Altered motor nerve excitability in end-stage kidney disease

Arun V. Krishnan,1,2 Richard K. S. Phoon,3 Bruce A. Pussell,3 John A. Charlesworth,3 Hugh Bostock4 and Matthew C. Kiernan1,2

1Institute of Neurological Sciences, Prince of Wales Hospital, Randwick, Sydney, Australia, 2Prince of Wales Medical Research Institute and Prince of Wales Clinical School, University of New South Wales, 3Department of Nephrology, Prince of Wales Hospital, Randwick, Sydney, Australia and 4Sobell Department of Neurophysiology, Institute of Neurology, Queen Square, London, UK

Correspondence to: Dr Matthew Kiernan, Prince of Wales Medical Research Institute, Barker Street, Randwick, Sydney, NSW 2031, Australia
E-mail: M.kiernan@unsw.edu.au

Although multiple toxins have been implicated in the development of uraemic neuropathy, no causative agent has been identified. In the present study, the excitability properties of lower limb motor nerves in patients with end-stage kidney disease treated with haemodialysis were measured before, during and after a standard 5 h haemodialysis session, in an attempt to explore the pathophysiology of uraemic neuropathy. Compound muscle action potentials were recorded from tibialis anterior and extensor digitorum brevis, following stimulation of the common peroneal nerve in 14 patients. Measures of excitability were assessed in relation to changes in serum levels of potential neurotoxins, including potassium, calcium, urea, uric acid, parathyroid hormone and \( \beta \)-2-microglobulin. Before dialysis, measures of nerve excitability were significantly abnormal in the patient group for axons innervating tibialis anterior and extensor digitorum brevis, consistent with axonal depolarization: refractoriness was increased and superexcitability and depolarizing threshold electrotonus were reduced. Pre-dialysis excitability abnormalities were strongly correlated with serum \( K^+ \). Correlation was also noted between the severity of symptoms and excitability abnormalities. Haemodialysis normalized the majority of nerve excitability parameters. In conclusion, lower limb motor axons in uraemic patients are depolarized before dialysis. The correlation between serum \( K^+ \) and excitability measures indicates that hyperkalaemia is primarily responsible for uraemic depolarization, and a likely contributing factor to the development of neuropathy.

Keywords: membrane potential; nerve excitability; potassium; threshold electrotonus; uraemic neuropathy

Abbreviations: \( \beta \)-2M = \( \beta \)-2-microglobulin; CMAP = compound muscle action potential; EDB = extensor digitorum brevis; ESKD = end-stage kidney disease; NCS = nerve conduction study; NSS = neuropathy symptom score; PTH = parathyroid hormone; SNAP = sensory nerve action potential; TA = tibialis anterior; TEd = depolarizing threshold electrotonus; TEh = hyperpolarizing threshold electrotonus; T-NSS = total neuropathy symptom score

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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). Previous studies of neuropathy in ESKD have demonstrated prevalence rates which vary from 60 to 100%, depending on the choice of nerve segments, the indices measured and the number of nerves studied (Nielsen, 1973; Bolton, 1980; Ackil et al., 1981; Van den Neucker et al., 1998; Laaksonen et al., 2002).

The pathophysiology of uraemic neuropathy has not been established. The finding that neurological complications of renal failure may be improved by dialysis (Hegstrom et al., 1962) and that patients receiving peritoneal dialysis had a lower incidence of neuropathy than 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. Examples of such molecules include parathyroid hormone (PTH) and β-2-microglobulin (β-2M), the levels of which are elevated in patients with ESKD (Vanholder et al., 1994). The hypothesis, however, remains unproven and the toxicity of a number of these middle molecules remains contentious (Vanholder et al., 1994; Bostock et al., 2004).

Measurements of nerve excitability, which provide information about membrane potential and biophysical properties of peripheral axons (Bostock et al., 1998; Burke et al., 2001), have been used to study peripheral nerves in patients with neuropathy and have provided information about disease pathophysiology (Cappelen-Smith et al., 2001; Kiernan et al., 2001a, 2002a, 2003; Kanai et al., 2003; Nodera et al., 2004). A preliminary study of motor nerve excitability in the upper limb of patients with ESKD demonstrated membrane potential changes—specifically membrane depolarization before haemodialysis (Kiernan et al., 2002b)—with subsequent improvement in nerve excitability after dialysis. Given the length–dependent predisposition of uraemic neuropathy, typically worse in the legs than in the arms, the present study has focused on lower limb motor nerve excitability. The aim of the study was to expand the original study by investigating the excitability properties of lower limb motor axons, before, during and after haemodialysis in patients with ESKD. In addition, correlations were explored between excitability changes and the clinical severity of neuropathy, related to changes in the serum levels of potential uraemic toxins and the severity of neuropathic symptoms.

**Methods**

Studies were undertaken on 14 patients with ESKD (8 men, 6 women: age range, 17–69 years; mean age, 50.3 years) receiving thrice-weekly haemodialysis, using a biocompatible low-flux polysulfone membrane (Fresenius, Bad Homburg, Germany). All patients were dialysed against a K⁺ concentration of 2 mmol/l. None of the patients had a history of other illnesses known to cause neuropathy such as diabetes or amyloidosis and there was no history of exposure to neurotoxic medications, including immunosuppressive therapy. The causes of ESKD in this group were glomerulonephritis (9 patients), polycystic kidney disease (1), medullary cystic kidney disease (2) and hypertensive vascular disease (2).

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. The studies were performed in accordance with the Declaration of Helsinki.

A neurological history and examination were initially undertaken and symptoms were graded using the neuropathy symptom score (NSS) (Dyck et al., 1980, 1987, 1992; Laaksonen et al., 2002). Patients were asked about the presence of motor symptoms in the limbs (subset IB) and sensory symptoms, both negative (subset IIA) and positive (subset IIB). Each symptom received a score of 1 and the number of symptoms present in each subset was added to give a total neuropathy symptom score (T-NSS). The maximum possible T-NSS was 9.

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., 2002). 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). Nerve stimulation was performed at a frequency of 1 Hz for motor nerves and 2 Hz for sensory nerves. Motor amplitudes were measured peak to peak and sensory amplitudes were measured as an average of the rising and falling phase amplitudes. Latency was measured to the onset of the compound potential. For sensory studies, a bipolar recording electrode configuration was used with a standard interelectrode distance of 4 cm (Eduardo and Burke, 1988). For tibial nerve F-wave studies, the latency was recorded as the mean of 10 responses following supramaximal stimulation of the nerve at the medial malleolus.

The excitability properties of lower limb motor nerves in patients with ESKD treated with haemodialysis were measured before, during and 1 h after a standard 5 h haemodialysis session using a previously described protocol (Kiernan et al., 2000; Krishnan et al., 2004). Recordings were obtained from tibialis anterior (TA) and extensor digitorum brevis (EDB), following stimulation of the peroneal nerve at the fibular neck. Skin temperature was monitored close to the site of stimulation for the duration of each study.

Serum electrolytes, urea, creatinine, PTH and β-2M were measured at the time of the excitability studies. Kt/V, a standard and commonly accepted measure of dialysis adequacy (Daugirdas, 1995, 2000), was also calculated according to the following formula, where K is the dialyser clearance, t is the length of the dialysis session (hours) and V is the urea distribution volume (litres), U1 is pre-dialysis urea (mmol/l); U2 is post-dialysis urea (mmol/l, 1 h after dialysis), BW is body weight, ΔBW is the change in body weight following dialysis.

\[
Kt/V = -\ln(U1/U2 - 0.008 \times t) + (4 - 3.5 \times U1/U2) \times \Delta BW/BW. \tag{1}
\]

The current required to produce the desired CMAP (compound muscle action potential) amplitude was determined using a computerized threshold-tracking program (QTRAC version 5.2a, Institute of Neurology, Queen Square, London, UK, with multiple excitability protocol TRONDXM2) that was run on a Pentium computer (Kiernan et al., 2000). Recordings were amplified (gain 1000, bandwidth 5–10 kHz) and digitized using an analogue-to-digital (A/D) board (DT2812, Data Translation, Marlboro, MA, USA), with a sampling rate of 10 kHz. Stimulus waveforms were converted to current using a purpose-built isolated linear bipolar constant-current simulator.

Stimulus–response curves were generated for test stimuli of 0.2 and 1 ms duration (Fig. 1). The slope of the 1 ms stimulus–response curve and the magnitude of the tracking ‘error’ (i.e. the difference between measured response and target response) were used to optimize the subsequent threshold tracking. The peak 1 ms response was also used to calculate the target response (40% of the supramaximal CMAP response). The ratio between the stimulus–response curves for the two different stimulus durations was used to calculate rheobase (Burke et al., 2001) and the strength–duration time constant (t50) of motor axons of different thresholds using Weiss’s formula (Weiss, 1901; Mogyoros et al., 1996).
The threshold changes that occur in response to subthreshold depolarizing and hyperpolarizing pulses, referred to as threshold electrotonus, were measured by altering nerve excitability using subthreshold polarizing currents of 100 ms duration, set to 40% of the unconditioned threshold current (Bostock and Baker, 1988; Kiernan et al., 2000; Burke et al., 2001). A current–threshold relationship, analogous to the conventional current–voltage (I/V) relationship (Kiernan et al., 2000), was obtained by tracking the changes in threshold of 1 ms test pulses that occurred following subthreshold polarizing currents of 200 ms duration. In the final part of the

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Fig. 1 Six plots of excitability parameters recorded from TA for a single representative patient (continuous lines with circles) before dialysis with 95% confidence intervals (broken lines) for a single subject (Krishnan et al., 2004). (A) Absolute stimulus–response relationship for stimuli of 0.2 ms duration (line without filled circle) and 1 ms duration (line with filled circle). The filled circle on the 1 ms response curve corresponds to the threshold for a CMAP 50% of maximum and the broken ellipse corresponds to the 95% confidence limits for a single subject. (B) Normalized stimulus–response relationship. The responses in (A) are expressed as a percentage of maximum and the stimuli as a percentage of the stimulus for a response 50% of maximum. (C) Current–threshold relationship, reflecting rectifying properties of the axon following polarizing currents, expressed as a percentage of resting threshold. Threshold changes to hyperpolarizing current are represented on the left and to depolarizing current on the right. (D) Distribution of strength–duration time constants of nine populations of axons, from 5 to 95% of maximal CMAP in groups of 10%. (E) Threshold electrotonus. Threshold changes to polarizing currents of ±40% of the resting threshold. Changes are plotted as threshold reductions, with depolarization represented in an upward direction and hyperpolarization in a downward direction. (F) Recovery cycle, showing refractoriness, superexcitability and late subexcitability. See text for further details.
protocol, the recovery cycle of excitability of motor axons was assessed by tracking the changes in threshold that occurred following a supramaximal conditioning stimulus of 1 ms duration.

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). Excitability results were corrected for age, temperature and gender (Kiernan et al., 2001b). Single comparisons in excitability parameters were analysed using Student’s unpaired t-test for comparisons with normative data (n = 25; age range, 22–60 years; mean age, 31 years) and Student’s paired t-test for comparisons before and after dialysis. Logarithmic conversions were undertaken to normalize certain variables. Correlations were analysed using Pearson’s correlation coefficient. A probability value of <0.05 was considered statistically significant. Results are expressed as mean ± standard error of the mean.

Results

Nerve conduction studies and neuropathy assessment

The amplitude of sural nerve SNAP (sensory nerve action potential) (Burke et al., 1974) was reduced in 71% of patients (Table 1) and in the entire group as a whole (mean sural SNAP amplitude, 5.8 ± 1.1 μV; n = 14), with a minor reduction in sural nerve conduction velocity (Table 1). Superficial radial SNAP amplitude (Ma et al., 1981) was reduced in 50% of patients (mean, 18.1 ± 2.4 μV), although conduction velocity was generally preserved (mean, 53.6 ± 0.9 m/s).

Abnormalities of lower limb motor conduction were also demonstrated in a number of patients with neuropathy (Table 1). Tibial CMAP amplitudes (Buschbacher, 1999) were reduced in 43% of patients and mean tibial CMAP amplitude was mildly reduced (mean tibial CMAP amplitude, 3.6 ± 0.7 mV). Tibial distal motor latency was prolonged in 38% of patients although mean latency was within the normal range (mean tibial distal motor latency, 5.2 ± 0.5 mV). Tibial F-wave minimum latency (Puksa et al., 2003) was prolonged in 43% of patients (Table 1) and persistence was reduced in 43% of patients. Peroneal CMAP amplitudes (Ma and Liveson, 1983) were reduced in 21% of patients and there was a mild reduction in mean CMAP amplitude (mean peroneal CMAP amplitude, 3.2 ± 0.5 mV). These changes were accompanied by a reduction in peroneal nerve conduction velocity in 36% of patients, although the mean value was within the normal range (mean peroneal nerve conduction velocity, 43.4 ± 1.6 m/s). Peroneal distal motor latency was normal in all patients in whom a CMAP response was evident.

All ESKD patients in the study reported symptoms of neuropathy (Table 2), with an average of approximately two symptoms per patient (mean NSS 1.9 ± 0.2). The severity of neuropathy in the present study (Table 2) was staged as follows using a modified form of a previously devised system (Dyck, 1988): Stage 0, no neuropathy [T-NSS < 2 with normal NCS (nerve conduction study)]; Stage 1, asymptomatic neuropathy (T-NSS = 0 with abnormalities on NCS); Stage 2, symptomatic neuropathy (T-NSS > 2 with normal NCS or T-NSS ≥ 1 with abnormal NCS; neuropathic symptoms non-disabling); Stage 3, disabling neuropathy (T-NSS ≥ 2 with normal NCS or T-NSS ≥ 1 with abnormal NCS; neuropathic symptoms reported to be disabling). Using this scale, 1 patient had no neuropathy (stage 0), 10 had Stage 2 neuropathy and 3 had Stage 3 neuropathy (Table 2).

Table 1  Nerve conduction parameters for each patient

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sural SNAP (μV)</th>
<th>Radial SNAP (μV)</th>
<th>Tibial CMAP (mV)</th>
<th>Tibial DML (ms)</th>
<th>Tibial F-wave min. latency (ms)</th>
<th>Tibial F-wave persistence (%)</th>
<th>Peroneal CMAP (mV)</th>
<th>Peroneal DML (ms)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>6.0*</td>
<td>15.0*</td>
<td>4.4</td>
<td>7.7*</td>
<td>51.9</td>
<td>100</td>
<td>4.7</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>2.0*</td>
<td>11.0*</td>
<td>2.8*</td>
<td>6.6*</td>
<td>59.8*</td>
<td>90</td>
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<tr>
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<tr>
<td>4</td>
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<td>13.0*</td>
<td>1.6*</td>
<td>6.2*</td>
<td>61.8*</td>
<td>60*</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12.0</td>
<td>24.0</td>
<td>8.6</td>
<td>3.3</td>
<td>43.1</td>
<td>60*</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
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<td>14.0</td>
<td>33.5</td>
<td>8.1</td>
<td>4.4</td>
<td>41.8</td>
<td>100</td>
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<td>36.0</td>
<td>4.0</td>
<td>5.2</td>
<td>30.6</td>
<td>90</td>
<td>4.2</td>
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<td>20.0</td>
<td>3.9</td>
<td>3.8</td>
<td>42.2</td>
<td>90</td>
<td>3.5</td>
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<td>9</td>
<td>2.5*</td>
<td>17.4</td>
<td>4.8</td>
<td>3.5</td>
<td>54.2*</td>
<td>100</td>
<td>3.5</td>
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<td>13.0*</td>
<td>3.8</td>
<td>5.2</td>
<td>58.3*</td>
<td>90</td>
<td>3.4</td>
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<tr>
<td>11</td>
<td>4.3*</td>
<td>24.3</td>
<td>0.3*</td>
<td>7.9*</td>
<td>49.2</td>
<td>40*</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
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<td>4.3*</td>
<td>20.7</td>
<td>4.2</td>
<td>6.1*</td>
<td>48.9</td>
<td>90</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>13</td>
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<td>6.0*</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
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<td>Mean</td>
<td>5.8 ± 1.1</td>
<td>18.1 ± 2.4</td>
<td>3.6 ± 0.7</td>
<td>5.2 ± 0.5</td>
<td>49.6 ± 2.5</td>
<td>80 ± 5</td>
<td>3.2 ± 0.5</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>Controls</td>
<td>13.6 ± 7.5</td>
<td>42.4 ± 14.9</td>
<td>12.9 ± 4.5</td>
<td>4.5 ± 0.8</td>
<td>45.9 ± 4.4</td>
<td>100%</td>
<td>8.9 ± 4.0</td>
<td>4.0 ± 0.7</td>
</tr>
</tbody>
</table>

Group data expressed as mean ± standard error of the mean for each parameter. Data include amplitudes for sensory nerve action potential (SNAP) and compound motor action potential (CMAP), distal motor latency (DML) and tibial F-wave minimum latency (min. latency). The patient number is the same as in Table 2. *Denotes an abnormal result. Normative data 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 y and 30–59 y, respectively.
Neuropathy staged according to the following scale: Stage 0, no neuropathy (T-NSS < 2); Stage 1, asymptomatic neuropathy (T-NSS = 2 with normal NCS); Stage 2, symptomatic neuropathy (T-NSS > 2 with normal NCS or T-NSS ≥ 2 with abnormal NCS; neuropathic symptoms non-disabling); Stage 3, disabling neuropathy (T-NSS ≥ 2 with normal NCS or T-NSS ≥ 1 with abnormal NCS; neuropathic symptoms reported to be disabling). The maximum possible T-NSS was nine.

Table 2: Demographic and clinical data: neuropathy stage, T-NSS, neuropathic symptoms and Kt/V for each patient

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Stage</th>
<th>T-NSS</th>
<th>Clinical symptoms</th>
<th>Kt/V</th>
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</thead>
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<tr>
<td>1</td>
<td>17</td>
<td>2</td>
<td>1</td>
<td>Lower limb paraesthesiae</td>
<td>1.67</td>
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<td>2</td>
<td>64</td>
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<td>4</td>
<td>Leg weakness, leg pain, numbness in hands and feet, unsteady gait</td>
<td>1.66</td>
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<td>3</td>
<td>57</td>
<td>2</td>
<td>2</td>
<td>Leg weakness, numbness in feet</td>
<td>2.37</td>
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<td>4</td>
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<td>2</td>
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<td>Upper limb paraesthesiae</td>
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<td>37</td>
<td>2</td>
<td>2</td>
<td>Lower limb paraesthesiae, leg weakness</td>
<td>1.43</td>
</tr>
<tr>
<td>8</td>
<td>62</td>
<td>2</td>
<td>2</td>
<td>Lower limb pain, paraesthesiae</td>
<td>1.62</td>
</tr>
<tr>
<td>9</td>
<td>52</td>
<td>2</td>
<td>1</td>
<td>Numbness in feet</td>
<td>1.69</td>
</tr>
<tr>
<td>10</td>
<td>55</td>
<td>2</td>
<td>2</td>
<td>Lower limb paraesthesiae, leg weakness</td>
<td>1.48</td>
</tr>
<tr>
<td>11</td>
<td>62</td>
<td>2</td>
<td>1</td>
<td>Numbness in feet</td>
<td>1.67</td>
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<td>Lower limb paraesthesiae, pain in feet</td>
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<td>Leg weakness, hand weakness</td>
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<td>2</td>
<td>2</td>
<td>Pain in feet, lower limb numbness</td>
<td>2.31</td>
</tr>
</tbody>
</table>

Neuropathy staged according to the following scale: Stage 0, no neuropathy (T-NSS < 2 with normal NCS); Stage 1, asymptomatic neuropathy (T-NSS = 2 with abnormalities on NCS); Stage 2, symptomatic neuropathy (T-NSS > 2 with normal NCS or T-NSS ≥ 1 with abnormal NCS; neuropathic symptoms non-disabling); Stage 3, disabling neuropathy (T-NSS ≥ 2 with normal NCS or T-NSS ≥ 1 with abnormal NCS; neuropathic symptoms reported to be disabling). The maximum possible T-NSS was nine.

Nerve excitability abnormalities before dialysis

Motor excitability studies were successfully obtained from TA in all patients and from EDB in 13 out of 14 patients. The recordings from a single, representative patient are compared with normal limits in Fig. 1, and mean electrotonus and recovery cycle data are illustrated in Fig. 2A and B. Patient 13 (Table 2) had no recordable response from EDB. Pre-dialysis abnormalities in excitability were noted in 12 out of 14 patients in the study. Stimulus–response curves for the group (e.g., Fig. 1A) were shifted to the right in pre-dialysis recordings from both TA and EDB when compared with previously established normative data (Krishnan et al., 2004), indicating that their axons were of high threshold before dialysis. The stimulus intensity needed to produce a CMAP 50% of maximal was significantly greater in the renal patients for both TA and EDB recordings (TA ESKD recordings, 7.3 ± 1.8 mA; controls, 3.8 ± 0.2 mA; P < 0.005; EDB ESKD recordings, 9.7 ± 1.2 mA; controls 4.5 ± 0.2 mA; P < 0.0005).

Reductions in threshold electrotonus (Figs 1E and 2A) were noted in both the depolarizing and hyperpolarizing directions, leading to a 'fanned in' appearance (Kiernan and Bostock, 2000; Kaji, 2003). Depolarizing threshold electrotonus (TEd) at the 90–100 ms interval (TEd 90–100 ms), previously established as a sensitive indicator of resting membrane potential (Kiernan and Bostock, 2000), was lower in the ESKD patients in both TA and EDB recordings (Table 3) than in the normal controls (TA, P < 0.005; EDB, P < 0.0005). There were similar reductions in TEd 10–20 ms (TA, P < 0.0005; EDB, P < 0.0005) and TEd 40–60 ms (TA, P < 0.0005; EDB, P < 0.0005). Hyperpolarizing threshold electrotonus (TEh) was also significantly reduced in the ESKD patients at the 10–20 ms (TA, P < 0.005; EDB, P < 0.0005) and 90–100 ms intervals (TA, P < 0.005; EDB, P < 0.0005). With respect to the current–threshold relationship (Fig. 1C), although the resting and minimum current–threshold slopes were not significantly different between renal patients and controls, there was a significant change in the hyperpolarizing current–threshold slope (TA ESKD recordings, 0.64 ± 0.02; controls 1.24 ± 0.30; P < 0.01; EDB ESKD recordings, 0.60 ± 0.03; controls 1.12 ± 0.12; P < 0.0005).

With respect to the recovery cycle (Figs 1F, 2B and D), refractoriness, owing to inactivation of voltage-gated transient Na+ channels, was significantly increased in the renal patients (TA, P < 0.05; EDB, P < 0.005). Superexcitability, related to the depolarizing afterpotential (Barrett and Barrett, 1982; David et al., 1992), was reduced in the ESKD patients (Table 3) before dialysis (TA, P < 0.0005; EDB, P < 0.0005). Late subexcitability was also reduced in pre-dialysis recordings (TA, P < 0.05; EDB, P < 0.05).
Nerve excitability changes following dialysis

The ‘fanned-in’ appearance of threshold electrotonus and the reduction in superexcitability noted before the commencement of haemodialysis indicate axonal depolarization (Kiernan and Bostock, 2000). In order to explore the hypothesis that these changes may be caused by a dialysable toxin (Bostock et al., 2004), excitability studies were repeated during and 1 h following haemodialysis. These studies revealed significant improvement in a number of excitability parameters (Figs 2 and 3). In particular, the original abnormalities in parameters of threshold electrotonus largely resolved during dialysis (Table 3). The initial pre-dialysis reduction in TEd 90–100 ms improved following dialysis in both TA and EDB recordings (TA, $P < 0.0005$; EDB, $P < 0.0005$). Qualitatively similar changes were also observed in TEd 40–60 ms (TA, $P < 0.005$; EDB, $P < 0.0005$) and TEh 90–100 ms (TA, $P < 0.05$; EDB, $P < 0.0005$).

Changes were also noted in parameters of the recovery cycle (Table 3). There was a reduction in the degree of refractoriness (TA, $P < 0.0005$; EDB, $P < 0.0005$) following dialysis and a shortening of the duration of the relative refractory period (TA, $P < 0.005$; EDB, $P = 0.06$). These changes were accompanied by an increase in superexcitability following dialysis (TA, $P < 0.05$; EDB, $P < 0.005$). An increase in late subexcitability following dialysis was also noted (TA, $P < 0.0005$; EDB, $P < 0.05$).

Comparison of the post-dialysis recordings with normative data (Krishnan et al., 2004) revealed complete resolution of the depolarization changes noted in the pre-dialysis recordings in all parameters of the recovery cycle (Table 3). There were, however, persistent abnormalities in TEd 10–20 ms (TA, $P < 0.005$; EDB, $P < 0.005$), TEh 10–20 ms (TA, $P < 0.0005$; EDB, $P < 0.0005$) and TEh 90–100 ms in TA recordings (TA, $P < 0.05$), indicating that, although improvement occurred with dialysis, some residual impairment persisted.
Correlation of symptoms, neurophysiological parameters and potential neurotoxins

There was a close correlation between the stage of neuropathy and nerve conduction parameters (Fig. 4A), both motor and sensory (sural amplitude, $r = 0.68$; $P < 0.01$; superficial radial amplitude, $r = 0.59$; $P < 0.05$; peroneal CMAP amplitude, $r = 0.74$; $P < 0.01$; tibial CMAP amplitude, $r = 0.54$; $P < 0.05$). Significant correlation was also noted between T-NSS and both pre-dialysis refractoriness ($r = 0.73$; $P < 0.01$) and pre-dialysis TEd 90–100 ms ($r = -0.53$; $P < 0.05$) in TA recordings (Fig. 4B).

In order to further explore the basis of the pre-dialysis excitability changes, excitability parameters were assessed in relation to levels of potential neurotoxins, in addition to
measures of dialysis adequacy (Table 4). With respect to pre-dialysis excitability parameters, changes in threshold electrotonus and superexcitability correlated strongly with pre-dialysis serum $K^+$ (Fig. 4C). The correlations between these excitability parameters and $K^+$ were far greater than those for other substances, including creatinine, urea, calcium, $\beta$-2M and PTH (Table 4). Recordings were also obtained from one patient on five separate occasions before dialysis and a similarly close correlation was noted between TEd 90–100 ms and serum $K^+$ (Fig. 4D). Correlation was also noted between pre-dialysis PTH and superexcitability in EDB recordings ($r = 0.60; P < 0.05$) and this correlation was further strengthened after allowing for the effect of $K^+$ (Table 4).

Although correlations were noted between excitability measures and both urea and $\beta$-2M, these correlations were not significant after allowing for $K^+$ (Table 4). $Kt/V$, a measure of dialysis adequacy, correlated poorly with changes in TEd 90–100 ms in recordings from both TA ($r = 0.30$) and EDB ($r = 0.37$). It is noteworthy that all patients in the present study had a $Kt/V > 1.2$ (Table 2), in keeping with current guidelines on dialysis adequacy (National Kidney Foundation, 2001).

**Table 4** Correlation coefficients for TEd 90–100 ms, TEh 90–100 ms and superexcitability against potential neurotoxins before dialysis

<table>
<thead>
<tr>
<th></th>
<th>Mean TEd 90–100 ms</th>
<th>TEd 90–100 ms</th>
<th>TEh 90–100 ms</th>
<th>Superexcitability</th>
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<tbody>
<tr>
<td></td>
<td>TA</td>
<td>EDB</td>
<td>TA</td>
<td>EDB</td>
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<tr>
<td>$K^+$ (3.6–5.1)</td>
<td>4.9 ± 0.3</td>
<td>−0.87***</td>
<td>−0.85***</td>
<td>0.90***</td>
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<tr>
<td>Creatinine (60–110)</td>
<td>877 ± 49.1</td>
<td>−0.26</td>
<td>−0.28</td>
<td>0.26</td>
</tr>
<tr>
<td>Urea (2.9–7.1)</td>
<td>24.2 ± 2.3</td>
<td>−0.68***</td>
<td>−0.55</td>
<td>−0.13</td>
</tr>
<tr>
<td>$\beta$-2M (0.7–1.8)</td>
<td>42.7 ± 4.8</td>
<td>−0.42</td>
<td>−0.66*</td>
<td>0.53*</td>
</tr>
<tr>
<td>PTH (0.5–5.0)</td>
<td>28.4 ± 10.2</td>
<td>−0.33</td>
<td>−0.36</td>
<td>0.37</td>
</tr>
<tr>
<td>Ca$^{2+}$ (2.25–2.58)</td>
<td>2.4 ± 0.06</td>
<td>−0.26</td>
<td>−0.36</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Correlation coefficients after allowing for the effect of $K^+$ are shown in bold font. Mean serum concentrations for the group are expressed as mean ± standard error of the mean. Normal ranges for serum concentrations are displayed in parentheses. Serum levels are expressed in mmol/l except for creatinine (μmol/l), $\beta$-2M (mg/l) and PTH (pmol/l). Significance levels: *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.001$; *****, $P < 0.0001$. 

**Fig. 4** Relationship between (A) sural amplitude and neuropathy stage, (B) pre-dialysis TEd 90–100 ms from TA and T-NSS and (C) pre-dialysis TEd 90–100 ms from TA and serum $K^+$. (D) Relationship between pre-dialysis TEd 90–100 ms from EDB and serum $K^+$ in a single subject, tested on five separate occasions before dialysis. All correlations were significant at the 0.05 level. Dotted lines in (C) and (D) represent 95% confidence intervals for normal controls.
Discussion
The present study investigated the excitability properties of lower limb motor axons in patients with ESKD. All patients complained of neuropathic symptoms, and in most patients nerve conduction studies revealed changes consistent with peripheral neuropathy. Axonal excitability was abnormal before dialysis, with reductions in TEd and THe at multiple intervals, leading to a ‘fanned in’ appearance, alteration in the hyperpolarizing current–threshold slope and less superexcitability and late subexcitability. There was marked improvement in excitability parameters following dialysis, and post-dialysis excitability recordings were either approaching or were within the normal range.

Clinical symptoms and nerve conduction abnormalities
The rate of neuropathy in the present study was 93%, in keeping with previous studies of uraemic neuropathy, which have demonstrated similarly high rates of neuropathy in patients with ESKD (Ackil et al., 1981; Angus-Leppan and Burke, 1992; Van den Neucker et al., 1998; Laaksonen et al., 2002). The most commonly affected parameter in the present study was sural SNAP amplitude, which was abnormal in a higher percentage of patients than radial SNAP amplitude, consistent with the lower limb predisposition of neuropathy. Previous studies have demonstrated that abnormalities in late responses, namely F-waves and H-reflexes, may be a sensitive marker of neuropathy in patients with uremia (Halar et al., 1979; Panayiotopoulos and Lagos, 1980; Van den Neucker et al., 1998; Laaksonen et al., 2002). In the present study, although tibial F-wave minimum latency was abnormal in 43% of patients, all but one of those patients had accompanying abnormalities of sensory amplitudes, suggesting that sural nerve SNAP amplitudes are a more sensitive neurophysiological indicator of neuropathy in patients with uremia than lower limb F-wave parameters.

Excitability abnormalities in ESKD
There were significant abnormalities in peroneal nerve excitability before dialysis. Maximal CMAP amplitude was reduced in EDB recordings but maintained in TA recordings, consistent with the length-dependent predisposition of uraemic neuropathy. In addition, there were changes in sensitive markers of membrane potential, including threshold electrotonus and recovery cycle parameters, suggestive of axonal depolarization. Threshold electrotonus refers to the threshold changes that occur in response to subthreshold depolarizing and hyperpolarizing pulses and is sensitive to changes in resting membrane potential (Bostock et al., 1998). Membrane depolarization causes a ‘fanning in’ of threshold curves (Kiernan and Bostock, 2000), as was noted in the present study in pre-dialysis recordings from both TA and EDB. Superexcitability, as a result of the depolarizing afterpotential, determined in part by the level of activation of paranodal voltage-dependent K⁺ channels (Barrett and Barrett, 1982; David et al., 1995), was significantly reduced before dialysis, which is also consistent with membrane depolarization (Kiernan and Bostock, 2000). Although a reduction in tSD (limited to EDB recordings) before dialysis would be unexpected for axonal depolarization, the inherent variability of this excitability measure, and its potential to vary with metabolic change, particularly acid-base disturbances, preclude further comment (Mogyoros et al., 1997; Baker and Bostock, 1999).

The clear improvements in excitability parameters following dialysis noted in the present study and in a previous study of upper limb nerve excitability in uraemic patients (Kiernan et al., 2002b) provide a sharp contrast to the conflicting results of studies utilizing standard nerve conduction techniques. Although some studies have documented post-dialysis improvements in a number of neurophysiological parameters, including sensory nerve conduction velocity (Nielsen, 1973; Lang and Forstrom, 1977) and sensory and motor action potential amplitudes (Mansouiri et al., 2001), others have found no significant changes in neurophysiological parameters following a single haemodialysis session (Laaksonen et al., 2002).

Contributing factors to pre-dialysis excitability changes
The present study detected correlations between serum K⁺ and threshold electrotonus parameters and superexcitability. There was also significant correlation between T-NSS and both pre-dialysis refractoriness and TEd 90–100 ms, with patients with a higher T-NSS manifesting greater excitability changes (Fig. 4B). This is the first evidence that altered axonal membrane potential, as recorded by nerve excitability testing, is directly related to neuropathic symptoms. Abnormalities of serum K⁺ will lead to changes in membrane potential as a result of the dependence of resting membrane potential on the concentration gradient for K⁺ (Bostock et al., 2004). The alterations in late subexcitability provide further evidence for the contribution of K⁺ to the excitability abnormalities, since subexcitability depends on the difference between the resting potential and the K⁺ equilibrium potential, and actually increases with depolarization if extracellular K⁺ is unchanged (Kiernan and Bostock, 2000). The reduction in late subexcitability in the pre-dialysis recordings from TA and EDB in the present study mirrors the findings of a previous study of median nerve excitability in patients with ESKD (Kiernan et al., 2002b). In total, these studies suggest that pre-dialysis axonal membrane depolarization in patients with ESKD is more probably caused through effects mediated by serum K⁺ than as a consequence of a reduction in Na⁺/K⁺ pump function (Kiernan and Bostock, 2000; Kiernan et al., 2002b).

Studies of sensory nerve excitability in ESKD are currently under way that may provide further insights into the role of K⁺ in uraemic neuropathy, given the sensory predilection of uraemic neuropathy and the differential effects of K⁺ on sensory and motor axons (Neumcke et al., 1980).
How do the reversible changes demonstrated in the present study lead to the more irreversible neurological changes that characterize uraemic neuropathy? It may be argued that the abnormalities of serum $K^+$ noted constitute a transient homeostatic disturbance that is rapidly corrected by dialysis and therefore unlikely to play a major role in the development of chronic irreversible neuropathy. Against such an argument, prolonged exposure to hyperkalaemia in ESKD patients seems likely, given that the post-dialysis rebound of $K^+$ is a well-recognized phenomenon (De Nicola et al., 2000; Ahmed and Weisberg, 2001), with hyperkalaemia typically occurring within 6 h of a dialysis session owing to re-equilibration between intracellular and extracellular fluid compartments (Blumberg et al., 1997). Such prolonged hyperkalaemia may cause disruption of normal ionic gradients, which are essential for axonal survival (see Bostock et al., 2004), activating damaging $Ca^{2+}$-mediated processes and leading to axonal loss (Craner et al., 2004). Excitability studies focusing on a more prolonged period between consecutive haemodialysis sessions may shed further light on the duration for which excitability remains truly 'normal' in ESKD patients.

It remains possible that $K^+$ acts synergistically with another toxin in mediating neurotoxicity, given that excitability changes occurred even when $K^+$ was in the upper limits of the normal range, rather than outside normative values (Fig. 4C). A possible candidate is PTH, which correlated with pre-dialysis superexcitability in EDB (Table 4). A number of studies have suggested a link between PTH and neurological complications in ESKD (Slatopolsky et al., 1980; Massry, 1987) and PTH has been shown to prolong motor nerve conduction velocities in animal studies (Goldstein et al., 1978). Human studies of the effect of PTH on peripheral nerves have yielded conflicting results, with variable changes in motor nerve conduction velocity in patients with ESKD (Avram et al., 1978; Di Giulio et al., 1978; Schaefer et al., 1980).

The correlation between pre-dialysis PTH and superexcitability in EDB must be interpreted with caution for the following reasons. First, there was no such correlation with superexcitability in TA, or in the combined TA and EDB data (whereas this combination strengthened the correlation with $K^+$). Second, there was no correlation between PTH and superexcitability in EDB when pre-dialysis and post-dialysis data were combined (whereas this combination again strengthened the correlation with $K^+$).

Apart from $K^+$ and PTH, there was no evidence for an effect on nerve excitability of the other potential toxins, including urea and creatinine. Although these substances are easily dialysed, they correlated poorly with excitability abnormalities, after allowing for the effect of $K^+$. The absence of any detectable neurotoxic effect of urea calls into question the suitability of $Kt/V$, a measure based on urea (Mallick and Gokal, 1999; Daugirdas, 2000), for determining the adequacy of a dialysis regimen to prevent neurotoxicity. All patients in the present study met the current guidelines for dialysis adequacy and yet excitability was significantly abnormal before dialysis in the majority, with all patients exhibiting symptoms of neuropathy. Our data suggest that, at least as far as axons are concerned, a better indication of adequate dialysis might be the maintenance of serum $K^+$ within normal limits between periods of dialysis. This may require more attention to dietary restriction of $K^+$ intake in some patients.

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References


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