Ischaemia induces paradoxical changes in axonal excitability in end-stage kidney disease

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Peripheral neuropathy is present in 65% of patients with end-stage kidney disease (ESKD). No cause is yet established: nerve excitability studies have shown that axons are chronically depolarized, primarily owing to hyperkalaemia, but in vitro studies have suggested a role for axonal Na+/K+ pump dysfunction. To investigate Na+/K+ pump activity in vivo, lower limb ischaemia was induced in five ESKD patients and six healthy controls by a sphygmomanometer cuff, inflated to 200 mm Hg and maintained for 13 min. The peroneal nerve was stimulated at the fibular neck and excitability parameters were recorded from tibialis anterior (TA) and extensor digitorum brevis (EDB) before, during and after the ischaemic period. Baseline excitability studies in ESKD patients demonstrated reductions in threshold electrotonus and superexcitability and increased refractoriness, consistent with membrane depolarization. During ischaemia, threshold increased in ESKD patients [+23.6 ± 5.0% (TA); +32.1 ± 7.3% (EDB)] in contrast to the persistent threshold reduction observed in normal controls [-2.4 ± 5.2% (TA); -13.0 ± 8.2% (EDB); P < 0.01]. These changes were accompanied by increased refractoriness and reduced superexcitability in both ESKD and control groups, consistent with ischaemic depolarization. Conversely, there was reduction in strength-duration time constant towards the end of ischaemia. Following release of ischaemia, the marked increase in threshold observed in normal controls was not evident in ESKD patients, but the rapid return of threshold to baseline argues against significant Na+/K+ pump dysfunction. The abnormal pattern of response to ischaemia in the ESKD patients was not fully explained by the hyperkalaemic membrane depolarization and suggests that another dialysable factor affects nerve excitability in ESKD patients, most likely H+ ions, but that this factor only becomes evident during ischaemia. Blockade of persistent Na+ conductances by H+ would also explain the reduction in strength-duration time constant observed during ischaemia.

Keywords: ischaemia; membrane potential; nerve excitability; potassium; uraemic neuropathy

Abbreviations: CMAP = compound muscle action potential; EDB = extensor digitorum brevis; ESKD = end-stage kidney disease; TA = tibialis anterior; TEd = depolarizing threshold electrotonus; τSD = strength-duration time constant

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Introduction

Peripheral neuropathy in end-stage kidney disease (ESKD) presents as a length-dependent, distal sensorimotor polyneuropathy with greater lower limb than upper limb involvement (Bolton, 1980; Asbury, 1993). Prevalence rates of 60–100% have been demonstrated in previous studies, using a combination of clinical examination and nerve conduction parameters (Nielsen, 1973; Ackil et al., 1981; Angius-Leppan and Burke, 1992; Van den Neucker et al., 1998; Laaksonen et al., 2002; Krishnan et al., 2005b).

In terms of the pathophysiology of uraemic neuropathy, the finding that neurological complications of ESKD may be improved by dialysis (Hegstrom et al., 1962), and that patients receiving peritoneal dialysis had a lower incidence of neuropathy compared with haemodialysis patients, gave
rise to the ‘middle molecule hypothesis’ (Babb et al., 1971). This hypothesis postulated that the higher rate of neuropathy in patients on haemodialysis was secondary to retention of toxic molecules in the middle molecular range of 300–12,000 Da (Vanholder et al., 1994), given that these substances were poorly cleared by haemodialysis membranes. This hypothesis remains unproven (Bostock et al., 2004).

Nerve excitability techniques, which provide information about axonal membrane potential and ion channel function (Bostock et al., 1998; Burke et al., 2001), have recently been applied to ESKD patients. These novel techniques have demonstrated alterations in membrane potential, specifically axonal depolarization prior to haemodialysis, with subsequent improvement in axonal function post-dialysis (Kiernan et al., 2002; Krishnan et al., 2005b; Krishnan et al., 2006a). In contrast to the unproven middle molecule hypothesis, comparison between levels of serum electrolytes and excitability indices indicated that hyperkalaemia was the principal cause of membrane depolarization prior to haemodialysis (Kiernan et al., 2002; Krishnan et al., 2005b; Krishnan et al., 2006a). However, depolarization may also be induced by nerve ischaemia, due to alteration in the function of the electrogenic energy-dependent Na⁺/K⁺ pump present on the axonal membrane (Bergmans, 1970; Bostock et al., 1991a, b; Bostock et al., 1994; Kiernan and Bostock, 2000). Furthermore, inhibition of the Na⁺/K⁺ pump by uraemic neurotoxins has been previously proposed as the mechanism underlying membrane depolarization and the subclinical slowing of nerve conduction in uraemic patients (Nielsen, 1973). Consequently, the present study was undertaken to explore the possibility that alterations in Na⁺/K⁺ pump function may contribute to axonal depolarization in ESKD and, thereby, the development of neuropathy.

**Methods**

Studies were performed on 5 male patients with ESKD (age range, 17–56 years; mean age 36.0 years) receiving haemodialysis three times per week, using a biocompatible low-flux polysulphone membrane (Fresenius, Bad Homburg, Germany). All patients had symptoms and signs of peripheral neuropathy. Patients with a history of peripheral vascular disease or of other illnesses known to cause neuropathy, such as diabetes, were excluded from the study. The causes of renal failure were glomerulonephritis (4) and medullary cystic kidney disease (1). The patients had been treated with haemodialysis for periods varying from 8 months to 6 years (mean 2.9 years). All patients gave informed consent to the procedures, which were approved by the South East Sydney Area Health Service Human Research Ethics Committee (Eastern Section) and the Committee on Experimental Procedures Involving Human Subjects of the University of New South Wales.

Baseline excitability properties of peroneal nerve motor axons were measured prior to a standard 5 h haemodialysis session. Excitability parameters were recorded from tibialis anterior following stimulation of the peroneal nerve. Stimulus–response behaviour using two stimulus durations, threshold electrotetonus to 100 ms polarizing currents, a current threshold relationship and the recovery of excitability following supramaximal stimulation were recorded, using previously devised protocols (Kiernan et al., 2000; Krishnan et al., 2004). The serum concentrations of putative neurotoxins including potassium, calcium, urea, uric acid, parathyroid hormone and beta-2-microglobulin were also measured.

After the baseline measurements, the peroneal nerve was stimulated 1 cm distal to the fibular neck (Fig. 1) using a bipolar electrode and the resultant compound muscle action potentials (CMAPs) were recorded simultaneously using surface electrodes overlying tibialis anterior (TA) and extensor digitorum brevis (EDB). The amplitude of the CMAP was measured from peak to peak. Skin temperature was monitored close to the stimulation site and maintained at >32°C for the entire duration of the recording by placing a blanket over the leg and using radiant heat if necessary. These recordings were continued until stable measurements were obtained for at least 5 min prior to the application of ischaemia.

Stimuli were delivered using a computerized threshold-tracking program (QTRAC© version 5.2a, Institute of Neurology, Queen Square, London) that was run by a Pentium computer. Recordings were amplified (gain 1000, bandwidth 5–10 Hz) and digitized using an analogue-to-digital (A/D) board (DT 2812, Data Translation) with a sampling rate of 10 kHz. Stimulus waveforms were converted to current with a purpose-built isolated linear bipolar constant-current stimulator. Stimuli were delivered at 1.25 Hz and were rotated sequentially through a series of five different combinations of test and conditioning stimuli for each recording site.

Channels 1–5 recorded excitability parameters from TA and channels 6–10 from EDB (Krishnan et al., 2005a). For TA, a fixed supramaximal stimulus of 0.2 ms duration was delivered on channel 1. Channels 2–5 used a proportional tracking system to achieve a target potential, set to 30% of the maximal amplitude and known as the threshold current (Bostock et al., 1998). On Channel 2, a stimulus of 0.2 ms duration was delivered and the threshold current was recorded. Channel 3 recorded the threshold for a stimulus of 1 ms duration. The values obtained for stimuli of 0.2 ms and 1 ms duration were used to calculate the strength–duration time constant (τSD) of motor axons in each study using Weiss’s formula (Weiss, 1901; Mogyoros et al., 1996). Channels 4 and 5 recorded specific periods of the recovery of excitability by tracking the changes in threshold that occurred following a supramaximal conditioning stimulus of 0.2 ms duration. On channel 4, the degree of refractoriness, expressed as the percentage change in current required to produce the target
CMAP, was recorded by applying a supramaximal conditioning stimulus 2.5 ms prior to the 0.2 ms test stimulus. Superexcitability, also expressed as the percentage change in threshold, was measured on channel 5 with a conditioning–test interval of 7 ms. On channels 4 and 5, the test response was measured after online subtraction of the conditioning stimulus obtained in isolation (using the response obtained from channel 1). A rotating sequence of conditioning–test stimuli combinations identical to that described above was also applied to EDB, using channels 6–10. Ischaemia was produced by placing a sphygmomanometer cuff over the thigh and inflating it to 200 mmHg. Ischaemia was maintained for 13 min in all studies and the changes in maximal CMAP, threshold, refractoriness, superexcitability and $\tau_{50}$ were tracked before, during and for 30 min following the ischaemic period. Patients were also asked to rate the intensity of paraesthesiae and numbness during and for 30 min following the ischaemic period. Patients were also asked to rate the intensity of paraesthesiae and numbness experienced during and after the ischaemic period on an 11-point Likert scale (0—no pins and needles; 10—unbearable pins and needles).

Routine nerve conduction studies were undertaken in all patients. Neurophysiological indices, which had previously been shown to be sensitive markers of uraemic neuropathy, were evaluated (Ackil et al., 1981; Laaksonen et al., 2000; Krishnan et al., 2005b). Studies were undertaken on the sural, tibial, common peroneal and superficial radial nerves using a Medelec Synergy system (Oxford Instruments, Surrey, UK) and conventional nerve conduction techniques (Burke et al., 1974; Kimura, 1983).

Abnormalities of nerve conduction and excitability were established by comparing the results with normative data from our unit (Burke et al., 1974; Krishnan et al., 2004) and other centres (Ma et al., 1981; Ma and Liveson, 1983; Buschbacher, 1999; Puksa et al., 2003). Baseline excitability measures were compared with previous normative values established using the same protocol (Krishnan et al., 2004). For ischaemia, values were compared with previously published data obtained from normal controls using identical stimulus protocols (Krishnan et al., 2005a). Single comparisons in excitability parameters were analysed using Student’s unpaired t-test. Repeated measures ANOVA was used for multiple comparisons. Correlations were analysed using Pearson’s correlation coefficient. A probability value of $p < 0.05$ was considered statistically significant. Results are expressed as mean ± standard error of the mean.

### Results

Excitability changes induced by ischaemia

During ischaemia there was a reduction in the threshold current recorded from both TA and EDB for stimuli of 0.2 and 1 ms duration in both ESKD patients and controls, as shown for a representative patient in Fig. 2. The maximal mean threshold reduction in ESKD patients (Fig. 3) was similar for TA and EDB for stimuli of both 0.2 and 1 ms durations (TA 0.2 ms stimulus 7.0 ± 1.5%; 1 ms stimulus 7.8 ± 2.6%; EDB 0.2 ms stimulus 7.5 ± 1.9%; 1 ms stimulus 6.2 ± 2.2%). However, these changes were short-lived, lasting

### Table 1 Nerve conduction parameters for each patient with serum K$^+$ concentration at time of study

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sural SNAP (μV)</th>
<th>Radial SNAP (μV)</th>
<th>Tibial CMAP (mV)</th>
<th>Tibial F-wave min latency (ms)</th>
<th>Tibial F-wave persistence (%)</th>
<th>Peroneal CMAP (mV)</th>
<th>Serum K$^+$ concentration at time of study (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (17)</td>
<td>6.0</td>
<td>15.0</td>
<td>4.4</td>
<td>51.9</td>
<td>100</td>
<td>4.7</td>
<td>4.0 (NR)</td>
</tr>
<tr>
<td>2 (28)</td>
<td>8.9</td>
<td>36.1</td>
<td>9.5</td>
<td>47.8</td>
<td>90</td>
<td>5.3</td>
<td>4.3 (NR)</td>
</tr>
<tr>
<td>3 (38)</td>
<td>6.1</td>
<td>36.0</td>
<td>3.2</td>
<td>51.3</td>
<td>90</td>
<td>4.1</td>
<td>5.9 (NR)</td>
</tr>
<tr>
<td>4 (40)</td>
<td>5.0</td>
<td>44.0</td>
<td>2.5</td>
<td>45.3</td>
<td>100</td>
<td>1.0</td>
<td>4.8 (NR)</td>
</tr>
<tr>
<td>5 (56)</td>
<td>2.5</td>
<td>22.0</td>
<td>10.7</td>
<td>60.8</td>
<td>100</td>
<td>1.2</td>
<td>4.2 (NR)</td>
</tr>
<tr>
<td>Mean (patients)</td>
<td>5.7 ± 1.0</td>
<td>30.6 ± 5.3</td>
<td>6.1 ± 1.7</td>
<td>51.4 ± 2.6</td>
<td>96 ± 3</td>
<td>3.3 ± 0.9</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>Normal controls</td>
<td>13.6 ± 7.5</td>
<td>42.4 ± 14.9</td>
<td>12.9 ± 4.5</td>
<td>45.9 ± 4.4</td>
<td>100%</td>
<td>8.9 ± 4.0</td>
<td>(NR) 3.6–5.1</td>
</tr>
</tbody>
</table>

Group data expressed as mean ± S.E.M for each parameter. Data include amplitudes for sensory nerve action potential (SNAP) and compound motor action potential (CMAP) and tibial F-wave minimum latency (min latency). Normative data for nerve conduction parameters are shown as mean ± standard deviation and are taken from the sources referred to in the text. Normative data for sural SNAP amplitudes (Burke et al., 1974) and tibial CMAP amplitudes (Buschbacher, 1999) were age-matched and are shown in the table for the age groups 41–60 years and 30–59 years respectively. Serum K$^+$ concentration is expressed in mmol/l for each patient, with normal laboratory range (NR).
4–7 min (mean 6.2 ± 0.7 min). Furthermore the threshold reductions were significantly smaller than those seen in normal controls (TA 0.2 ms stimulus 9.0 ± 2.4%; 1 ms stimulus 11.9 ± 2.6%; EDB 0.2 ms stimulus 25.8 ± 5.4%; 1 ms stimulus 29.3 ± 7.7%) when analysed as both the maximal mean reduction in threshold per patient ($P < 0.05$ for both TA and EDB) and as the threshold change at 5 min of ischaemia for EDB recordings ($P < 0.05$ for 0.2 and 1 ms stimuli).

Following the brief threshold reduction, ESKD patients demonstrated a rapid increase in threshold during the ischaemic period in both TA and EDB recordings, with a maximal threshold increase of 20.5 ± 6.1% and 32.0 ± 10.5% for a 0.2 ms stimulus and 23.6 ± 5.0% and 32.1 ± 7.3% for a 1 ms stimulus, for TA and EDB, respectively. This pattern of threshold changes was not observed in healthy controls in whom there was persistent threshold reduction at the conclusion of the ischaemic period (TA 0.2 ms stimulus 2.8 ± 4.2%; 1 ms stimulus 2.4 ± 5.2%; EDB 0.2 ms stimulus 14.2 ± 5.4%; 1 ms stimulus 13.0 ± 8.2%; $P < 0.01$ for ESKD compared with controls), with a threshold increase only occurring in the post-ischaemic period (Fig. 3). Cuff release in ESKD led to a rapid reduction in threshold rather than the expected increase seen in normal controls (threshold increase in controls for TA 0.2 ms stimulus 18.0 ± 4.1%; 1 ms stimulus, 24.9 ± 6.5%; EDB 0.2 ms stimulus, 39.7 ± 10.8%; 1 ms stimulus 61.2 ± 15.6%). The threshold changes were associated with a reduction in maximal CMAP amplitude, although this was not significantly different from that observed in normal controls (ESKD patients for TA 26.7 ± 11.7%; for EDB 18.0 ± 11.0%; controls for TA 23.5 ± 11.4%; for EDB 38.4 ± 16.5%). There was no correlation between the extent of reduction in maximal CMAP amplitude and the ischaemic threshold increase in ESKD patients.

These differential threshold changes were accompanied by differences in the symptom profiles of normal controls and ESKD patients. While normal controls reported that paraesthesiae, common symptoms of neuropathy, were maximal in the post-ischaemic period, ESKD patients reported

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**Fig. 2** Excitability parameters recorded from tibialis anterior for a single ESKD patient, before, during and after 13 min of lower-limb ischaemia (filled horizontal bar indicates period of ischaemia). (A) Normalized threshold (NT) for stimulus of 1 ms duration. Thresholds were normalized to pre-ischaemic values. (B) Corresponding changes in strength-duration time constant ($\tau_{SD}$); (C) refractoriness, expressed as the percentage increase in threshold at a conditioning–test interval of 2.5 ms; and (D) superexcitability, expressed as the percentage decrease in threshold, measured at a conditioning–test interval of 7 ms.

**Fig. 3** Excitability parameters recorded from TA for ESKD patients and controls before, during and after the ischaemic period (indicated by filled horizontal bar). Mean data ± standard error of the mean. During ischaemia refractoriness increased and superexcitability decreased in both groups, consistent with axonal depolarization. The reversal of these changes in the post-ischaemic period is consistent with axonal hyperpolarization. There is a smaller threshold reduction during ischaemia in ESKD patients and a prominent ischaemic threshold increase beyond baseline values, a feature not observed in control subjects.
prominent paraesthesiae during ischaemia, with a paradoxical reduction in symptoms following release of ischaemia.

The changes in threshold in ESKD patients were accompanied by changes in $\tau_{SD}$, a voltage-dependent property of the nodal membrane, which provides an indirect measure of a nodal persistent Na$^+$ conductance (Mogyoros et al., 1996; Bostock and Rothwell, 1997). Prior to ischaemia, $\tau_{SD}$ was 0.23 ± 0.04 ms for TA recordings and 0.24 ± 0.03 ms for EDB recordings. During ischaemia there was initially a small increase in $\tau_{SD}$ for both TA and EDB in ESKD patients (TA 0.28 ± 0.06 ms; EDB 0.25 ± 0.02 ms), with a reduction towards the end of the ischaemic period (Fig. 2B). This was followed by a further reduction during the post-ischaemic period (TA 0.22 ± 0.02 ms; EDB 0.23 ± 0.02 ms). While the changes in $\tau_{SD}$ were qualitatively similar in controls (Krishnan et al., 2005a), pooling of data from TA and EDB recordings revealed that the magnitude of these changes during ischaemia was significantly less in ESKD patients ($P < 0.05$). Refractoriness, due to inactivation of voltage-gated transient Na$^+$ channels, increased during the period of ischaemia, consistent with membrane depolarization (refractoriness pre-ischaemia for TA 39.0 ± 7.2%; during ischaemia 108.3 ± 44.5%; pre-ischaemia for EDB 23.4 ± 6.8%; during ischaemia 46.1 ± 13.3%) and was reduced in the post-ischaemic phase, consistent with membrane hyperpolarization (Fig. 3). Superexcitability was reduced during ischaemia (superexcitability pre-ischaemia for TA −6.4 ± 4.1%; during ischaemia −4.4 ± 4.2%; pre-ischaemia for EDB −13.6 ± 4.8%; during ischaemia −0.02 ± 5.1%), thereby providing further evidence for ischaemic depolarization, and increased in the post-ischaemic period (maximal superexcitability for TA −12.1 ± 6.8%; for EDB 19.9 ± 6.0%), consistent with post-ischaemic hyperpolarization.

In baseline recordings, there was significant correlation between serum K$^+$ and sensitive measures of membrane potential such as superexcitability ($r = 0.79$, $P < 0.05$) and $\tau_{ED}$ 90–100 ms ($r = 0.69$, $P < 0.05$), consistent with the results of previous studies (Kiernan et al., 2002; Krishnan et al., 2005b). However, no correlation was noted between threshold changes during ischaemia and either serum K$^+$ concentrations, serum bicarbonate as a measure of acid–base balance or baseline excitability values. All patients in the study manifested abnormal responses to ischaemia, even those in whom serum K$^+$ was within the normal range. Similarly, no correlation was noted between excitability parameters and any of the other potential neurotoxins that were assayed (see Methods).

To further explore the effects of dialysis on nerve excitability, and specifically the changes that were induced by ischaemia, excitability recordings were obtained before and after dialysis in recordings in a single subject (Patient 2, Table 1). While the pre-dialysis recording again demonstrated a significant increase in threshold during ischaemia, the post-dialysis study revealed resolution of these abnormalities, more akin to ischaemic changes observed in controls (Fig. 4). This improvement could not be explained on the basis of changes in serum K$^+$ concentration, which was coincidentally 4.3 mmol/l in both pre-dialysis and post-dialysis recordings, or alterations in baseline membrane potential given the lack of significant change in superexcitability (pre-dialysis −15.3%; post-dialysis −11.1%) and refractoriness (pre-dialysis 36.8%; post-dialysis 27.8%).

**Discussion**

The present study has demonstrated complex abnormalities in the response of lower limb motor axons to ischaemia in ESKD patients. Baseline excitability recordings undertaken prior to dialysis (i.e. steady-state) established that axons were depolarized, with significant correlation to serum K$^+$, as previously reported (Krishnan et al., 2005b). With the onset of ischaemia, a short-lived threshold reduction was followed by a rapid increase in threshold. In the post-ischaemic period, there was a reduction in threshold, rather than the expected threshold increase. This paradoxical pattern of threshold change is the converse of that documented for normal controls during and after ischaemia.

Ischaemia alters resting membrane potential through effects on energy-dependent processes, particularly the energy-dependent Na$^+$/K$^+$ pump, present on the axonal membrane. Paralysis of Na$^+$/K$^+$-ATPase abolishes its direct, electrogenic contribution to the membrane potential, leading to extracellular accumulation of K$^+$ and further depolarization (Ritchie and Straub, 1957; Kaji and Sumner, 1989; Felts et al., 1995). Studies of nerve excitability in healthy subjects have demonstrated reduction in threshold during the period of ischaemia, consistent with axonal depolarization, with appropriate changes in other excitability variables, namely an increase in refractoriness, a reduction in superexcitability and an increase in $\tau_{ED}$ (Bostock et al., 1994; Kiernan and Bostock, 2000; Krishnan et al., 2005a). Changes in the opposite direction occur in the
post-ischaemic period, indicative of membrane hyperpolarization (Lin et al., 2002).

The present study addressed excitability changes during ischaemia in motor axons rather than sensory axons primarily because studies based on the CMAP were more reproducible, given the difficulties with threshold tracking small sensory potentials in patients with uraemic neuropathy. Furthermore, recent studies have demonstrated that the pre-ischaemia excitability changes are similar in both fibre types (Krishnan et al., 2005b; Krishnan et al., 2006b).

The findings of the present study indicate a different pattern of ischaemic threshold changes in ESKD patients to those observed in normal controls. While the changes in refractoriness, superexcitability and $\tau_{SD}$ were similar to those in normal controls and consistent with ischaemic membrane depolarization, the threshold changes were markedly different, consisting of a brief reduction in threshold followed by a rapid increase (Fig. 3). In contrast, normal controls showed a prolonged threshold reduction during ischaemia with a rise towards but not exceeding baseline levels at the end of the ischaemic period. The ischaemic threshold increase in ESKD patients could not be explained by a reduction in maximal CMAP amplitude, given that quantitatively similar reductions in CMAP amplitude occurred in control subjects but without any ischaemic threshold increase (Krishnan et al., 2005a).

What then underlies the rapid increase in threshold during ischaemia in ESKD patients? Previous studies that explored the relationship between threshold current and membrane potential documented a ‘U-shaped’ relationship, such that threshold increase occurred with either hyperpolarization or severe membrane depolarization (Bostock and Grafe, 1985), with the threshold increase during severe depolarization explained primarily by the inactivation of voltage-dependent Na$^+$ channels (Baker and Bostock, 1989). During prolonged ischaemia, the nerves of normal subjects also show an increase in threshold after the initial fall, before conduction fails (Bostock et al., 1991b). Prior to dialysis, axons of ESKD patients have been shown to exist in a chronically depolarized state (Kiernan et al., 2002; Krishnan et al., 2005b), and the pre-ischaemia recordings in this study were consistent with membrane depolarization. The threshold increase noted in ESKD patients during ischaemia may, therefore, reflect in part the development of severe depolarization caused by the combined effects of baseline depolarization with the superadded effects induced by ischaemic Na$^+$/K$^+$ pump paralysis and consequent strong Na$^+$ channel inactivation.

The abnormal ischaemic threshold increase in ESKD patients could not, however, be accounted for simply by the tendency of their axons to be chronically depolarized by hyperkalaemia, since all patients showed the abnormal threshold increase, but not all their nerves started off depolarized. Despite the significant correlation between baseline excitability abnormalities and serum K$^+$, no significant association was noted between these measures and the magnitude of threshold changes induced by ischaemia. Furthermore, the recordings in Fig. 4 show a typical ESKD pattern of ischaemic threshold increase before dialysis, and a normal pattern of ischaemic threshold decrease from the same nerve after dialysis, although serum K$^+$ was identical in the two cases, and superexcitability measurements showed that the axons in this particular patient were no more depolarized before dialysis than after dialysis.

This study, therefore, provides the first indication that there is another dialysable factor (apart from potassium ions), or process, that affects nerve excitability in ESKD patients but that this factor only becomes evident during ischaemia. Of relevance, ischaemia is thought to result in even more Na$^+$ channel inactivation than can be accounted for by the change in membrane potential alone (Grosskreutz et al., 2000; Lin et al., 2002). The mechanism is unknown, although indirect evidence suggested that during ischaemia, the depolarizing change in membrane potential was limited by the accumulation of an ischaemic metabolite, possibly H$^+$ ions, that produced a voltage-dependent block of Na$^+$ channels, with unblocking on release of ischaemia (Grosskreutz et al., 2000; Lin et al., 2002).

H$^+$ ions alter channel gating and ion permeation either by binding within the channel pore (Wanke et al., 1980; Begenisich and Danko, 1983) or by altering the surface charge at the pore entrance (Hille et al., 1975). Studies of persistent Na$^+$ channels have revealed that a lowering of pH leads to reduction in the number of contributing channels and the single channel conductance (Baker and Bostock, 1999). Consequently, during ischaemia, two opposing effects alter $\tau_{SD}$: ischaemic depolarization tendencies to increase $\tau_{SD}$; and blockade of persistent Na$^+$ channels tending to decrease $\tau_{SD}$. This opposition would account for the small changes in $\tau_{SD}$ on average and, particularly, the reduction in $\tau_{SD}$ during ischaemia. Blockade of persistent Na$^+$ channels, due to effects mediated by H$^+$ ions, would also explain the lower than expected increase in $\tau_{SD}$ during the ischaemic period in ESKD patients compared with controls. A reduction in the overall Na$^+$ conductance may also explain the prominent threshold increase noted in ESKD patients during the ischaemic period. These patients may be particularly prone to such an accumulation of H$^+$ ions given that metabolic acidosis, due in part to reduced excretion of H$^+$ ions, is a prominent feature of ESKD (Kraut, 2000). The results in ESKD patients could, therefore, be explained by higher than normal pre-existing levels of this metabolite acting at a local level, or factors affecting its build-up during ischaemia, which are corrected by dialysis.

The primary Na$^+$ channel expressed at the node of Ranvier of myelinated axons is Na$\alpha_1.6$ (Caldwell et al., 2000). In cerebellar Purkinje cells, Na$\alpha_1.6$ is responsible for a surge of Na$^+$ current occurring, paradoxically, as repolarization after a step depolarization (Raman and Bean, 1997; Pan and Bean, 1999; Raman and Bean, 2001; Grieco et al., 2002). Depolarization, which may occur during ischaemia-induced depolarization of ESKD axons, may be expected
to cause the channel to enter a blocked state preferentially. If unbinding of the putative ischaemic metabolite then occurred, this would allow resurgent currents to pass, similar to cerebellar Purkinje cells, as axons repolarize from a depolarized state.

In the post-ischaemic period, increased activity of the Na\(^+\)/K\(^+\) pump induces membrane hyperpolarization (Bergmans, 1970; Bostock et al., 1991a; Bostock et al., 1994). While this process resulted in an increase in threshold in control subjects (Krishnan et al., 2005a), a paradoxical reduction in threshold occurred in ESKD patients. The rapid return of threshold towards baseline in the post-ischaemic period would be consistent with a stabilizing effect of the axonal Na\(^+\)/K\(^+\) pump, attempting to return membrane potential towards baseline from the highly depolarized levels of the ischaemic period. This rapid return of threshold to baseline levels (Fig. 3), therefore, provides further evidence that the Na\(^+\)/K\(^+\) pump is functioning well in ESKD and would argue against any significant contribution of Na\(^+\)/K\(^+\) pump dysfunction to the development of uremic neuropathy (Krishnan et al., 2006a).

The pattern of ischaemic excitability changes noted in ESKD patients in the present study differ from those observed in previous studies of diabetic neuropathy, another common metabolic neuropathy (Seneviratne and Peiris, 1968; Weigl et al., 1989; Strupp et al., 1990). In diabetic patients, nerves develop a resistance to ischaemic conduction block, which may be reproduced in model systems either by chronic endoneural hypoxia (Low et al., 1986; Low et al., 1989; Hohman et al., 2000) or by hyperglycaemia (Strupp et al., 1990). The use of threshold tracking excitability techniques has been advocated as a sensitive indicator of ischaemic resistance and early neuropathy (Weigl et al., 1989). Although these studies demonstrated a threshold reduction during ischaemia that was significantly less in diabetic nerves compared with controls, there was no evidence of the paradoxical changes observed in ESKD patients from the present study.

In conclusion, this study has provided evidence that the axonal Na\(^+\)/K\(^+\) pump functions well in ESKD, and is unlikely to contribute to the development of uremic neuropathy. However, it has also provided evidence that depolarization by hyperkalaemia is not the only factor responsible for abnormal nerve excitability in these patients: another dialysable substance appears to contribute to the abnormal ischaemic threshold increase during ischaemia.

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