Nerve excitability changes in chronic renal failure indicate membrane depolarization due to hyperkalaemia

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Summary
Multiple nerve excitability measurements were used to investigate axonal membrane properties in patients with chronic renal failure (CRF). Nine patients were studied during routine haemodialysis therapy. The median nerve was stimulated at the wrist and compound muscle action potentials recorded from abductor pollicis brevis. Stimulus–response behaviour, strength–duration time constant, threshold electrotonus, current–threshold relationship and recovery cycle (refractoriness, superexcitability and late subexcitability) were recorded using a recently described protocol. In six patients, sequential studies were performed before, during and after haemodialysis. All patients underwent standard electrolyte and renal function tests before and after haemodialysis. Before dialysis, there were significant abnormalities in axonal excitability: reduced superexcitability; increased accommodation to depolarizing and hyperpolarizing currents; and a steeper current–threshold relationship compared with normal controls. These excitability parameters are the most sensitive to membrane potential and the abnormalities, which were all reduced by haemodialysis, closely resembled those in normal axons depolarized by ischaemia. Before dialysis, the excitability parameters correlated significantly with serum potassium (range 4.3–6.1 mM), but not with other markers of renal dysfunction: patients with normal axonal resting potentials had normal serum potassium, although urea and creatinine were elevated. We conclude that nerves are depolarized in many CRF patients and that the depolarization is primarily due to hyperkalaemia.

Keywords: chronic renal failure; haemodialysis; potassium; uraemic neuropathy

Abbreviations: CMAP = compound muscle action potentials; CRF = chronic renal failure; RRP = relative refractory period; TEd = depolarizing threshold electrotonus; TEh = hyperpolarizing threshold electrotonus

Introduction
Nerve dysfunction is a common accompaniment of uraemia, with neuropathy estimated to be present in 60% of patients commencing haemodialysis (Bolton, 1980). This complication may decrease with earlier treatment (Manis and Friedman, 1979) or reverse following renal transplantation (Bolton et al., 1971; Nielsen, 1974; Oh et al., 1978). However, despite the high incidence of neurological problems due to uraemia, the pathophysiology remains unknown. Since uraemic neuropathy improves with dialysis, it is attributed to the accumulation of dialysable metabolites, but the nature of these uraemic toxins remains obscure (Bolton and Young, 1990). Substances in the 300–5000 range of molecular weights were first implicated on the basis of the supposed superiority of peritoneal dialysis in preventing neuropathy (Scribner, 1965), and this ‘middle molecule’ theory (Babb et al., 1981) is still considered the most plausible (Bolton, 1993; Raskin, 2001). All attempts to identify the putative middle molecular weight neurotoxins have, however, been unsuccessful (Merrill, 1979; Bolton and Young, 1990).
As to the mechanism whereby uraemic neurotoxins may cause nerve damage, the prevailing hypothesis appears to be that inhibition of Na+/K+-ATPase by the toxin causes membrane depolarization (Nielsen, 1973) and that maintenance of a normal membrane potential and ionic gradients is considered essential for axonal survival (Stys et al., 1995). Supporting evidence for a depolarizing dialysable neurotoxin was provided by the observation of Lowitzsch et al. (1981) that of 18 patients with chronic renal failure (CRF), nine had prolonged axonal refractory periods prior to dialysis but, in eight of these, the refractory period was normalized by dialysis.

Refactory period is just one of several measures of nerve excitability that are now being used clinically to provide information about biophysical properties of peripheral axons in disease states (Kiernan et al., 2000). These in vivo techniques are particularly sensitive to changes in axonal membrane potential and the use of multiple excitability measures provides information about the involvement of different ions and ion channels (Bostock et al., 1998; Kiernan and Bostock, 2000; Burke et al., 2001). The present study was therefore undertaken to test the hypothesis that axons in CRF patients are depolarized, and that this depolarization is rapidly reversed by dialysis. It was anticipated that, if this hypothesis were confirmed, excitability studies should be able to help in the identification of the uraemic neurotoxins responsible for the depolarization. The results have not only confirmed that axonal membranes are depolarized in CRF and that this depolarization is rapidly reduced by dialysis, but provided good evidence that potassium—rather than a toxin acting on Na+/K+-ATPase—is the dialysable substance responsible.

**Methods**

Recordings were made on nine patients (aged 32–78 years; mean 58.6 years; seven males, two females) from the haemodialysis unit of a major London teaching hospital. All patients suffered from CRF and had been on regular haemodialysis for at least 3 years. The thrice-weekly haemodialysis was adequate, as verified by regular clinical examination, constant weight, subjective well-being and satisfactory normalized whole body urea clearance (delivered Kt/V ≥ 1.4), where K = dialyser clearance; t = time on dialysis; V = volume of distribution (Hakim et al., 1992). Of the nine patients, four had reached end stage renal failure with polycystic kidney disease, three with interstitial nephritis and two with glomerulonephritis. No patient had signs or symptoms of neurological disease and none had diabetes. All patients gave informed consent and the study was approved by the St Mary’s Local Research Ethics Committee of the Kensington, Chelsea and Westminster Health Authority.

Studies were performed using a recently described protocol (Kiernan et al., 2000) designed to measure a number of different nerve excitability parameters rapidly. The nine patients were studied prior to or soon after the commencement of a haemodialysis session. In addition, the time course of the changes in these excitability parameters with dialysis was followed in six of the patients by completing the protocol near the start, middle and end of a session of haemodialysis. Serum electrolytes (sodium, potassium, calcium and phosphate), renal function (urea and creatinine), acid–base balance (bicarbonate), magnesium and uric acid were measured in all patients before and after haemodialysis.

Compound muscle action potentials (CMAPs) were recorded from thenar muscles using surface electrodes over abductor pollicis brevis, with the active electrode at the motor point and the reference on the proximal phalanx. The EMG signal was amplified (gain 1000, bandwidth 1.6 Hz to 2 kHz) and digitized by a computer (486 PC) with an A/D board (DT2812, Data Translation Inc., 100 Locke Drive, Marlboro, MA 01752-1192, USA) using a sampling rate of 10 kHz. Stimulus waveforms generated by the computer were converted to current with a purpose-built isolated linear bipolar constant current stimulator (maximum output ±50 mA). The stimulus currents were applied via non-polarizable electrodes (Red Dot, 3M Health Care, D-46325 Borken, Germany), with the active electrode over the median nerve at the wrist and the reference electrode ~10 cm proximal over muscle. Stimulation and recording were controlled by QTRAC software (version 5.2, copyright Institute of Neurology, London, with multiple excitability protocol TRONDXM).

Test current pulses of 0.2 or 1 ms were applied at 0.8 s intervals, and combined with supra-threshold conditioning stimuli or sub-threshold polarizing currents as required. The polarizing, conditioning and test current pulses were all delivered through the same electrodes. The amplitude of the CMAP was measured from baseline to negative peak. For all tracking studies, the target CMAP was set to 40% of maximum. Skin temperature was recorded using an adhesive probe over the nerve, adjacent to the stimulation electrode, to monitor temperature close to the site where axonal excitability was tested.

The sequence of recordings followed that previously described (Kiernan et al., 2000). Stimulus–response curves were recorded separately for test stimuli of durations 0.2 and 1 ms (Fig. 1A). The stimuli were increased in 6% steps, with two responses averaged for each step, until three averages were considered maximal. The ratio between the 0.2 and 1 ms stimuli required to evoke the same response was used to estimate rheobase and strength–duration time constant of axons of different threshold (Fig. 1D). A target response was then set at 40% of the maximum and the 1.0 ms test stimuli adjusted automatically by the computer to maintain this peak CMAP amplitude. ‘Proportional tracking’ was used whereby the change in stimulus amplitude from one trial to the next was made proportional to the ‘error’, or difference between the last response and the target response (Bostock et al., 1998). The slope of the stimulus–response curve was used to
set the constant of proportionality and to optimize the tracking efficiency.

Prolonged sub-threshold currents were used to alter the potential difference across the internodal as well as the nodal axonal membrane. The changes in threshold associated with these electrotonic changes in membrane potential normally have a similar time course and are known as threshold electrotonus (Bostock et al., 1998). Threshold tracking was
used to record the changes in threshold induced by 100 ms polarizing currents, set to 40% (depolarizing) and −40% (hyperpolarizing) of the control threshold current. Three stimulus combinations were tested in turn: (i) test stimulus alone (to measure the control threshold current); (ii) test stimulus + depolarizing conditioning current; and (iii) test stimulus + hyperpolarizing conditioning current. Threshold was tested at 26 time points (maximum separation 10 ms) before, during and after the 100 ms conditioning currents (Fig. 1E). Each stimulus combination was repeated until three valid threshold estimates were recorded, as judged by the response being within 15% of the target response or alternate responses being either side of the target.

The current–threshold relationship (Fig. 1C) was tested with 1 ms pulses at the end of 200 ms polarizing currents, which were altered in 10% steps from +50% (depolarizing) to −100% (hyperpolarizing) of the control threshold. As with the conventional threshold electrotunnus protocol, stimuli with conditioning currents were alternated with test stimuli alone, and each stimulus combination was repeated until three valid threshold estimates were obtained.

The final part of the protocol recorded the recovery of excitability following a supramaximal conditioning stimulus (Fig. 1F). These changes were recorded at 18 conditioning (1/n) test intervals, decreasing from 200 ms to 2 ms in approximately geometric progression. Three stimulus combinations were tested in turn: (i) unconditioned test stimulus (of 1 ms duration) tracking the control threshold; (ii) supramaximal conditioning stimulus (1 ms duration) alone; and (iii) conditioning + test stimuli. The response to (ii) was subtracted on-line from the response to (iii) before the test CMAP was measured, so that the conditioning maximal CMAP did not contaminate the measured response when the conditioning-test interval was short. Each stimulus combination was repeated until four valid threshold estimates were obtained.

Data analysis

The following excitability parameters were derived from each recording such as the one in Fig. 1: **Resting current–voltage (IV) slope** is the slope of the current–threshold relationship (as in Fig. 1C), calculated from the polarizing currents between −10% and +10% of the resting threshold; **Minimum IV slope** is the minimum slope, calculated by fitting a straight line to each three adjacent points in turn; **RRP** is the relative refractory period, calculated from the recovery cycle data (as in Fig. 1F) as the first intercept on the x-axis; **Superexcitability** is also calculated from the recovery cycle data (as in Fig. 1F), as the minimum mean of three adjacent points; and **Subexcitability** as the maximum mean of three adjacent points after 10 ms; the threshold electrotunnus parameters TEd (10–20 ms), TEd (90–100 ms) and TEd (90–100 ms) were the mean threshold reductions, calculated from the data (as in Fig. 1E) between the specified latencies for the 40% depolarizing (TEd) and hyperpolarizing (TEd) currents.

Values for these excitability parameters obtained in the current study were compared with normative data established in a previous study of 29 normal control subjects (Kiernan et al., 2000) and with data from a previous study of the effects of polarizing currents and ischaemia on 14 nerve excitability parameters (Kiernan and Bostock, 2000). Pre- and post-dialysis data were compared using paired two-tailed t-tests, while pre-dialysis data were compared with normal controls using unpaired two-tailed t-tests. Because RRP measurements are very sensitive to skin temperature (Kiernan et al., 2000, 2001a), individual measurements were compensated for temperature using the relationship found in normal control subjects (Kiernan et al., 2000) before any statistical tests were applied.

Theoretical dependence of resting potential on potassium ions

The resting potentials of myelinated axons depend on the diffusion of permeable ions and on the electrogenic sodium pump (Na+/K+ ATPase). According to the Goldman–Hodgkin–Katz constant field theory (Goldman, 1943; Hodgkin and Katz, 1949), the passive sodium diffusion current (I_{Na}) is given by:

\[ I_{Na} = P_{Na} E F 2/RT [Na_o - Na_i \exp(ERF/RT)]/[1 - \exp(ERF/RT)] \]

where \( P_{Na} \) is the membrane permeability to sodium ions, \( E \) is the membrane potential, \( F \) is Faraday’s constant, \( R \) is the gas constant, \( T \) is the absolute temperature, and \( Na_o \) and \( Na_i \) are the outside and inside sodium concentrations. Similarly, the potassium diffusion current is given by:

\[ I_{K} = P_{K} E F 2/RT [K_o - K_i \exp(ERF/RT)]/[1 - \exp(ERF/RT)] \]

In the resting state, these passive currents of sodium and potassium ions must be equal and opposite to the currents pumped by Na+/K+ ATPase. The pump is electrogenic and couples the extrusion of three sodium ions to the inward movement of two potassium ions, so that:

\[ I_K = -\frac{3}{2} I_{Na} \]

\[ P_{K} [K_o - K_i \exp(ERF/RT)] = \frac{3}{2} P_{Na} [Na_o - Na_i \exp(ERF/RT)] \]

\[ (P_{K} K_o + \frac{3}{2} P_{Na} Na_o) / (P_{K} K_i + \frac{3}{2} P_{Na} Na_i) = \exp(ERF/RT) \]

Therefore the resting potential (ER) is given by:

\[ E_r = (RT/F) \log[e \{(K_o + k)/(K_i + k2)\}] \]

where \( k = \frac{3}{2} Na_o \cdot P_{Na}/P_{K} \), and \( k2 = \frac{3}{2} Na_i \cdot P_{Na}/P_{K} \).

With realistic values of \( E_r \) and ion concentrations, \( k2 \ll K_o \) so that equation 1 simplifies to:

\[ E_r = (RT/F) \log[e \{(K_o + k)/K_i\}] \]

Equation 2 predicts how \( E_r \) should depend on potassium concentrations, provided that the changes are sufficiently small that changes in \( k \) can be ignored. The value of \( k \) can be estimated by rearranging equation 2 as:

\[ k = K_i \exp(ERF/RT) - K_o \]
For example, if $E_t = -84$ mV (Schwarz et al., 1995) at 32°C, $K_o = 4.5$ mM and $K_i = 155$ mM, then $k = 1.84$ mM. Estimates given by equation 2 are necessarily rough, since the constant field equations are only approximations (Jack, 1976), and $k$ is not known accurately for human axons.

**Results**

The full sequence of excitability measurements described above was recorded prior to haemodialysis in each patient, and the data from a single patient are plotted in a standard format (Kiernan et al., 2000) in Fig. 1. The six standard plots of Fig. 1A–F are described in turn. In each plot, the recording from a single CRF patient is compared with the 95% confidence limits for a single subject drawn from a normal population, based on previously published data from 29 normal controls (Kiernan et al., 2000).

Stimulus–response curves for the test stimuli of duration 0.2 and 1 ms are plotted on log–log coordinates in Fig. 1A. The filled circle on the 1 ms response curve corresponds to the threshold for a CMAP 50% of maximum. This point lies outside the ellipse corresponding to the 95% confidence limits established previously for normal control subjects (Kiernan et al., 2000), indicating that thresholds were abnormally high in this subject.

The two stimulus–response curves in Fig. 1A are replotted in Fig. 1B on linear axes, normalized by plotting the responses as a percentage of maximum and the stimuli as percentages of the stimulus for a response 50% of maximum. The curves for the 0.2 and 1 ms stimuli are very similar and well within normal limits, indicating a normal spread of axon thresholds.

Figure 1C shows plots of the normalized threshold changes at the end of 200 ms current pulses. The plot is orientated such that depolarization occurs to the right and hyperpolarization to the left. The current–threshold relationship reflects the rectifying properties of the axon (both nodal and internodal axolemma), and the slope of the curve can be used to provide an estimate of the threshold analogue of input conductance. The steepening of the curve towards the top right results from outward rectification, due to activation of fast and slow $K^+$ channels, while the less prominent steepening towards the bottom left indicates inward rectification, due to activation of the hyperpolarization-activated conductance ($I_h$). The data appear on the limit of the normal range, but the slope at zero current (resting I/V slope) is abnormally steep, indicating a high input conductance at rest.

Strength–duration time constants were calculated for different fractions of the compound action potential (Fig. 1D). Strength–duration time constant is an apparent membrane time constant, derived from the relationship between threshold current and stimulus duration, which depends on active as well as passive membrane properties (Bostock et al., 1998). The values are well within the normal range for this parameter, which is not very sensitive to membrane potential (Kiernan and Bostock, 2000).

The threshold electrotonus changes in excitability occurring during and after 100 ms sub-threshold depolarizing and hyperpolarizing currents are plotted in Fig. 1E. After the initial step changes in threshold, corresponding to the ±40% polarizing currents, the threshold electrotonus responses are abnormally flat in this subject, indicating an absence of the normal slow changes in excitability due to changes in potential of the internodal axon. Threshold electrotonus waveforms are particularly sensitive to changes in membrane potential (Baker and Bostock, 1989; Bostock et al., 1998; Kiernan and Bostock, 2000). In depolarized axons, internodal potassium channels are activated which short-circuit the normal slow components of electrotonus.

The recovery cycle of excitability changes following an action potential are plotted in Fig. 1F. The recovery cycle normally comprises clear periods of refractoriness, super-excitability and late subexcitability—each phase reflecting predominantly different properties of the axonal membrane (Bostock et al., 1998). In this CRF patient, however, only a relative refractory period is evident.

Mean data from all nine patients (±standard error of measurement; SEM) recorded prior to haemodialysis are presented in Fig. 2 where they are compared with the mean data (±SEM) for 29 normal control subjects. All the abnormalities seen in the single patient in Fig. 1 were reproduced in the average recordings and found to be highly statistically significant. Thus, patients had markedly reduced CMAPs (4.0 ± 0.8 mV for patients; 9.0 ± 0.6 mV for controls; $P < 0.0001$; Fig. 2A) and their axons were of high threshold, as indicated by a shift to the right of the stimulus–response curve (stimulus current 6.3 ± 0.84 mA for patients; 4.6 ± 0.21 mA for controls; $P < 0.01$) and an increase in rheobase current (4.31 ± 0.60 mA for patients; 3.14 ± 0.15 mA for controls; $P < 0.01$). The high threshold may reflect a combination of local factors such as peripheral oedema and fluid accumulation, present to some degree in all patients prior to dialysis, in addition to some degree of axonal loss. Stimulus response slope was not significantly different between patients and control subjects (Fig. 2B).

The current–threshold relationship was abnormal in patients (Fig. 2C), with smaller changes in threshold for the same currents indicating a reduced input impedance. Strength–duration time constant (estimated for a 40% of maximal CMAP) was, however, remarkably similar (0.42 ± 0.03 ms for patients; 0.42 ± 0.02 ms for controls; Fig. 2D).

The most striking abnormalities in excitability parameters are revealed by the recordings of threshold electrotonus (Fig. 2E) and the recovery cycle (Fig. 2F), both properties being strongly sensitive to membrane potential. Threshold electrotonus waveforms from the patients show a marked increase in accommodation relative to controls; the 100 ms depolarizing and hyperpolarizing currents produced smaller changes in excitability after the initial step changes, as occurs in depolarized axons (Baker and Bostock, 1989; Bostock et al., 1998; Kiernan and Bostock, 2000). In the recovery cycles (Fig. 2F), the relative refractory period was markedly prolonged in patients (4.7 ± 0.6 ms for patients; 3.1 ± 0.1 ms...
for controls; \( P < 0.0001 \)); superexcitability was reduced
\((13.9 \pm 4.2\% \text{ for patients}; 25.5 \pm 1.0\% \text{ for controls}; \ P < 0.0005)\); the late subexcitability was also reduced
\((5.8 \pm 0.7\% \text{ for patients}; 14.6 \pm 0.7\% \text{ for controls}; \ P < 0.000005)\).

**Change in axonal excitability properties during dialysis**

The excitability protocol was repeated during and following completion of dialysis in six patients from the original group. A striking improvement in the excitability recordings,
especially those most closely related to membrane potential, was observed. As indicators of membrane potential, we have used the six excitability parameters which were previously found to correlate the best (out of 14) with small polarizing currents (all correlation coefficients > 0.75), and were therefore considered to provide the most accurate indices of membrane potential (Kiernan and Bostock, 2000). Figure 3 compares pre-dialysis values of these six excitability parameters with the corresponding post-dialysis values and with normal control values (Kiernan et al., 2000). All these potential-sensitive excitability parameters were significantly abnormal prior to dialysis (all \( P < 0.0001 \)) and all the abnormalities were significantly reduced by the dialysis, although normalization was not complete in most cases.

The time course of the improvements in these six excitability parameters is charted for each subject in Fig. 4. Repeated excitability measurements on the same subject, especially if the electrodes are not moved, show very little variation with time (Kiernan et al., 2000); the horizontal dashed lines indicate the mean values for normal controls. Figure 4 shows that the averaged data in Fig. 3 conceals a wide degree of variation. In some subjects, the excitability parameters were relatively normal to start with and changed little, but when the parameters were initially very abnormal they improved rapidly during dialysis.

**Comparison of excitability abnormalities with those in depolarization and ischaemia**

Figure 5 shows scatter plots of the data, taking two excitability parameters at a time (compare with Fig. 4 in Kiernan and Bostock, 2000), to compare the excitability abnormalities in CRF patients with those previously found in normal nerves depolarized by applied currents and by...
ischaemia. The dashed ellipses (lines in Fig. 5B) indicate the normal ranges of the pairs of parameters, allowing for the correlation between them (i.e. the 95% confidence limits for recordings from a single individual drawn from the normal control group). The filled circles (R) indicate the mean predialysis values of the six CRF patients, and the open circles the mean post-dialysis figures. The tendency of dialysis to bring the excitability parameters within the normal range recapitulates the data in Figs 3 and 4. Figure 5 also shows the effects on the same excitability parameters in normal subjects of a depolarizing current of 1 mA (D and triangles; mean of four subjects) and the effects of 5 min of ischaemia (I and
In Fig. 5A–C, it can be seen that the excitability abnormalities in CRF, depolarization and ischaemia are qualitatively very similar. Thus, CRF and ischaemia resemble depolarizing currents in increasing the resting I/V slope—increasing the refractory period and reducing the absolute size of depolarizing and hyperpolarizing electrotonus in similar proportions. These data therefore provide good evidence that the excitability abnormalities in CRF, like those in ischaemia, are primarily due to membrane depolarization.

In Fig. 5D, however, the changes in subexcitability show qualitative differences, which provide a clue to the different mechanisms of depolarization in the three cases. Subexcitability depends on the activation of nodal slow potassium channels and on the difference between the resting membrane potential ($E_r$) and the potassium equilibrium potential ($E_K$) (Kiernan and Bostock, 2000). The increase in subexcitability on depolarization by applied current (D in Fig. 5D) occurs because $E_r$ is depolarized, but $E_K$ is unchanged, so that $E_r - E_K$ increases. During ischaemia (I in Fig. 5D), depolarization occurs partly because of the block of the electrogenic sodium pump, and partly because of the consequent extracellular accumulation of potassium ions. Depolarization of $E_K$ as well as $E_r$ can account for the lack of change in subexcitability. The reduction of subexcitability in CRF patients (R in Fig. 5D) suggests that, in this case, $E_K$ depolarizes more than $E_r$, i.e. that the depolarization is driven by an increase in extracellular potassium and not by a failure of the electrogenic sodium pump. This evidence is only suggestive, since subexcitability could also be reduced by a block of slow potassium channels. More direct evidence of the importance of extracellular potassium changes was provided by comparing serum electrolytes with the excitability changes.

**Fig. 5** Plots of selected excitability parameters to compare effects of depolarization by applied currents, ischaemia and CRF. In each plot, the dashed ellipse or dashed lines indicate 95% confidence limits for a single individual drawn from the normal population (Kiernan et al., 2000a). Triangles indicate the effects of depolarization by applied currents: filled triangles = controls, open triangles = 1 mA depolarizing current (D) (means of four subjects, data from Kiernan and Bostock, 2000). Squares indicate the effects of depolarization by proximal pressure cuff: filled squares = controls; open squares = 5 min ischaemia (I) (means of four subjects, data from Kiernan and Bostock, 2000). Circles indicate new data on CRF and haemodialysis: filled circles = pre-dialysis (R), open circles = end of dialysis (means of six subjects). (A) Minimum versus resting current–threshold slope. (B) RRP versus skin temperature. (C) Early depolarizing versus late hyperpolarizing threshold electrotonus. (D) Late subexcitability versus superfexcitability. CRF induces abnormalities in excitability properties which are qualitatively similar to those induced by depolarizing currents and ischaemia, except for subexcitability.
Influence of serum electrolytes and renal function on excitability

To further explore the cause of the axonal membrane depolarization in CRF patients, pre-dialysis excitability measures in all nine patients were compared with serum levels of sodium, potassium, urea, creatinine, calcium, magnesium, bicarbonate, phosphate and uric acid. Comparisons were made with pre-dialysis serum concentrations only, based on the assumption that prior to dialysis serum concentrations would be close to equilibrium with those in endoneurial fluid (given that patients had not undergone dialysis for the preceding 2–3 days). Table 1 lists the correlations between the six potential-sensitive excitability parameters illustrated in Figs 3 and 4, and the serum concentrations of potassium, urea and creatinine. Notably, all six excitability parameters were significantly related to the potassium levels, but none of them to urea or creatinine. It is also notable that the potassium concentrations overlapped with the normal range, whereas those of urea and creatinine were abnormally elevated in all subjects, in accordance with their diagnosis of CRF. Among the other six serum constituents tested, the only significant correlations with the six excitability parameters were between sodium and RRP, sodium and TEd (90–100 ms), and between bicarbonate and resting I/V slope (all 0.01 < P < 0.05). The correlations with sodium (range 136–142 mM) most likely reflected the significant inverse relationship between sodium and potassium (R = −0.67) only.

The relationships between serum potassium and the six potential-dependent excitability parameters are plotted in Fig. 6. On each plot, the horizontal dashed line indicates the mean value of the excitability parameter in normal controls (as in Fig. 4). The regression lines are of potassium on the excitability parameter (i.e. of y on x, rather than the more normal x on y), to provide an estimate, from the intercept with the dashed line, of the serum potassium concentration at which a pre-dialysis CRF patient would have a normal axonal resting potential. The six intercepts fall in the range 4.3–4.6 mM (mean 4.43 mM), very close to the expected value for serum potassium in normal subjects. This result indicates that when CRF patients have normal serum potassium, they also have normal axonal excitability parameters in spite of raised serum levels of urea, creatinine and other metabolites. It is unlikely, therefore, that any toxic metabolite apart from potassium contributes to membrane depolarization in CRF patients.

Discussion

This study has provided the clearest evidence so far that axons in many patients with CRF are chronically depolarized, and provided the first indication that this membrane depolarization is fully accounted for by hyperkalaemia. We found that multiple measures of axonal excitability were strikingly abnormal prior to dialysis (Figs 1 and 2), with increased refractory period, increased accommodation in threshold electrotonus and reduced superexcitability. All these changes are indicative of axonal depolarization, and in the opposite direction to those recently described in multifocal motor neuropathy and attributed to axonal hyperpolarization (Kiernan et al., 2002). Haemodialysis produced rapid and significant normalization of these excitability parameters (Figs 3 and 4). Compared with nerves depolarized by applied currents or by ischaemia, the excitability changes in CRF were more like those in ischaemia, but differences in subexcitability suggested that the increase in endoneurial potassium was relatively more important in CRF than in ischaemia (Fig. 5). Direct comparison between serum levels of potassium and other electrolytes and markers of renal function showed that differences in membrane potential between the nine pre-dialysis recordings were strongly correlated with serum potassium, but not with other serum constituents (Fig. 5 and Table 1). Moreover, CRF patients with highly abnormal serum levels of urea and creatinine, but

Table 1 Correlations between selected plasma constituents and excitability parameters recorded in nine CRF patients before dialysis

<table>
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<tr>
<th></th>
<th>Potassium</th>
<th>Urea</th>
<th>Creatinine</th>
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<tbody>
<tr>
<td></td>
<td>Range: 4.3–6.1 mM</td>
<td>Range: 15.4–32.9 mM</td>
<td>Range: 510–1109 μM</td>
</tr>
<tr>
<td></td>
<td>Normal: 3.5–5.0 mM</td>
<td>Normal: 2.9–8.9 mM</td>
<td>Normal: 53–133 μM</td>
</tr>
<tr>
<td>Resting I/V slope</td>
<td>0.873</td>
<td>0.005**</td>
<td>0.556</td>
</tr>
<tr>
<td>RRP (ms)</td>
<td>0.736</td>
<td>0.036*</td>
<td>0.620</td>
</tr>
<tr>
<td>Superexcitability (%)</td>
<td>0.769</td>
<td>0.025*</td>
<td>0.292</td>
</tr>
<tr>
<td>TEd (10–20 ms) (%)</td>
<td>−0.866</td>
<td>0.003**</td>
<td>−0.417</td>
</tr>
<tr>
<td>TEd (90–100 ms) (%)</td>
<td>−0.837</td>
<td>0.005**</td>
<td>−0.306</td>
</tr>
<tr>
<td>TEd (90–100 ms) (%)</td>
<td>0.771</td>
<td>0.015*</td>
<td>0.293</td>
</tr>
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The six excitability parameters were those that correlated best with current-induced changes in membrane potential in normal subjects (Kiernan and Bostock, 2000). These indices of membrane depolarization correlated well with potassium levels (see also Fig. 6), but not with the other indices of renal dysfunction. R = correlation coefficient between excitability parameter and logarithm of plasma concentration; P = probability of obtaining such correlation by chance; *P < 0.05; **P < 0.01; ns = not significant.

Axonal depolarization in CRF 1375
with normal potassium, had normal nerve excitability properties (Fig. 5). These data all point to hyperkalaemia as the primary cause of membrane depolarization in these subjects.

If the changes in resting potential \((E_r)\) were due to hyperkalaemia only, they should correspond in amplitude with the predictions of ionic theory (see Methods). Assuming normal potassium concentrations of 4.5 mM (external) and 155 mM (internal), a temperature of 32°C, and a normal \(E_r\) at human nodes of −84 mV (Schwarz et al., 1995), equation 2 predicts that an increase in external potassium to 6 mM would cause a depolarization of 5.6 mV. For comparison, the effects of hyperkalaemia on \(E_r\) can be estimated from the dependence

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**Fig. 6** Relationships between axonal excitability properties and serum potassium concentration in nine CRF patients prior to dialysis. The six excitability parameters are the same indices of membrane potential as in Figs 3 and 4. Dashed lines are mean values of excitability parameters in normal controls, as in Fig. 4. Solid lines are regression lines of potassium on the excitability parameter, so that intercept with dashed line provides estimate of serum potassium in CRF patient with normal axonal excitability. Correlation coefficients and \(P\) values are given in Table 1. (A) Resting I/V slope. (B) Depolarizing threshold electrotonus from 10–20 ms after current onset. (C) Relative refractory period. (D) Depolarizing threshold electrotonus from 90–100 ms. (E) Superexcitability. (F) Hyperpolarizing electrotonus from 90–100 ms.
of the six potential-dependent excitability parameters on serum potassium illustrated in Fig. 6, and the previous study of the effects of polarizing currents on these parameters (see table 1 in Kiernan and Bostock, 2000). For example, the resting I/V slope was found in that study to increase by 0.43 per mA depolarization, and it was suggested that 1 mA was roughly equivalent to 4 mV. In this study, regression of resting I/V slope on log(potassium) indicates that a change in potassium from 4.5 to 6 mM corresponds to a change in slope from 0.64 to 1.19, an increase of 0.55. The membrane depolarization estimated from the change in resting I/V slope is therefore \((4 \times 0.55)/0.43\), or 5.1 mV. Corresponding estimates from the other five parameters were: 10.1 mV from RRP, 2.3 mV from TEh (90–100 ms), 7.4 mV from TEd (10–20 ms), 3.7 mV from superexcitability and 3.4 mV from TEd (90–100 ms). These values give a mean estimated depolarization at 6 mM serum potassium of 5.3 mV. This crude estimate is consistent with the depolarization predicted by ionic theory and supports the idea that hyperkalaemia alone causes the abnormal nerve excitability properties in CRF patients.

In a previous study of sensory nerves in patients undergoing dialysis, Lowitzsch et al. (1981) also found increased RRRs, which they attributed to membrane depolarization, and that these correlated significantly with serum potassium levels. They related this abnormality to their earlier finding of shortened refractory periods (attributed to membrane hyperpolarization) in patients with hypokalaemia (Maurer et al., 1977). However, since they could not show that the changes in refractory period produced by dialysis correlated with the changes in serum potassium, they doubted a causal relationship. We also found that correlation between the changes in potassium during dialysis and the changes in nerve excitability parameters were not as significant as the pre-dialysis correlations in Table 1. Our interpretation is that it is the potassium concentration of the endoneurial fluid bathing the axons that directly affects membrane potential, and that this is closely related to serum potassium only in the relatively stable state prior to dialysis. During dialysis, serum potassium was changing, and no longer in equilibrium with the endoneurial potassium or with the large pool of intracellular potassium. For this reason, we used pre-dialysis serum concentrations for correlation only with the excitability data.

Little information is available about the endoneurial potassium concentration in humans or its relationship to serum potassium. Animal studies have shown that there is a diffusion barrier for sodium and potassium ions between blood and endoneurial fluid (i.e. the blood–nerve barrier), but that this is not appreciably selective between sodium and potassium ions (Manery and Bale, 1941; Krnjevic, 1954; Weerasuriya, 1987). There is therefore no mechanism for stabilizing the potassium concentration in the peripheral nerve microenvironment within a narrow range, as there is for the cerebral microenvironment (Bradbury et al., 1963; Weerasuriya, 1987). This may be related to the lack of polarity of Na+/K+–ATPase activity in peripheral nerve endothelial cells and perineurium, which contrasts with a marked polarity at the blood–brain barrier (Allt and Lawrenson, 2000). Our data, and that of Lowitzsch and colleagues (Maurer et al., 1977; Lowitzsch et al., 1981), indicate that the blood–nerve barrier behaves similarly in human nerves: the peripheral axons appear to be exposed to the full variation in steady-state potassium levels measured in serum, albeit slowly. It follows that much of the previously documented inter-subject variability in nerve excitability properties (Kiernan et al., 2000, 2001a) may be due to the fact that serum potassium can vary between ~3.5 and 5.0 mM in normal subjects. If confirmed, this would demonstrate that nerve excitability measurements provide a sensitive new technique for estimating endoneurial potassium concentrations.

In conclusion, our data indicate not only that axons in CRF patients are often depolarized, but also that the depolarization is mainly caused by hyperkalaemia. Since axons require adequate membrane polarization to maintain normal biochemical homeostasis for survival (Stys et al., 1995), this chronic hyperkalaemic depolarization should be considered as a possible aetiological factor in uraemic neuropathy.

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