Altered nerve excitability properties in established diabetic neuropathy

Arun V. Krishnan and Matthew C. Kiernan

Institute of Neurological Sciences, Prince of Wales Hospital; Prince of Wales Medical Research Institute and Prince of Wales Clinical School, University of New South Wales, Randwick, Sydney, NSW, Australia

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

Summary
The underlying cause of diabetic neuropathy remains unclear, although pathological studies have suggested an ischaemic basis related to microangiopathy, possibly mediated through effects on the energy-dependent Na$^+$/K$^+$ pump. To investigate the pathophysiology of diabetic neuropathy, axonal excitability techniques were undertaken in 20 diabetic patients with neuropathy severity graded through a combination of quantitative sensory testing (QST) using a vibratory stimulus, assessment of symptom severity using the Total Neuropathy Symptom Score (T-NSS) and measurement of glycosylated haemoglobin as a marker of disease control. To assess axonal excitability, compound muscle action potentials were recorded at rest from abductor pollicis brevis following stimulation of the median nerve, and stimulus–response behaviour, threshold electrotonus, a current–threshold relationship and the recovery of excitability were recorded in each patient. All patients had established neuropathy, with abnormalities of T-NSS present in all patients and QST abnormalities present in 65%. Compared with controls, diabetic neuropathy patients had significant reduction in maximal CMAP amplitude ($P < 0.0005$), accompanied by a ‘fanning in’ of threshold electrotonus. In addition, the strength–duration time constant was decreased in diabetic neuropathy patients and recovery cycles were altered with reductions in refractoriness, the duration of the relative refractory period, superexcitability and subexcitability. It is proposed that while the changes in threshold electrotonus with supportive findings in the current–threshold relationship are consistent with axonal depolarization, possibly mediated by a decrease in Na$^+$/K$^+$ pump activity, the alterations in the recovery cycle of excitability could be explained on the basis of a smaller action potential, reflecting a limitation on the nodal driving current imposed by a reduction in Na$^+$ conductances.

Keywords: diabetic neuropathy; membrane potential; Na$^+$/K$^+$ pump; nerve excitability; threshold electrotonus

Abbreviations: CMAP = compound muscle action potential; HbA1C glycosylated haemoglobin; HCO$_3^-$ = bicarbonate ion; NSS = Neuropathy Symptom Score; OHA = oral hypoglycaemic agents; QST = quantitative sensory testing; TE = threshold electrotonus; TE$_{d}$ = depolarizing threshold electrotonus; TE$_{h}$ = hyperpolarizing threshold electrotonus; T-NSS = total neuropathy symptom score; VDT = vibration detection threshold


Introduction
Diabetes may be complicated by the development of a symmetrical length-dependent polyneuropathy presenting initially with sensory symptoms of paraesthesias and pain (Lozeron et al., 2002). The incidence of diabetic neuropathy increases with duration of disease (DCCT Research Group, 1988), affecting up to 50% of diabetic patients after 25 years of disease (Pirart, 1978) and leading to considerable morbidity (Gordois et al., 2003, 2004; Shearer et al., 2003).

The pathophysiology of diabetic neuropathy has not been established, although pathological studies have suggested an ischaemic basis, possibly related to microangiopathy (Sima, 1996). Nerve biopsies in diabetic neuropathy demonstrate multifocal fibre loss, most prominent distally and similar in nature to the alterations seen in animal models of experimental ischaemic neuropathy (Sugimura and Dyck, 1982; Dyck et al., 1986b; Johnson et al., 1986). Diabetic nerves have also been shown to exhibit increased pathological vulnerability to ischaemia (Nukada, 1986, 1992).

As part of an ischaemic hypothesis, considerable attention has been focused on the role of metabolic derangements in
diabetic neuropathy, mediated by decreased activity of the energy-dependent Na\(^+\)/K\(^+\) pump on the axonal membrane (Greene et al., 1987; Sima, 1996). Although ischaemia may lead to alterations in Na\(^+\)/K\(^+\) pump function, the abnormalities in Na\(^+\)/K\(^+\) pump function in diabetic neuropathy have also been linked to metabolic changes occurring as a result of hyperglycaemia (Greene, 1986a, 1988; Stevens et al., 1993) and C-peptide deficiency (Wahren et al., 2000; Sima et al., 2004). Regardless of the cause, impairments of Na\(^+\)/K\(^+\) pump function would be expected to produce an alteration in membrane potential, specifically membrane depolarization, due to retention of intra-axonal Na\(^+\) (Bostock et al., 1991, 1994; Grosskreutz et al., 1999; Kierman and Bostock, 2000; Lin et al., 2002b).

It is now possible to investigate changes in axonal membrane potential in vivo using novel nerve excitability techniques, which have recently provided information about disease pathophysiology in a number of neuropathic conditions (Kiernan et al., 2001a, 2002a, b; Isbister et al., 2002; Kanai et al., 2003). Initial excitability studies in patients with diabetic neuropathy, using a limited set of excitability parameters, demonstrated abnormalities including shortened refractory periods and alterations of inwardly rectifying currents (Horn et al., 1996; Mackel and Brink, 2003). To further investigate the pathophysiology of diabetic neuropathy, axonal excitability in diabetic patients was assessed in the present study using multiple excitability measures, and correlated with symptoms of diabetic neuropathy, abnormalities of quantitative sensory testing (QST) for a vibratory stimulus and glycosylated haemoglobin (HbA1C), as a marker of diabetic control.

**Methods**

Studies were undertaken in 20 diabetic patients (12 male, eight female, age range 38–67 years, average age 53.8 years) referred for neuropathy screening. All patients had established diabetes and gave informed consent to the procedures, which had been 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. Patients with concurrent neuropathy-related comorbidities were excluded from the study. All patients had non-insulin-dependent diabetes mellitus (NIDDM), 15 patients were controlled on oral hypoglycaemic agents (OHA), and five patients were receiving insulin therapy for secondary failure.

A neurological history and examination were initially undertaken and symptoms were graded using the Neuropathy Symptom Score (NSS) (Dyck et al., 1980, 1987, 1992). 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). The number of symptoms present in each subset was added to give a Total Neuropathy Symptom Score (T-NSS).

Patients underwent automated vibration detection threshold testing (VDT) using a CASE IV\textsuperscript{TM} quantitative sensory testing system (WR Electronics, Stillwater, USA), as a marker of severity for generalised neuropathy. For VDT testing, the vibration stimulus was placed over the left big toe in all patients and VDT was estimated using a 4–2–1 stepping algorithm (Dyck et al., 1993b). After an estimated VDT was established, the automated protocol was used to determine the just noticeable vibration threshold using the forced choice testing method (Dyck et al., 1993b). Serum electrolytes including bicarbonate levels (HCO\textsubscript{3}\textsuperscript{-}) were assayed due to the potential effect of acid–base abnormalities on excitability parameters, particularly the strength–duration time constant (Baker and Bostock, 1999), as was glycosylated haemoglobin (HbA1C), an indicator of diabetic control over the previous 3 months (Gabbay et al., 1977).

The severity of neuropathy in the present study was staged using a modified form of a previously devised system (Dyck, 1988). In the present study, severity was staged as follows: Stage 0, no neuropathy (T-NSS <2 with normal VDT); Stage 1, asymptomatic neuropathy (T-NSS = 0 with abnormal VDT); Stage 2, symptomatic neuropathy (T-NSS ≥2 with normal VDT or T-NSS ≥1 with abnormal VDT; neuropathic symptoms non-disabling); Stage 3, disabling neuropathy (T-NSS ≥2 with normal VDT or T-NSS ≥1 with abnormal VDT; neuropathic symptoms reported to be disabling).

Nerve excitability studies were performed using a recently described protocol designed to measure a number of different nerve excitability parameters (Kiernan et al., 2000). The median nerve was stimulated at the wrist with the reference electrode placed proximally over the forearm. Compound muscle action potentials (CMAPs) were recorded from abductor pollicis brevis (APB) using surface electrodes. The active recording electrode was placed over the motor point of APB and the reference was placed over the proximal phalanx. In all studies the amplitude of the CMAP was measured from baseline to the initial negative peak. Skin temperature was monitored close to the site of stimulation for the duration of each study.

The current required to produce the desired CMAP was determined using a computerized threshold-tracking program (QTRAC version 5.2a, Institute of Neurology, Queen Square, London, UK) that was run using a Pentium PC. Recordings were amplified 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 with a purpose-built isolated linear bipolar constant current stimulator.

Stimulus–response curves were generated using test current impulses of 0.2 ms and 1 ms. The peak 1 ms response was 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 (Bostock et al., 1998) and the strength–duration time constant using Weiss’s formula (Weiss, 1901).

The threshold changes that occur in response to subthreshold depolarizing and hyperpolarizing pulses, referred to as threshold electrotonous (TE), were measured by altering nerve excitability using prolonged 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 protocol, the recovery cycle of excitability was assessed by tracking the changes in threshold that occurred following a supramaximal conditioning stimulus of 1 ms duration.
In the case of excitability studies, individual measurements were corrected for age and temperature before statistical tests were applied, using relationships established by normative data (Kiernan et al., 2000, 2001b, c). Statistical significance was determined by $P < 0.05$ using Student’s unpaired $t$-test and correlations were analysed using Pearson’s correlation coefficient. An abnormal VDT was defined as a computed threshold to vibration $\geq 95$th percentile for age, sex and stimulus site (Dyck et al., 1993a). Data are presented as mean $\pm$ SEM.

**Results**

All 20 diabetic patients had neuropathic symptoms, when assessed using the T-NSS, with a mean of two symptoms per patient (mean T-NSS, $2.05 \pm 0.2$). On quantitative sensory testing, 65% of patients had an abnormal VDT, with a mean VDT of $21.7 \pm 0.7$ (Dyck et al., 1993a). Based on the neuropathy staging system (see Methods), all 20 patients had established neuropathy, 13 patients having stage 2 neuropathy and seven patients having stage 3 neuropathy (Table 1). HbA1C was elevated in the majority of subjects (mean HbA1C $7.7 \pm 0.4\%$; normal range $4.4–6.4\%$), indicating poor diabetic control underlying the development of neuropathy.

Excitability studies revealed significant differences between the diabetic patients and normal controls. The amplitude of the maximal CMAP was significantly lower in the diabetic patients (diabetic patients $4.7 \pm 0.6$ mV; normal controls $9.1 \pm 0.6$ mV; $P < 0.0005$). Motor latency, measured from onset to peak, was prolonged in the diabetic group (diabetic patients $7.5 \pm 0.4$ ms; normal controls $6.7 \pm 0.1$ ms; $P < 0.05$).

Axons in diabetic patients were of high threshold (Figs 1 and 2), with a significantly higher mean stimulus intensity for a 1 ms stimulus in the diabetic patients (diabetic patients $7.9 \pm 0.8$ mV; normal controls $4.6 \pm 0.2$ mV; $P < 0.00005$), a rightward shift in stimulus–response curves for stimuli of 0.2 and 1 ms durations (Figs 1A and 2A) and a significant increase in rheobase (diabetic patients $6.3 \pm 0.6$ mA; normal controls $3.2 \pm 0.2$ mA; $P < 0.0005$). These combined changes reflect a degree of axonal loss in the population of diabetic patients studied. Stimulus–response slope was greater in the diabetic patients compared with controls (diabetic patients $6.4 \pm 0.7$; normal controls $5.0 \pm 0.2$, $P < 0.05$).

With respect to the current–threshold relationship (Fig. 2C), the hyperpolarizing current–threshold slope was greater in diabetic patients (diabetic patients $0.41 \pm 0.03$; normal controls $0.38 \pm 0.01$), although the difference between the two groups was not significant. The strength–duration time constant, a voltage-dependent property of the nodal membrane that provides an indirect measure of a nodal persistent Na$^+$ conductance (Mogyoros et al., 1996; Bostock and Rothwell, 1997), was lower in the diabetic patients (diabetic patients $0.38 \pm 0.05$ ms; normal controls $0.43 \pm 0.02$ ms) (Fig. 2D). There was no significant metabolic or acid–base disturbance (mean serum HCO$_3$–, $25.0 \pm 0.6$ mmol/l; normal range $22–32$ mmol/l) that may have contributed to such a change (Baker and Bostock, 1999).

Differences were noted in parameters of TE, which tracked the changes in resting threshold in response to subthreshold

**Table 1** Demographic and clinical data of diabetic neuropathy patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Neuropathy stage</th>
<th>T-NSS</th>
<th>VDT</th>
<th>VDT Percentile</th>
<th>HbA1C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>2</td>
<td>2</td>
<td>20.0</td>
<td>89</td>
<td>7.6</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>2</td>
<td>2</td>
<td>25.0</td>
<td>98</td>
<td>6.6</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>2</td>
<td>2</td>
<td>17.4</td>
<td>55</td>
<td>7.5</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>2</td>
<td>2</td>
<td>19.4</td>
<td>73</td>
<td>6.4</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>3</td>
<td>4</td>
<td>25.0</td>
<td>98</td>
<td>8.1</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>3</td>
<td>2</td>
<td>25.0</td>
<td>99</td>
<td>11.6</td>
</tr>
<tr>
<td>7</td>
<td>55</td>
<td>2</td>
<td>2</td>
<td>19.8</td>
<td>94</td>
<td>6.6</td>
</tr>
<tr>
<td>8</td>
<td>58</td>
<td>2</td>
<td>1</td>
<td>19.0</td>
<td>96</td>
<td>9.9</td>
</tr>
<tr>
<td>9</td>
<td>46</td>
<td>2</td>
<td>2</td>
<td>25.0</td>
<td>98</td>
<td>9.9</td>
</tr>
<tr>
<td>10</td>
<td>56</td>
<td>3</td>
<td>2</td>
<td>24.3</td>
<td>98</td>
<td>9.2</td>
</tr>
<tr>
<td>11</td>
<td>54</td>
<td>2</td>
<td>2</td>
<td>17.9</td>
<td>88</td>
<td>6.1</td>
</tr>
<tr>
<td>12</td>
<td>46</td>
<td>3</td>
<td>3</td>
<td>25.0</td>
<td>99</td>
<td>8.0</td>
</tr>
<tr>
<td>13</td>
<td>67</td>
<td>2</td>
<td>2</td>
<td>21.3</td>
<td>84</td>
<td>5.9</td>
</tr>
<tr>
<td>14</td>
<td>63</td>
<td>2</td>
<td>2</td>
<td>21.6</td>
<td>88</td>
<td>7.7</td>
</tr>
<tr>
<td>15</td>
<td>51</td>
<td>3</td>
<td>3</td>
<td>22.1</td>
<td>99</td>
<td>10.3</td>
</tr>
<tr>
<td>16</td>
<td>65</td>
<td>3</td>
<td>2</td>
<td>25.0</td>
<td>98</td>
<td>7.0</td>
</tr>
<tr>
<td>17</td>
<td>38</td>
<td>2</td>
<td>1</td>
<td>14.4</td>
<td>95</td>
<td>7.3</td>
</tr>
<tr>
<td>18</td>
<td>57</td>
<td>3</td>
<td>2</td>
<td>24.6</td>
<td>99</td>
<td>7.0</td>
</tr>
<tr>
<td>19</td>
<td>50</td>
<td>2</td>
<td>1</td>
<td>19.3</td>
<td>96</td>
<td>7.9</td>
</tr>
<tr>
<td>20</td>
<td>59</td>
<td>2</td>
<td>2</td>
<td>23.6</td>
<td>96</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Neuropathy severity was staged using a previously established three-stage system (Dyck et al., 1987) according to total neuropathy symptom score (T-NSS) and vibration detection threshold (VDT) (see text for further details). VDT percentile refers to normative data for age, sex and stimulus site (Dyck et al., 1993a) and abnormality was defined as $\geq 95$th percentile. Normal range for glycosylated haemoglobin (HbA1C) is 4.4–6.4%.
polarizing currents, reflecting the activity of internodal conductances (Bostock and Baker, 1988). Threshold electrotonus waveforms from the patients showed a marked increase in accommodation relative to normal controls (Figs 1E and 2E); the 100 ms depolarizing and hyperpolarizing currents produced smaller changes in excitability, as occurs in depolarized axons (Bostock and Baker, 1988; Kiernan and Bostock, 2000). Depolarizing threshold electrotonus at the 90–100 ms interval (TEd 90–100 ms) was significantly lower (Fig. 3A) in the diabetic patients (TEd 90–100 ms, diabetic patients 38.7 ± 1.0%; normal controls 42.0 ± 0.8%; P < 0.05), as was the hyperpolarizing threshold electrotonus at the same time interval (TEh 90–100 ms, diabetic patients −79.8 ± 7.6%; normal controls −117.7 ± 2.9%; P < 0.0005) (Fig. 3B), leading to a ‘fanned in’ appearance of the threshold curve (Kiernan and Bostock, 2000; Kaji, 2003).

Refractoriness (Figs 1F and 2F), due to inactivation of nodal voltage-gated transient Na⁺ channels, was reduced in the diabetic patients (Fig. 3C) when corrected for temperature (Kiernan et al., 2001b) and expressed as a percentage of 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 the relative refractory period (RRP), superexcitability and late subexcitability.
also shorter in the diabetic patients (diabetic patients 3.4 ± 0.2 ms; normal controls 3.7 ± 0.1 ms). Mean superexcitability (Figs 1F and 2F), due to spontaneous discharging of electrical charge stored in the myelin sheath and dependent on paranodal fast K⁺ channels (Barrett and Barrett, 1982; David et al., 1995), was reduced in the diabetic group (diabetic patients −19.0 ± 1.7%; normal controls −25.3 ± 1.0%; P < 0.005) (Fig. 3D). Late subexcitability, measured as the maximum mean of three adjacent points at interstimulus intervals >15 ms and determined by nodal slow K⁺ channels, was also lower in the diabetic group (diabetic patients 11.8 ± 1.0%; normal controls 14.8 ± 0.7%; P < 0.02). There were no significant differences in parameters of threshold electrotonus or the recovery cycle between the patients receiving OHA and those who were treated with insulin.

Correlation between neuropathy severity scores and axonal excitability

In terms of assessing neuropathy severity, there was significant correlation between VDT and T-NSS (Fig. 4A), although T-NSS was abnormal in a greater proportion of diabetic neuropathy patients (100%) compared with VDT (65%), consistent with a previous study (Dyck et al., 1987). The present study would therefore support the view that both NSS and VDT are sensitive methods for neuropathy detection in diabetic patients (Dyck et al., 1991). Correlation was also noted between VDT and the degree of superexcitability (Fig. 4B), known to be a sensitive marker of membrane potential (Kiernan and Bostock, 2000), with reduction in the magnitude of superexcitability documented for an
Altered nerve excitability in established diabetic neuropathy

Increase in VDT and thereby a worsening in neuropathy severity. In turn, there was a significant correlation between superexcitability and depolarizing threshold electrotonus (TEd 90–100 ms), another sensitive marker of resting membrane potential (Fig. 4C). Of further interest, there was also a significantly greater reduction in TEd 90–100 ms in patients with Stage 3 neuropathy (Fig. 5A) compared with Stage 2 (TEd 90–100 ms, Stage 2 patients 40.2 ± 0.7; Stage 3 patients 36.4 ± 1.6; \( P < 0.05 \)) and normal controls (Fig. 5B). Measures of neuropathy severity were similar in the group receiving OHA therapy and the insulin group with regard to both VDT (OHA patients 21.9 ± 0.6; insulin patients 21.1 ± 0.6) and T-NSS (OHA patients 2.1 ± 0.1; insulin patients 2.0 ± 0.4).

Relationships between refractoriness, the duration of the relative refractory period, rheobase and \( r_{SD} \) could not be established with neuropathy severity based on NSS and VDT measures. Furthermore, somewhat unexpectedly, HbA1C correlated poorly with both VDT and NSS. The weak association between HbA1C and both VDT and NSS may reflect the short-term nature of HbA1C, given that this indicator of diabetic control is limited to assessment of the preceding 1–3 months (Gabbay et al., 1977). Although previous studies have demonstrated that elevated HbA1C is a risk factor for the development of peripheral neuropathy (Maser et al., 1989; Franklin et al., 1994; Partanen et al., 1995) and that nerve conduction parameters and quantitative sensory testing may improve with better glycaemic control (Graf et al., 1981; Holman et al., 1983; Kitano et al., 2004), these studies have been confined to patients with mild degrees of neuropathy (Parry, 1999). The short-term nature of HbA1C may therefore preclude any correlation with long-standing diabetic neuropathy (Cohen et al., 1998) and the results of these previous studies cannot be extrapolated to patients in the present study, all of whom had established diabetic neuropathy.

Discussion

The present study has documented nerve excitability changes in patients with diabetic neuropathy, established on the basis of abnormalities in clinical neurological examination, T-NSS and quantitative sensory testing. Patients with diabetic neuropathy (stages 2 and 3) had significantly lower CMAPs and prolonged motor latencies. Axons of diabetic patients were of higher threshold compared to normal controls with an increased rheobase. In addition there were significant reductions in TE both in the depolarizing and hyperpolarizing directions, resulting in a ‘fanned-in’ appearance. These changes in threshold electrotonus, suggestive of membrane depolarization, were accompanied by appropriate changes in current–threshold relationships. In terms of other excitability parameters, there was reduction in the strength–duration time constant, refractoriness, the duration of the relative refractory period, superexcitability and late subexcitability in the diabetic patients. Reductions in each of these parameters is the converse of what would be expected with axonal depolarization, and this contrast in excitability measures will form the basis of discussion.
Axonal excitability and Na\(^+\)/K\(^+\) pump function in diabetic neuropathy

The present study addressed excitability changes in motor axons primarily because studies based on the CMAP were technically easier and more reproducible, given the difficulties with threshold tracking small sensory potentials in patients with severe diabetic neuropathy. Of the 20 patients studied, all had symptoms of neuropathy and 65% had abnormalities of VDT, confirming significant involvement of afferent conduction pathways. The significant reduction in CMAP in the present study confirms a similar involvement of motor axons, and although the length-dependent form of diabetic neuropathy is initially a sensory condition, motor nerve involvement is well documented (Younger et al., 1998; Andersen, 1999; Lozeron et al., 2002).

The severity of diabetic neuropathy, as graded using T-NSS and QST, was correlated with changes in a number of excitability parameters, particularly measures of TE. Reductions in TE were noted in both the depolarizing and hyperpolarizing directions (Fig. 2E), leading to a 'fanned-in' appearance (Kaji, 2003). In particular, the reduction in TEd at the 90–100 ms interval, previously established as a sensitive indicator of resting membrane potential (Kiernan and Bostock, 2000), provides supportive evidence for membrane depolarization. The alterations in TEd 90–100 ms were significantly greater in patients with Stage 3 neuropathy, consistent with a greater change in membrane potential in more severely affected patients (Fig. 5).

These findings differ from those of a previous study in patients with diabetic neuropathy, in which abnormalities of TE were only noted in the hyperpolarizing direction, possibly reflecting changes in inwardly rectifying currents rather than an alteration in membrane potential (Horn et al., 1996), although the severity of neuropathy was not quantitated in that patient population. In contrast, changes in TE in diabetic neuropathy patients from the present study, all of whom had established neuropathy, would support other studies that postulated decreased Na\(^+\)/K\(^+\) pump activity underlying the pathophysiology of diabetic neuropathy (Greene, 1986b; Santini et al., 1996; Sima, 1996, 2004; Quasthoff, 1998; Kitano et al., 2004). Specifically, the changes in TE in diabetic neuropathy patients in the present study are similar to those that occur with membrane depolarization and nerve ischaemia (Kiernan and Bostock, 2000). Nerve ischaemia is known to alter axonal membrane potential, producing membrane depolarization, through impairments of Na\(^+\)/K\(^+\) pump function, leading to an increase in intra-axonal Na\(^+\) concentration.

Abnormalities of Na\(^+\)/K\(^+\) pump function have been demonstrated in animal models of diabetic neuropathy (Greene and Lattimer, 1984; Greene, 1986b; Greene et al., 1987) and may therefore underlie the axonal depolarization demonstrated in the present study. In vitro studies have suggested that abnormalities of Na\(^+\)/K\(^+\) pump function occur due to C-peptide deficiency (Wahren et al., 2000) and the biochemical consequences of high glucose levels for metabolites of the polyol pathway, rather than as a consequence of nerve ischaemia per se (Greene et al., 1988; Stevens et al., 1995; Sima, 1996). Hyperglycaemia leads to increased levels of sorbitol with consequent myoinositol depletion (Greene et al., 1988; Stevens et al., 1993). A reduction in the concentration of myoinositol causes a reduction in protein kinase C activation (Zhu and Eichberg, 1990a, b), necessary for adequate functioning of the Na\(^+\)/K\(^+\) pump. Further support for decreased Na\(^+\)/K\(^+\) pump function induced by ischaemia comes from pathological studies of diabetic neuropathy which have shown multifocal loss of nerve fibres (Dyck et al., 1986a, b), a hallmark of nerve ischaemia, and have demonstrated that diabetic nerves are exquisitely sensitive to ischaemia when examined pathologically following an ischaemic insult (Nukada, 1986, 1992).
The recovery cycle and other measures of axonal excitability in diabetic neuropathy

In the present study, the 20 diabetic neuropathy patients demonstrated changes in the recovery cycle of excitability following a single impulse. Specifically, there was reduction in refractoriness, the duration of the relative refractory period, superexcitability and late subexcitability.

The reduction in refractoriness, due to the inactivation of nodal transient voltage-gated Na⁺ channels, although consistent with previous human studies (Mackel and Brink, 2003), was nevertheless unexpected, given the changes in threshold electrotonus which supported membrane depolarization. Refractoriness has been previously shown to increase with axonal depolarization, induced either through the application of ischaemia or direct depolarizing current (Grosskreutz et al., 1999; Kiernan and Bostock, 2000; Lin et al., 2001, 2002a), the converse of what occurred in diabetic neuropathy patients in the present study.

The differences in the recovery cycles from diabetic neuropathy patients, namely reduced refractoriness, superexcitability and late subexcitability, have a similar time course and pattern to recovery cycles of normal controls, suggesting that the cause does not lie with any simple parameter that determines the separate periods of the cycle. While axonal size has been reported to correlate with relative refractory period (Brink and Mackel, 1993) recovery cycles for axonal populations of different thresholds are identical (Kiernan et al., 1996), suggesting that differences in axonal diameter in the functional surviving fibres is unlikely to be the underlying cause. Rather, these variations in axonal excitability may represent a difference in the amplitude of the initial disturbance to the resting equilibrium state. It is possible that this difference could result from a reduced nodal driving current, with smaller action potentials in diabetic neuropathy axons, compared with controls (Vogel and Schwarz, 1995; Kiernan et al., 1996). In addition, there would be less charging of the internode, and consequently less superexcitability. A smaller action potential would also lead to less activation of slow K⁺ channels, thereby reducing the late subexcitability period.

How can we explain such a reduction in driving currents in diabetic neuropathy patients? One explanation would be that axons in diabetic neuropathy patients have an overall reduction in the concentration of nodal Na⁺ channels, a finding which has been highlighted in previous in vitro studies which have shown a reduction in Na⁺ current even in situations of membrane hyperpolarization, possibly due to a reduction in functional Na⁺ channels in diabetic neuropathy (Brismar and Sima, 1981; Brismar et al., 1987; Brismar, 1993). Such a reduction in Na⁺ conductances may also underlie the reduction in the strength–duration time constant in the present study. Furthermore, a recent study has shown that nodal and paranodal structural changes which may underlie the alterations in both refractoriness and strength–duration time constant are correctable with C-peptide replacement (Sima et al., 2004). Reductions in the strength–duration time constant and parameters of the recovery cycle, changes that would not be expected to develop with pure alteration in membrane potential, such as axonal depolarization or hyperpolarization (Kiernan and Bostock, 2000), have also recently been established to occur in patients exposed to tetrodotoxin, a potent inhibitor of Na⁺ channel function (Isbister et al., 2002). Such reductions in refractoriness, superexcitability and late subexcitability, as occurred in patients following ingestion of tetrodotoxin, were successfully reproduced using a mathematical model of the human axon in which Na⁺ conductances were reduced by 50% (Bostock et al., 2005; Kiernan et al., 2005). Further support for reduction in Na⁺ conductances underlying the changes in recovery cycles in diabetic neuropathy patients in the present study may be derived from the improvement in refractoriness, duration of the relative refractory period, and strength–duration time constant with intensive insulin treatment and thereby presumably better diabetic control (Kitano et al., 2004). Of further relevance, Na⁺/K⁺ pump dysfunction may also be reversed with insulin therapy (Zhang et al., 2001). It therefore seems likely that long-term excitability studies of diabetic neuropathy, incorporating assessment of Na⁺/K⁺ pump function (Vagg et al., 1998; Lin et al., 2002b), will provide further information about the biophysical abnormalities in human diabetic neuropathy and their potential for reversibility.

Acknowledgements

A.K. received grant support from the National Health and Medical Research Council of Australia and the Australian Association of Neurologists. Grant support from the Australian Brain Foundation and the Sylvia and Charles Viertel Charitable Foundation is also gratefully acknowledged.

References


