Nerve function and dysfunction in acute intermittent porphyria

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Acute intermittent porphyria (AIP) is a rare metabolic disorder characterized by mutations of the porphobilinogen deaminase gene. Clinical manifestations of AIP are caused by the neurotoxic effects of increased porphyrin precursors, although the underlying pathophysiology of porphyric neuropathy remains unclear. To further investigate the neurotoxic effect of porphyrins, excitability measurements (stimulus-response, threshold electrotonus, current–threshold relationship and recovery cycle) of peripheral motor axons were undertaken in 20 AIP subjects combined with the results of genetic screening, biochemical and conventional nerve conduction studies. Compared with controls, excitability measurements from five latent AIP patients were normal, while 13 patients who experienced acute porphyric episodes without clinical neuropathy (AIPWN) showed clear differences in their responses to hyperpolarizing currents (e.g. reduced hyperpolarizing I/V slope, \( P < 0.01 \)). Subsequent mathematical simulation using a model of human axons indicated that this change could be modelled by a reduction in the hyperpolarization-activated, cyclic nucleotide-dependent current (\( I_H \)). In contrast, in one patient tested during an acute neuropathic episode, axons of high threshold with reduced superexcitability, consistent with membrane depolarization and reminiscent of ischemic changes. It is proposed that porphyrin neurotoxicity causes a subclinical reduction in \( I_H \) in AIPWN axons, whereas porphyric neuropathy may develop when reduced activity of the Na\(^+\)/K\(^+\) pump results in membrane depolarization.

Keywords: porphyria; haem; nerve excitability; inward rectification (\( I_H \)); ischaemia

Abbreviations: AIPWN = acute intermittent porphyria without neuropathy; AIPN = acute intermittent prophyric neuropathy; ALA = \( \delta \)-aminolevulanic acid; ATP = adenosine triphosphate; APB = abductor pollicis brevis; CMAP = compound muscle action potential; Cr = creatinine; GBS = Guillain Barré syndrome; GH = inward rectifying conductance; HMBS = hydroxymethylbilane synthase gene; \( I_H \) = hyperpolarizing activated conductance/inward rectification; PBGD = porphobilinogen deaminase; PN = porphyric neuropathy; TA = tibialis anterior; TE = threshold electrotonus


Introduction

Porphyrins (derived from the ancient Greek word porphura, purple pigment) are the main precursors of heme, an essential constituent of haemoglobin, myoglobin, catalase, peroxidase, respiratory and P450 liver cytochromes. Human porphyrinas are inherited metabolic disorders resulting from deficiency of the enzymes involved in the biosynthesis of heme, the principal product of porphyrin metabolism. Of these, acute intermittent porphyria (AIP), an autosomal dominant inherited disorder, represents the most common form of the neuroporphyrinas.

AIP is characterized by deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the porphyrin–heme biosynthetic pathway, which occurs due to mutations in the hydroxymethylbilane synthase gene (HMBS). To date, over 300 different mutations within PBGD have been identified (Hrdinka et al., 2006). Acute episodes are biochemically characterized by overproduction and tissue...
accumulation of the porphyrin precursors, delta-aminolevulinic acid (ALA) and PBG.

Clinical expression of AIP is highly variable and up to 90% of heterozygotes may remain asymptomatic throughout life. In those clinically affected, acute episodes usually manifest after puberty, more commonly in females. AIP symptoms comprise a variety of neurological (severe pain, sensory loss, motor weakness, convulsions) and more general manifestations (fever, tachycardia, nausea, vomiting, constipation), as well as respiratory failure that can lead to coma and death (Kauppinen, 2005). Porphyria has also been linked to the madness that affected King George III of Great Britain (Cox et al., 2005) and the illness of Vincent van Gogh (Arnold, 2004). Symptoms of AIP do not generally develop unless a person with the enzymatic deficiency encounters a trigger such as hormonal substances, medications, sun exposure or dietary factors (Herrick and McColl, 2005).

Porphyric neuropathy (PN) typically presents as a motor neuropathy of the axonal type (Albers and Fink, 2004). The pathophysiology underlying axonal dysfunction in AIP remains unknown. Heme is an essential component of the mitochondrial electron transport chain and critical to aerobic metabolism and ATP production. Fast axonal transport is highly energy dependent, and diminished ATP availability would disrupt this process. The accumulation of neurotoxic heme precursors may lead to impaired function of the axonal Na⁺/K⁺ pump with consequent alteration in membrane potential to cause neuronal cell death and axonal degeneration (Albers and Fink, 2004). Changes in axonal ion channel function and membrane potential may now be investigated in vivo using novel nerve excitability methods, recently adapted for clinical use (Kiernan et al., 2000, 2001b, 2004). In the present study, multiple excitability parameters were recorded to investigate axonal properties in patients with AIP, and also in asymptomatic carriers of genetic mutations linked to AIP. The aim was to test the hypothesis that the development of porphyric neuropathy may be linked to axonal membrane depolarization.

Methods
Clinical assessment and neurophysiological investigations including nerve conduction studies (NCS) and nerve excitability studies were undertaken in 15 AIP patients (10 with previous clinical episodes and confirmed genetic mutations; 5 with previously confirmed clinical episodes without detected mutation) and 5 asymptomatic latent gene carriers. The clinical and biochemical characteristics of this cohort are summarized in Table 1. Clinical diagnosis was confirmed through assessment of serum biochemistry indices, enzyme activity and mutation screening of patients and their families. Patients were recruited from both the Prince of Wales Hospital and the National Taiwan University Hospital. All subjects gave informed consent to the procedures, which were approved by the ethics committee of National Taiwan University Hospital and Human Research Ethics Committees of the University of New South Wales and the South Eastern Sydney Area Health Service Human Research Ethics Committee. The studies were performed in accordance with the Declaration of Helsinki.

Routine nerve conduction studies used conventional techniques (Kimura, 1983). Excitability studies were undertaken on the median and peroneal nerves. Compound muscle action potentials (CMAPs) were recorded using surface electrodes from the abductor pollicis brevis (APB) and tibialis anterior (TA) with the active electrode at the motor point and the reference electrode 4 cm distal (Eduardo and Burke, 1988). The signal was amplified, filtered (3 Hz–3 kHz) and digitized by computer (PC) with an analog-to-digital board (National Instrument DAQCard for PCMCIA). Stimulus current was applied via non-polarizable electrodes with the cathode over the median nerve at the wrist and the anode 10 cm proximal over muscle. Stimulus waveforms were generated by the computer and converted to current by an isolated linear bipolar constant-current source (maximal output ±50 mA). Stimulation and recording were controlled by software (QTRAC version 8.2, Institute of Neurology, London). Motor axons were studied using the multiple excitability protocol, TRONDCM (Z’Graggen et al., 2006). Test current pulses of 1-ms duration were applied to produce a target potential that was on the fast-rising phase of the stimulus–response curve, ~40% of the maximal CMAP. These stimuli were combined with suprathreshold conditioning stimuli or subthreshold polarizing currents. The amplitude of the CMAP was measured from baseline to the negative peak. The automated excitability protocols incorporated the following measures:

(i) 'Stimulus-response curves' recorded the response as the stimulus was increased from zero to where it produced supramaximal potentials.

(ii) Threshold was then recorded using a 1-ms test stimuli applied 200 ms after the onset of a long-lasting subthreshold polarizing current, the strength of which was altered in 10% steps from +50% (depolarizing) to ~100% (hyperpolarizing) of the control threshold. This produced a 'current–threshold relationship' (I/T), analogous to the current–voltage relationship that depends on the rectifying properties of the nodal and internodal axolemma. The slope of this curve may be used to provide an estimate of the threshold analog of input conductance (Kiernan and Bostock, 2000). Values of the hyperpolarizing I/V slope were derived from the slope of the most hyperpolarized three points of the I/V curve (Kiernan et al., 2001a) as depicted in Fig. 1B.

(iii) Changes in threshold associated with electrotonic changes in membrane potential, termed 'threshold electrotonus' (TE), have a time course similar to the underlying changes in membrane potential (Bostock and Baker, 1988; Bostock et al., 1998; Kiernan et al., 2005). Prolonged subthreshold currents were used to alter the potential difference across the internodal axonal membrane. The subthreshold polarizing currents were of 100-ms duration and set to be +40% (depolarizing, TEp) and ~40% (hyperpolarizing, TEh) of the control threshold current (i.e. the current required to produce the unconditioned target CMAP, which was ~40% of maximum). Threshold was tested at different time points during and after the 100-ms polarizing currents.

(iv) The final part of the protocol measured the recovery of axonal excitability following delivery of a supramaximal
conditioning stimulus with conditioning-test-intervals from 2 to 200 ms. The ‘recovery cycle’ of excitability consisted of refractory period at short conditioning-test-intervals due to inactivation of transient Na⁺ channels with a resultant increase in threshold. This was followed by a period of superexcitability due to the depolarizing afterpotential (Barrett and Barrett, 1982; Blight, 1985; Bowe et al., 1987). The late-subexcitable period during which axonal excitability was reduced reflected the kinetics of voltage-dependent slow K⁺ channels activated by the conditioning stimulus (Baker et al., 1987; Stys and Waxman, 1994; Kiernan and Bostock, 2000; Schwarz et al., 2006).

Electrical model of nerve excitability

To model the excitability changes in human motor axons induced by porphyria, mathematical simulations were undertaken using a model of the human axon (Bostock et al., 1991, 1995; Kiernan et al., 2005b, c; Jankelowitz et al., 2007). Transient Na⁺ channels were modelled using the voltage clamp data (Schwarz et al., 1995), and persistent Na⁺ currents were added (Bostock and Rothwell, 1997). The equations for a single node and internode, representing a spatially uniform axon, were evaluated by integration over successive small time steps (Van Euler’s method, Press et al., 1992), using a maximal integration interval of 3 μs. At times corresponding to those in human nerve excitability recordings, the excitability of the model nerve was tested repeatedly to determine threshold with an accuracy of 0.5%. The discrepancy between the thresholds determined for the model and those determined from a sample of real nerves was scored as the weighted sum of the error terms: \[ (x_m-x_i)/s_i \], where \( x_m \) is the threshold of the model, \( x_i \) the mean and \( s_i \) the standard deviation of the thresholds for the real nerves. The weights were the same for all threshold measurements of the same type (e.g. recovery cycle), and chosen to give an equal total weight to the different types of threshold measurement: current/threshold relationship, threshold electrotonus and the recovery cycle. The standard model was obtained by minimizing the discrepancy between the model and the normal control data with an iterative least squares procedure, so that alternation of any of the above parameters would make the discrepancy worse.

Statistical analysis

Values obtained in this study were compared to established normative data for NCS for this unit (Burke et al., 1974) and elsewhere (Ma and Liveson, 1983). For the excitability studies, a separate group of normal Taiwanese controls (11 healthy females, 2 males) was recruited to match the gender ratio of the AIP patients (ages: AIPWN 19–54 years, mean 33.1 years; controls 19–58 years, mean 37.7 years). Recordings were compared using the Student’s t-test. All data are expressed as mean ± SE, and P-values < 0.05 were regarded as statistically significant.

Results

Demographic, biochemical and genetic data for the 20 subjects are detailed in Table 1. After clinical and excitability testing, one patient was found to be diabetic and his data were excluded from the analysis given that diabetes mellitus may affect axonal excitability (Kitano et al., 2004; Krishna and Kiernan, 2005). None of the other subjects had a history of other medical conditions known to affect nerve function other than AIP.

The results presented in this study were categorized according to three groups: (i) 13 patients with AIP without neuropathy (AIPWN); the diagnosis of porphyria was confirmed by elevated ALA levels, positive porphyrin urine tests and in 10 cases a confirmed PBGD mutation; however, each of these patients had normal NCS, further confirming that they did not have neuropathy; (ii) five asymptomatic latent AIP gene carriers; (iii) one patient with AIP who developed clinical neuropathy (AIPN) was studied during an acute neuropathic relapse.

Table 1: Summary of clinical and biochemical data from AIP patients (either clinically affected or latent gene carrier)

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Clinical episodes</th>
<th>PBGD Mutation identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>F</td>
<td>+</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>F</td>
<td>+</td>
<td>Y</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>F</td>
<td>+</td>
<td>Y</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>F</td>
<td>+</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>F</td>
<td>+</td>
<td>Y</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>F</td>
<td>+</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>54</td>
<td>F</td>
<td>+</td>
<td>Y</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>F</td>
<td>+</td>
<td>Y</td>
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<tr>
<td>9</td>
<td>19</td>
<td>F</td>
<td>+</td>
<td>Y</td>
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<tr>
<td>10</td>
<td>24</td>
<td>F</td>
<td>+</td>
<td>Y</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>M</td>
<td>+</td>
<td>N</td>
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<tr>
<td>12</td>
<td>22</td>
<td>M</td>
<td>+</td>
<td>N</td>
</tr>
<tr>
<td>13</td>
<td>27</td>
<td>F</td>
<td>+</td>
<td>N</td>
</tr>
<tr>
<td>14</td>
<td>69</td>
<td>M</td>
<td>+/DM</td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>9</td>
<td>F</td>
<td>-</td>
<td>Y</td>
</tr>
<tr>
<td>16</td>
<td>23</td>
<td>F</td>
<td>-</td>
<td>Y</td>
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<tr>
<td>17</td>
<td>18</td>
<td>M</td>
<td>-</td>
<td>Y</td>
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<tr>
<td>18</td>
<td>47</td>
<td>M</td>
<td>-</td>
<td>Y</td>
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<tr>
<td>19</td>
<td>54</td>
<td>F</td>
<td>-</td>
<td>Y</td>
</tr>
<tr>
<td>20</td>
<td>38</td>
<td>F</td>
<td>-</td>
<td>NT</td>
</tr>
</tbody>
</table>

PBGD mutation screening was undertaken in most patients, and the mean PBG level for 13 symptomatic patients was < 25 nm/h/mlRBC (normal > 30). Patient #14 was excluded from the subsequent data analysis due to presence of co-existent diabetes mellitus (DM). Patient #20 developed acute porphyric neuropathy.

Multiple excitability measurements in AIPWN

Comparison of mean data from AIPWN patients with control subjects established no significant difference in the stimulus–response curves (Fig. 1A). During threshold electrotonus (Fig. 1C), the threshold changes to the depolarizing conditioning currents were similar. However, AIPWN patients had greater threshold changes to hyperpolarizing conditioning currents. This became more apparent in the hyperpolarizing part of the current threshold (I/V) relationship (Fig. 1B), which showed that the threshold increase induced by strong hyperpolarizing currents of 200 ms duration in AIPWN was significantly greater than that in control subjects. The I/V relationship
reflects the rectifying properties of the axon, both nodal and internodal (Baker et al., 1987). The steeper the curve (i.e. the greater the current required to produce a change in threshold) the higher the input conductance. The steepening towards the top indicates outward rectification, an accommodative response to the depolarizing current associated with the activation of fast and slow K+ channels. In contrast, the steepening of the curve towards the bottom represents inward rectification, an accommodation to the hyperpolarizing current due to an increase in the hyperpolarization-activated conductance, \( I_H \) (Pape, 1996). These conductance properties are reflected by the slope of the I/V curves, shown in Fig. 2A: the minimum I/V slope was lower in AIPWN than in NC (AIPWN: 0.195 ± 0.004; NC: 0.232 ± 0.009; \( P < 0.005 \)), and the hyperpolarizing I/V slope, calculated from the most hyperpolarized three points of the I/V curve (Kiernan et al., 2001a), was significantly reduced (AIPWN: 0.276 ± 0.017, controls: 0.350 ± 0.016; Fig. 2B, \( P < 0.01 \)).

The findings for the recovery cycle in AIPWN are depicted in Fig. 1D. There were no significant differences between AIPWN and control subjects for the excitability parameters derived from their recovery cycles (refractoriness, AIPWN: 44.4 ± 10.3%, controls: 28.7 ± 5.8%; superexcitability, AIPWN: −24.1 ± 1.63%, controls: −25.7 ± 1.53%; subexcitability, AIPWN: 14 ± 1.59%, controls: 15.5 ± 1.44%).

**Fig. 1** Excitability findings from patients who had previously experienced porphyric attacks (n = 13) without evidence of neuropathy (AIPWN). Mean data (±SE) of excitability parameters recorded from AIPWN patients (filled circles) and normal controls (n = 13, open circles). (A) Stimulus-response curves; (B) Current–voltage (I/V) relationship demonstrating outward and inward rectification and the hyperpolarizing I/V slope was calculated with the three most hyperpolarizing points; (C) Threshold electrotonus; (D) Recovery cycle; no significant difference found in refractoriness, superexcitability and subexcitability.

**Fig. 2** Changes in the slope of the current–threshold (I/V) relationship: (A) The slopes of the I/V relationship (derived from Fig. 1B), mean data of AIPWN patients (filled circles) superimposed on normal controls (open circles). No difference was shown in the depolarizing direction, in contrast to the significant difference established with greater hyperpolarization. (B) Histograms of the hyperpolarizing I/V slope: the slope is significantly reduced \((P < 0.01)\) in AIPWN patients (±SE) (filled) in comparison to normal controls (open) indicating a reduction in the expression of inward-rectifying conductances (\( I_H \)).
Of the 13 clinically affected patients with AIPWN, 10 had confirmed PBGD gene mutations, and subgroup analyses of these 10 again confirmed significant differences in the minimum and hyperpolarizing I/V slopes ($P < 0.05$).

To investigate whether these changes in nerve excitability occurred independently of clinical episodes, excitability studies were also undertaken in a group of asymptomatic subjects who expressed PBGD genetic mutations linked to the development of porphyria. Findings in these asymptomatic carriers were almost identical to normal controls ($P > 0.05$ for all parameters).

**Reproducibility**

To investigate the reproducibility of these excitability changes, repeat testing was undertaken on patient #1 on seven different occasions. This series of studies confirmed that excitability parameters were highly repeatable, with small variability between multiple sessions of testing (Fig. 3). The stimulus–response curve of this patient was consistently normal (Fig. 3A), but the I/V relationship (Fig. 3B) showed a consistent abnormality, similar to that of the grouped patient data (Fig. 1B). Thus, although the mean minimum I/V slope (0.19) was within the range of normal control values (0.185–0.29), it was consistently below the normal mean of 0.23 ($P < 0.001$). The consistency of the repeated superexcitability measurements in patient #1 (Fig. 3D), which had a standard deviation of ±1.90%, is relevant to the interpretation of the observations on patient #20, described in the next section. Previous data on intra-individual variation in superexcitability in normal subjects (Kiernan et al., 2000) indicated a similar standard deviation of 1.97%.

**Acute porphyric neuropathy during a relapse**

Patient #20, a previously undiagnosed 38-year-old female, presented acutely with a 3-day history of generalized weakness, manifested by difficulty in walking up stairs and also in raising her arms. In addition, the patient complained of intermittent abdominal pain and change in urine colour (Fig. 4). Cranial nerve examination revealed a lower motor neuron pattern of facial weakness bilaterally. Limb power was initially graded at Medical Research Council grade 4/5 in most groups and progressed to grade 3/5 over 3 days. Deep tendon reflexes remained symmetrically normal and plantar responses were flexor. Sensory examination was normal.

In this acutely symptomatic patient, nerve conduction studies demonstrated reductions in compound motor action potential (CMAP) amplitudes, with relative preservation of conduction velocities, consistent with a motor neuropathy of the axonal type (Table 2). Sensory

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**Fig. 3** Reproducibility of excitability recordings in a single AIPWN patient (patient #1, Table I). This patient had several porphyric attacks and seven repeated excitability studies were undertaken on different days (mean ± SE; filled circle), superimposed with normal controls (mean ± SE; open circle).
values were within normal limits as were proximal H-reflex and F-wave parameters. Electromyography demonstrated widespread reduction in interference patterns in upper and lower limb muscles, with minor evidence of spontaneous activity, limited to the right deltoid.

Biochemical testing demonstrated an increase in plasma porphyrin levels and elevations in the ratios of urinary δ-aminolevulanic acid (ALA) and porphobilinogen (PBG) to creatinine ratio, consistent with AIP. Treatment with a high glucose diet (400 g daily) and infusions of heme arginate (150 mg daily) led to reduction in plasma porphyrin indices, associated with gradual clinical improvement.

In this symptomatic acute porphyric neuropathy patient, excitability studies established that axons were of high threshold in both upper (median nerve) and lower limb (peroneal nerve) recordings, with stimulus–response curves shifted to the right, and rheobase increased (median nerve 6.6 mA, normal 1.3–5.0 mA; peroneal nerve 8.8 mA, normal 1.5–6.0 mA).

Recordings on this patient were repeated 4 months after the initial presentation when the patient had clinically improved and demonstrated significant improvement in stimulus–response measures. Furthermore, superexcitability, a sensitive marker of membrane potential (Kiernan and Bostock, 2000), demonstrated marked improvement and had increased from 17.7% during the acute phase to 31.0% during the recovery phase (Fig. 5). Although the value of 17.7% is within the 95% confidence limits for superexcitability in the control group (14.7–36.7%), because of the normal intra-individual consistency of this measure, as indicated by the repeatability study on patient #1, the difference of 12.3% between the two recordings from patient #20 in Fig. 5 is statistically significant ($P \leq 0.0001$, estimated on the basis of the above SD of 1.9%). In light of the established reduction of superexcitability with membrane depolarization (e.g. Kiernan and Bostock, 2000), the results in Fig. 5 suggest that there is axonal depolarization during the acute porphyric relapse, with subsequent normalization of membrane potential during the period of clinical recovery. The improvement in clinical symptoms and nerve excitability findings was reflected in biochemical markers of disease activity, with a marked improvement in the urinary porphobilinogen/creatinine ratio (PBG/Cr).

**Fig. 4** Urine sample obtained from patient #20 during the porphyric attack, with dark port wine colour evident after the sample was left standing in sunlight, in comparison to a control sample.

**Table 2** Nerve conduction parameters from patient #20, who presented with acute neuropathy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Amplitude</th>
<th>Conduction velocity/distal motor latency (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R sural</td>
<td>21.5 μV</td>
<td>41</td>
</tr>
<tr>
<td>R radial</td>
<td>48 μV</td>
<td>53</td>
</tr>
<tr>
<td>R tibial (to AH): ankle</td>
<td>5.6 mV</td>
<td>5.1</td>
</tr>
<tr>
<td>R deep peroneal (to EDB): ankle</td>
<td>2.8 mV</td>
<td>4.2</td>
</tr>
<tr>
<td>fibular neck</td>
<td>2.3 mV</td>
<td>42</td>
</tr>
<tr>
<td>L deep peroneal (to EDB): ankle</td>
<td>0.9 mV</td>
<td>4.3</td>
</tr>
<tr>
<td>R median (to APB): wrist</td>
<td>3.8 mV</td>
<td>3.8</td>
</tr>
<tr>
<td>R ulnar (to ADM): wrist</td>
<td>4.3 mV</td>
<td>2.9</td>
</tr>
<tr>
<td>R soleus H reflex</td>
<td>31.7</td>
<td></td>
</tr>
<tr>
<td>F-waves R. AH</td>
<td>50.9–53.7</td>
<td>(100% persistence)</td>
</tr>
</tbody>
</table>

NCS established reduction in motor amplitudes, most prominent for left EDB, right EDB and right APB. Sensory amplitudes were within normal limits as were proximal H-reflex and F-wave parameters. EDB = extensor digitorum brevis; AH = abductor hallucis; APB = abductor pollicis brevis; ADM = abductor digiti minimi.

**Fig. 5** Recovery cycles of acute phase of the illness (open circle) and after glucose and heme treatment (filled circle) demonstrated improvement after treatment, particularly an increase in superexcitability (block arrow).
during the recovery period (acute phase, 80 mol/mmol: recovery phase, 39 mol/mmol).

Mathematical simulations of abnormal excitability properties in AIPWN

To clarify the changes in nerve excitability induced by porphyria, the mathematical model of a human motor axon was first adjusted to provide a close match to the recordings from the control group. The model was used to explore whether changes in any membrane parameter could reproduce the changes seen in the AIPWN patients. The best fit was obtained by reducing the inward rectifying conductances (GH) by 27%, which reduced the discrepancy by 61%. This fitting procedure, which takes into account the effects of conductances on threshold electrotonus, current threshold relationships and the recovery cycle, supported the interpretation that inward rectifying conductance ($I_{H}$) was reduced in AIPWN patients (Fig. 6).

Discussion

The present study has combined clinical, biochemical and genetic findings with assessment of conventional neurophysiological and nerve excitability parameters across a spectrum of AIP patients and asymptomatic gene mutation carriers. Nerve excitability properties were normal in asymptomatic AIP patients, whereas AIPWN patients who suffered porphyrin attacks demonstrated consistent differences in their responses to hyperpolarizing currents. These abnormalities in nerve excitability were significant and reproducible in a symptomatic patient who underwent repeat studies on seven different occasions.

In contrast, in a patient who presented with weakness and abdominal pain due to AIP complicated by neuropathy, peripheral axons in the upper and lower limbs were of high threshold, and nerve excitability findings were consistent with axonal membrane depolarization. Reduction in the levels of heme precursors induced by treatment with a high glucose diet and heme arginate led to significant clinical improvement and normalization of nerve excitability parameters. This combination of changes in nerve excitability was not predicted, but is reminiscent of apparently contradictory changes previously described in diabetic neuropathy. Thus, Horn et al. (1996), in an early clinical study of threshold electrotonus, found evidence for reduced inward rectification in motor and sensory axons in diabetic patients, which was related to age and neuropathy. More recently, Krishnan and Kiernan (2005) have found evidence of membrane depolarization in patients with established diabetic neuropathy. Mathematical modelling of the changes in axonal excitability has supported the interpretation that the changes are associated with a reduction in the hyperpolarization-activated current $I_{H}$.

Mechanisms of neurotoxicity in porphyria

Despite increased understanding of the molecular and cellular biology of AIP, the mechanisms of neurotoxicity remain unclear. Previous studies have suggested that up to 40% of patients with porphyria will develop clinical features of neuropathy (Albers and Fink, 2004). Patients typically present with predominantly motor involvement, characterized by weakness and areflexia, which may mimic Guillain Barre syndrome (GBS). Nerve conduction studies are of prime importance in differentiating these two conditions, as demonstrated by patient #20 from the present series with porphyrin neuropathy who manifested features of an axonal neuropathy but lacked the demyelinating features of GBS (Albers and Fink, 2004) and maintained preserved reflex function. Nerve excitability abnormalities in this acutely symptomatic neuropathy patient were consistent with axonal depolarization and resembled those in ischaemia (Kiernan and Bostock, 2000). In further comparison, studies undertaken on patients with axonal GBS have established that threshold electrotonus (TE) was normal in acute motor axonal neuropathy (AMAN) (Kuwabara et al., 2002). Nerve excitability studies may therefore provide the opportunity to examine neurotoxic mechanisms in greater detail.

For instance, changes in membrane potential may be induced by impairment of the axonal Na⁺/K⁺ pump (Kiernan and Bostock, 2000). This pump is electrogenic, with three Na⁺ ions being pumped out for every two K⁺ ions pumped into the axon, leading to a net deficit of positive charge on the inner aspect of the axonal membrane. Paralysis of the Na⁺/K⁺ pump results in the loss of its electrogenic contribution to the membrane potential and leads to further depolarization due to extracellular accumulation of K⁺ ions (Kaji and Sumner, 1989; Kaji, 2003). In vitro studies of porphyria provide a theoretical basis for impairment of Na⁺/K⁺ pump function in PN (Becker et al., 1971, 1975). These previous studies demonstrated membrane depolarization in muscle fibers.
(Becker et al., 1975) and impairment of Na⁺/K⁺ pump function in brain and red blood cells, induced by high levels of ALA (Becker et al., 1971). It appears that the blood–nerve barrier does not afford peripheral axons as good a protection from high levels of ALA in the blood as that provided to the central nervous system by the blood–brain barrier (Meyer et al., 1998). Other studies have suggested that ischaemia, which would also impair Na⁺/K⁺ pump function, may play a role in the development of other complications of porphyria, including abdominal pain and optic atrophy (DeFrancisco et al., 1979; Lithner, 2000).

**Interpretation of excitability changes in AIPWN patients**

The excitability changes in AIPWN patients imply a reduction in the inward rectifying conductance \( I_{H} \). The hyperpolarization-activated, cation non-selective (HCN) channels underlying \( I_{H} \) are regulated by neurotransmitters and metabolic stimuli, so that several mechanisms could contribute to the change in \( I_{H} \) in AIPWN patients. Changes in the intracellular metabolism acting through changes in the activity of adenylylate cyclase will alter \( I_{H} \) (McCormick and Pape, 1990; Tokimasa and Akasu, 1990). Intracellular acidosis also shifts the activation curves for \( I_{H} \) to the left (Munsch and Pape, 1999). As mentioned above, \( I_{H} \) appears to be decreased in diabetes mellitus (Horn et al., 1996) and this has also been found in animal models of diabetes (Yang et al., 2001), a change that reflects a parallel change in cAMP and responds to agents that boost intracellular cAMP (Quasthoff, 1998). More recently, nerve excitability studies supported by modelling have provided evidence of a selective reduction in \( I_{H} \), very similar to that in the AIPWN patients, on the affected side in motor axons of stroke patients (Jankelowitz et al., 2007). In that case, the change presumably results from a loss in descending input to the motoneurons, and was attributed to the consequent lack of impulse activity in the axons.

In the case of porphyria, as in diabetes, it seems that a likely common factor underlying both the reduction in \( I_{H} \) and membrane depolarization may be cellular hypoxia. In porphyria, this may result from impairment of energy-dependent cellular functions by porphyrin metabolites (Herrick et al., 1989; Peters and Deacon, 2003). Previous studies have suggested that alterations in \( I_{H} \) may occur as an early manifestation of hypoxia and impaired cellular metabolism (Duchen, 1990). The reductions in \( I_{H} \) noted in AIPWN patients in the current study, and in diabetics by Horn et al. (1996) may therefore represent a subclinical manifestation of metabolic deficiency.

The potential effects of more severe energy depletion on axonal Na⁺/K⁺ pump function appear self-evident, given that 50% of axonal energy requirement is consumed by the Na⁺/K⁺ pump. Such a reduction in energy delivery would be expected to cause axonal Na⁺/K⁺ pump dysfunction, with consequent membrane depolarization, as noted in the AIPN patient. Similarly, impaired Na⁺/K⁺-pump activity has been implicated by many studies in diabetic neuropathy (Krishnan and Kiernan, 2005; Krishnan et al., 2008).

In conclusion, the new evidence for membrane depolarization in an AIP patient with neuropathy supports previous suggestions that impaired Na⁺/K⁺ pump function is involved in the pathogenesis of the neuropathy. In contrast, AIPWN patients exhibit a specific deficit in \( I_{H} \), which may be a subclinical sign of impaired metabolism.

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**References**


Appendix

Glossary of specialist terms

Depolarization
When the membrane potential becomes less negative, as occurs during anoxia or ischaemia.

Electrotonus
Changes in membrane potential evoked by subthreshold depolarizing or hyperpolarizing current pulses.

Hyperpolarization
When the membrane potential becomes more negative.

Inward rectification
The passing of more current in the inward (as opposed to the outward direction).

Membrane potential
Voltage difference across the axonal membrane (inside–outside).
Refractoriness
The decrease in excitability during the relative refractory period after a nerve impulse. It may be expressed quantitatively as the percentage increase in threshold when a test stimulus is preceded by a conditioning stimulus at an interval within the relative refractory period (e.g. 2 ms).

Relative refractory period
The period between the end of the absolute refractory period and the start of the superexcitable period, when an axon may be excited but its threshold is increased.

Subexcitability
A decrease in excitability (increase in threshold) that occurs after the superexcitability phase.

Subthreshold
(A stimulus) too weak to trigger an action potential.

Superexcitability
An increase in excitability (or reduction in threshold current) commonly observed shortly after a nerve impulse. It may be expressed quantitatively as the maximum percentage reduction in threshold when a test stimulus is preceded by a conditioning stimulus at an interval within the superexcitable period.

TEd
Depolarizing threshold electrotonus.

TEh
Hyperpolarizing threshold electrotonus.

Threshold (current)
The stimulus current required to excite a single unit, or to evoke a compound potential that is a defined fraction of the maximum.

Threshold electrotonus
Threshold changes produced by prolonged subthreshold depolarizing or hyperpolarizing currents, normally corresponding to the induced change in membrane potential (i.e. electrotonus).