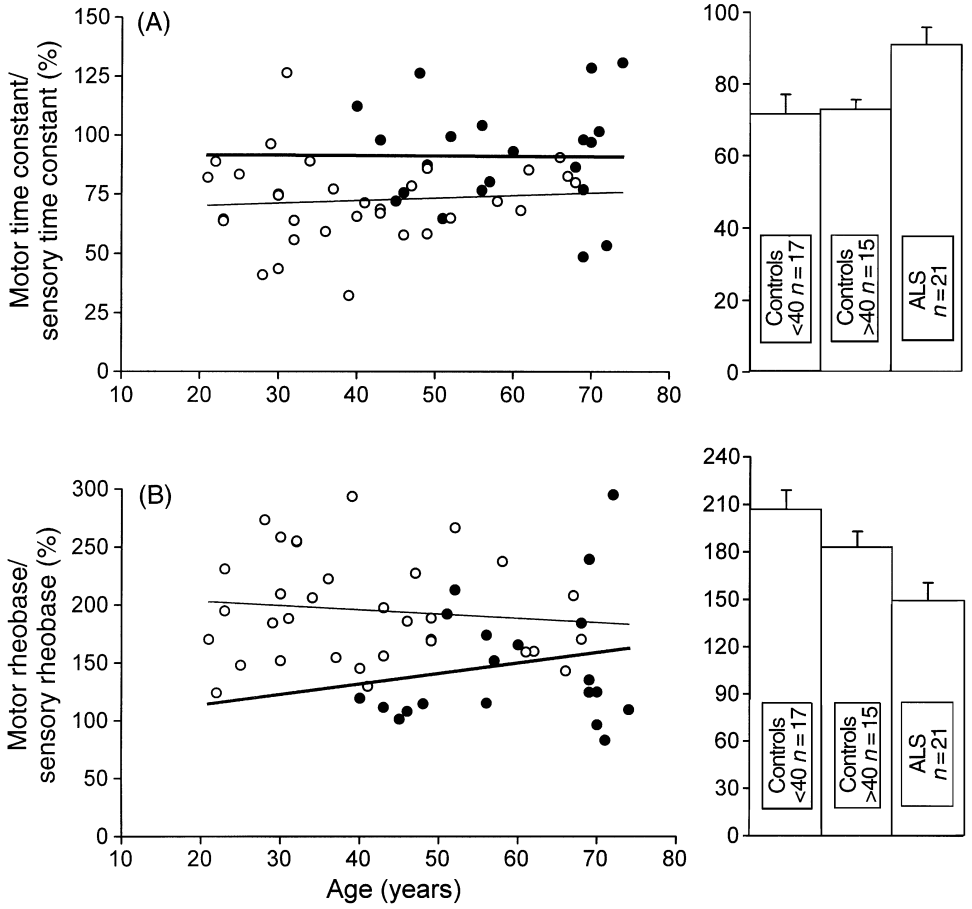

Fig. 4 Motor time constant (expressed as a percentage of sensory time constant) and motor rheobase (expressed as a percentage of sensory rheobase) are plotted against age in scattergrams in **A** and **B**. Patient data are shown as filled symbols, with the thick regression line; the control data are shown as open symbols, with the thin regression line. The histograms in **A** and **B** present the data (mean + SEM) from the scattergrams for control subjects aged <40 years, control subjects aged >40 years and the patients.

significant difference in the slopes of the relationships for control subjects and patients. The regression equation for the control subjects was $y = –1.0107x + 0.8637$, and when the patients were added, it was $y = –0.9641x + 0.8596$ (Fig. 5). The similarity of the relationships suggests that the difference in time constant data in the patients and the control subjects may be explained by that same factor.

Supernormality and refractoriness as measures of resting membrane potential

If the differences in strength–duration time constant and rheobase between cutaneous afferents and motor axons are primarily due to a voltage-dependent non-inactivating Na^+ conductance (Mogyoros *et al.*, 1996; Bostock and Rothwell, 1997; Mogyoros *et al.*, 1997*b*), a decreased difference in the patients would occur if (i) the motor axons of patients with ALS express more persistent Na^+ channels than normal, or (ii) the motor axons of patients with ALS are more depolarized, or (iii) sensory axons of patients with ALS express the conductance less (or are more hyperpolarized) than control subjects. The third possibility is not supported by the data detailed earlier and in Fig. 3. To determine whether the motor axons were more depolarized in patients with ALS, measurements were made of two parameters of axonal excitability that are strongly dependent on membrane potential but for different reasons (Kiernan *et al.*, 1997; Mogyoros *et al.*, 1997*b*), and these measures were compared for the patients and the older control group (Fig. 6).

Supernormality of cutaneous afferents and motor axons was measured as the percentage reduction in threshold when the test stimulus was preceded by a supramaximal conditioning stimulus using a conditioning–test interval of 7 ms. The data for patients were compared with those for the older control subjects, neglecting the slight age difference, because preliminary data suggest that neither refractoriness nor supernormality change significantly with age. There was no significant difference from normal in supernormality of axons in patients with ALS (for cutaneous afferents, $P = 0.477$, and for motor axons, $P = 0.383$; Fig. 6A). Similarly,

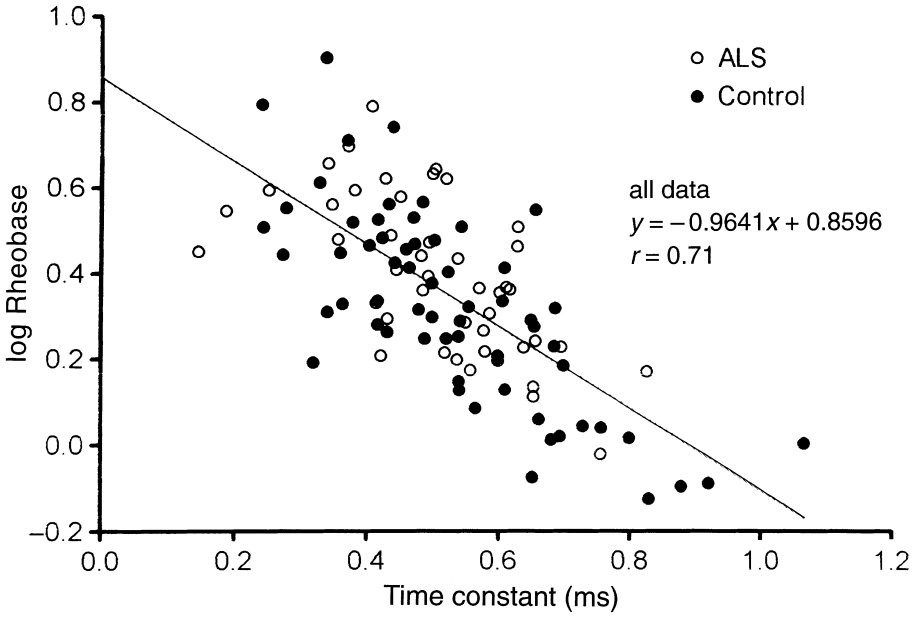

Fig. 5 The reciprocal relationship between strength–duration time constant and rheobase demonstrated by plotting time constant against the logarithm of rheobase. Open symbols: data from patients (n = 23 for sensory data; n = 21 for motor data). Filled symbols: data from control subjects (n = 32). For each group, data for sensory and motor axons are plotted. The regression line is for all data points.

refractoriness was measured as an increase in threshold at the 2-ms conditioning–test interval. Again, the differences between patients and control subjects were not significant (for cutaneous afferents, P = 0.174 , and for motor axons, P = 0.426; Fig. 6B). Indeed, the trend towards greater supernormality and less prominent refractoriness of motor axons in patients with ALS is the opposite of that which would be expected if the axons were depolarized. Equally the trends for cutaneous afferents do not favour their being hyperpolarized, as would be required to produce a smaller difference in sensory and motor time constants [as in alternative (iii) above]. It is therefore concluded that the changes in strength–duration time constant are unlikely to be due to a difference in resting membrane potential.

Correlation with disease severity and duration

Because the involvement of different motor neuron pools is not usually uniform in ALS, the size of the CMAP was used as an index of the severity of involvement of the test motor neuron pool. There was no significant correlation between the strength–duration properties of motor axons or cutaneous afferents and the size of the maximal CMAP. Similarly there was no correlation with disease duration.

Discussion

In this study it has been demonstrated that axons in patients with ALS have abnormal strength–duration properties. Additionally, the influence of age on these properties has been analysed and has been found to be an important variable. The data suggest that the strength–duration time constant of

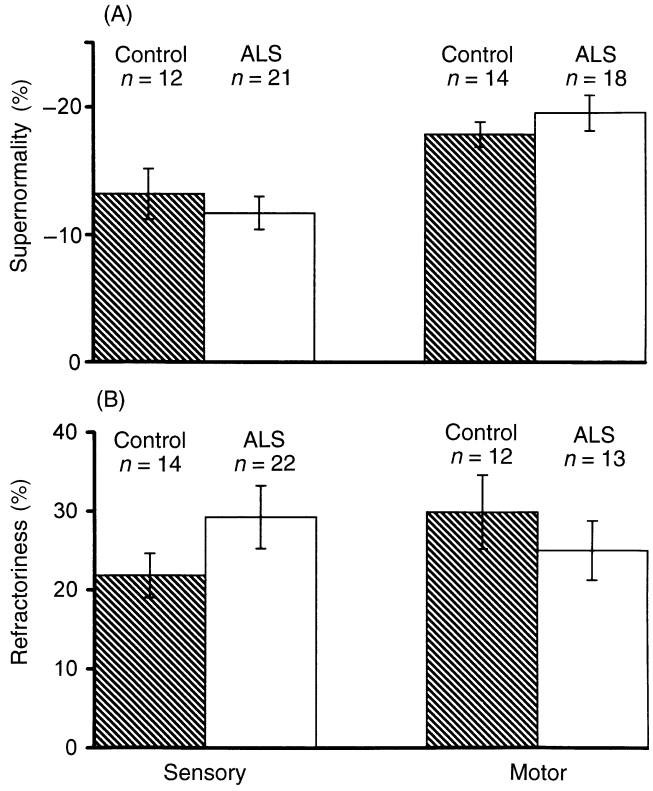

Fig. 6 Resting supernormality (**A**) and refractoriness (**B**), as indicators of membrane potential, in the patients and age-matched control subjects. Supernormality and refractoriness are expressed as percentages of the control threshold. Note that not all parameters were (or could be) measured in each subject.

motor axons is longer and rheobase lower in patients with ALS, and that this is not due to a change in resting membrane potential. It will be argued below that motor axons have an ion channel defect that could contribute to their greater tendency to become ectopically active.

Strength–duration properties as a measure of persistent Na^+ conductance

The differences in strength–duration time constant and rheobase of normal sensory and motor axons are thought to reflect differences in expression of a persistent Na^+ conductance (Bostock and Rothwell, 1997). Increases in strength–duration time constant are observed when this conductance is activated by depolarization (Bostock and Rothwell, 1997; Mogyoros *et al*., 1997*b*) or by hyperventilation (Mogyoros *et al*., 1997*b*). However, demyelination, which exposes internodal membrane with a higher membrane time constant than that of the original node (Brismar, 1981), can also increase strength–duration time constant (Bostock *et al*., 1983).

Change in strength–duration properties in ALS

As mentioned above, the strength–duration time constant can be increased by demyelination. The characteristic neuropathological features of ALS are neuronal degeneration and loss of anterior horn cells in the spinal cord, with subsequent shrinkage and pyknosis of the cells, neuronophagia and glial replacement (Hughes, 1982). Loss of cells in the dorsal root ganglia (Kawamura and Dyck, 1981) and loss of cutaneous axons in the peripheral nerves have been found (Dyck *et al*., 1975; Bradley *et al*., 1983; Heads *et al*., 1991), but demyelination has not been described.

Changes in the geometry of the nerve due to loss of axons and subsequent fibrosis within the peripheral nerve could theoretically contribute to changes in strength–duration properties in patients with ALS, but they are more likely to affect rheobase than time constant. In normal subjects strength–duration time constants are the same at the wrist and elbow despite differences in rheobase values (Mogyoros *et al*., 1996) and, in carpal tunnel syndrome, the time constant is normal but the rheobase high (Mogyoros *et al*., 1997*a*). These findings suggest that that the geometric relationship between nerve and stimulating electrodes may not be a critical factor for time constant (Mogyoros *et al*., 1997*a*). Importantly, preservation of the normal inverse relationship between time constant and rheobase in the patients argues against a significant role for altered nerve geometry in the present findings. It is also unlikely that the abnormal strength–duration properties resulted from loss of low-threshold motor axons such that the axons tested in patients were axons of biologically higher threshold than those in the control subjects. First, motor axons of different threshold have similar strength–duration time constants (Mogyoros *et al*., 1996) and, secondly, the longer time constant was associated with a lower, not higher, rheobase.

A logical conclusion of the present data is that there is a greater persistent Na^+ conductance at rest in motor axons of patients with ALS than normal. When membrane potential is changed experimentally to produce the differences in time constants seen in the present study, refractoriness increases markedly, by 50–100% (our unpublished data; see also Kiernan *et al*., 1997). Refractoriness (and supernormality) are sensitive indicators of membrane potential. The normal values for these parameters suggest that the increased conductance is not secondary to membrane depolarization, but due to a greater channel density or availability. Using the model of Bostock and Rothwell (1997), it can be estimated that a 10–20% increase in the number of persistent Na^+ channels would be required to produce the observed changes in the strength–duration properties of motor axons. This increase is quantitatively small, but the greater channel density would result in a greater tendency of these axons to discharge ectopically, thereby producing fasciculation. However, an abnormal persistent Na^+ conductance cannot be the sole cause of fasciculation because, on average, the sensory time constant is still longer than the motor time constant in ALS. Presumably the genesis of fasciculation in ALS depends on the interaction of a number of biophysical abnormalities (see below).

Age-related changes in strength–duration properties

The strength–duration time constant of cutaneous afferents decreases with age, and there was a similar trend for motor axons. There are at least two possible reasons for an age-related effect. First, nerve geometry might change with age because of axonal loss and neural fibrosis and, secondly, the persistent Na^+ conductance might decrease with maturation. There are three arguments against the first possibility. The decrease in time constant was associated with an appropriate increase in rheobase (Fig. 5). As discussed above, the time constant does not change significantly with a different nerve geometry, even though the rheobase may increase (Mogyoros *et al*., 1996, 1997*a*). Finally, most age-related neural changes, such as paranodal demyelination, would increase the time constant, not decrease it (Brismar, 1981; Bostock, 1983), and would alter other parameters of nerve excitability such as supernormality and refractoriness.

There have been no studies of the expression of ion channels in old age, but changes in some conductances have been documented between the neonatal and ‘mature’ ages (Bowe *et al*., 1985; Waxman *et al*., 1994; Waxman, 1995). Specifically, in pyramidal neurons of the rat, the density of persistent Na^+ channels increases about threefold and the density of transient Na^+ channels increases six- to tenfold between birth and maturity (Alzheimer *et al*., 1993). Subjects aged >60 years experience fewer paraesthesiae during

ischaemic compression or after its release than young subjects (Poole, 1956). If the expression of persistent Na^+ channels on cutaneous afferents decreases with age, this could explain why the elderly people experience less, or no, paraesthesiae. An alternative explanation would be that cutaneous afferents develop a more hyperpolarized resting membrane potential with age. However, the degrees of supernormality and refractoriness in elderly subjects in the present study are similar to those in other studies from this laboratory (Kiernan *et al*., 1996, 1997), and this suggests that there is no significant age-related change in the resting membrane potential of cutaneous afferents.

In a preliminary report, we have suggested that the strength–duration time constant of cutaneous afferents was shorter in patients with ALS (Burke *et al*., 1997). It is now clear that this difference was due to the greater age of patients, and that there is no such disease-related change. It is conceivable that the failure of patients with ALS to experience paraesthesiae during or after the release of ischaemia (Poole, 1956, 1957; Shahani and Russell, 1969; Shahani *et al*., 1971) is also age- rather than disease-related.

Pathophysiological implications

Most fasciculations probably arise from the axon, particularly its terminals (Wettstein, 1979; Roth, 1982, 1984; Conradi *et al*., 1982; Layzer, 1994), and an increased persistent Na^+ conductance in motor axon terminals would increase the possibility of ectopic activity. This would be more likely if it was associated with reduced K^+ conductances, activity of which might oppose the depolarizing effect of the inward Na^+ leak. Indeed, studies using threshold electrotonus suggest that there may be fewer functioning K^+ channels in motor axons of patients with ALS (Bostock *et al*., 1995; Kodama *et al*., 1995; Horn *et al*., 1996).

An increase in the persistent Na^+ conductance could result from an increased number of channels or from modulation of channel function (Catterall, 1992; Bulatko and Greeff, 1995; Crill, 1996). For example, changes in intracellular Ca^{2+} concentration result in changes in the functional availability of Na^+ channels in cultured mammalian neurones (Bulatko and Greeff, 1995), and there is direct evidence for increased intracellular Ca^{2+} in motor nerve terminals in ALS (Siklós *et al*., 1996). Accordingly, a combination of raised intracellular Ca^{2+} concentration, enhanced Na^+ conductance and reduced K^+ conductance could provide the appropriately abnormal biophysical environment in the motor terminals.

Acknowledgement

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