Excitability changes in human cutaneous afferents induced by prolonged repetitive axonal activity

Matthew C. Kiernan, Ilona Mogyoros, John P. Hales, Jean-Michel Gracies and David Burke

The Prince of Wales Medical Research Institute and the Department of Neurology, Prince of Wales Hospital, Randwick, Sydney, NSW 2031, Australia

1. The present study was undertaken to document the excitability changes produced by prolonged high-frequency trains of impulses in cutaneous afferents of six human subjects.

2. Trains of supramaximal stimuli at 200 Hz for 2 min or less produced a prolonged depression in excitability, consistent with activation of the electrogenic Na^+–K^+ pump. Trains of longer duration resulted in an initial period of hyperexcitability which, with 10 min trains, was associated with the sensation of paraesthesiae in all subjects. This transient hyperexcitability gradually gave way to a long-lasting period of hypoexcitability.

3. The excitability changes were reproducible, and were accompanied by corresponding changes in supernormality, refractoriness, strength–duration time constant and rheobase current, suggesting that the changes in axonal excitability reflected a change in membrane potential.

4. The transient increase in excitability that follows tetanic trains of 10 min had qualitatively similar effects on cutaneous axons as ischaemia or application of a depolarizing current. The post-tetanic changes in the supernormal period of sensory axons were those expected from the changes in excitability, without evidence of a gross distortion in its time course, as has been previously demonstrated in a hyperstimulated human motor axon.

5. It is concluded that the post-tetanic hyperexcitability of human sensory axons is probably driven by increased K^+ accumulation in the restricted diffusion space under the myelin sheath, much as in motor axons, the differences in behaviour of sensory and motor axons being explicable by greater inward rectification in sensory axons.

Conduction of prolonged trains of impulses in cutaneous afferent fibres results in long-lasting changes in excitability. The primary change is a profound depression in axonal excitability lasting more than 60 min, dependent on train length, due to activation of the electrogenic Na^+–K^+ pump (Ritchie & Straub, 1957; Bergmans, 1970, 1982; Schoepfle & Katholi, 1973; Raymond, 1979; Barrett & Barrett, 1982; Bostock & Grafe, 1985; Applegate & Burke, 1989; Gordon, Kocsis & Waxman, 1990; Morita, David, Barrett & Barrett, 1993; Bostock & Bergmans, 1994) and possibly to activation of Na^+-dependent K^+ channels (Koh, Jonas & Vogel, 1994). However these processes are not the only ones operating: for example, repetitive activity will result in increased extracellular K^+ concentration, and at the peak of each action potential there may be reversed action of the Na^+–Ca^{2+} exchanger (Tatsumi & Katayama, 1995). Both phenomena would have increasingly significant consequences the longer the impulse train. Indeed following prolonged impulse trains, cutaneous afferents can become spontaneously active (Ochoa & Torebjörk, 1980; Burke & Applegate, 1989) and subjects experience paraesthesiae (Bergmans, 1970; Ng, Burke & Al-Shehab, 1987; Applegate & Burke, 1989; Macefield & Burke, 1991*a*). Similarly, fasciculation can result from hyperstimulation of motor axons (Bergmans, 1970; Bostock & Bergmans, 1994).

Post-tetanic paraesthesiae are associated with a transient increase in axonal excitability that may last 10 min and is superimposed on the profound long-lasting, presumably pump-induced decrease in excitability (Ng *et al.* 1987; Applegate & Burke, 1989; Macefield & Burke, 1991*a*). Recently, a similar post-tetanic increase in excitability has been documented for a single motor axon (Bostock & Bergmans, 1994) and this was found to be associated with a markedly enhanced supernormal period lasting not the usual 10–20 ms but as long as 4 s, and attributed to an increase in extracellular $[K^+]$ in the restricted diffusion space under the myelin sheath (see David, Barrett & Barrett, 1993). Such an accumulation can be associated with ectopic impulse activity (David *et al.* 1993; Kapoor, Smith, Felts & Davies, 1993*a*, *b*).

The present study addresses the mechanisms responsible for the post-tetanic changes in excitability of human cutaneous afferents and, in particular, whether the mechanisms

invoked by Bostock & Bergmans (1994) for a single motor axon are equally applicable to human cutaneous afferents. Human sensory axons differ from motor axons in a number of properties: greater inward rectification (Bostock, Burke & Hales, 1994); longer strength–duration time constants (Panizza, Nilsson, Bradley, Rothwell & Hallett, 1994; Mogyoros, Kiernan & Burke, 1996); greater expression of a non-inactivating threshold Na^+ conductance (Bostock & Rothwell, 1997); and lesser supernormality and late subnormality (Kiernan, Mogyoros & Burke, 1996). Differences might reasonably be expected in their post-tetanic behaviour.

The present studies were designed to determine whether the post-tetanic changes in excitability were accompanied by parallel changes in refractoriness, supernormality and strength–duration time constant, as would be expected for a change in membrane potential. Given the disproportionate changes in supernormality reported for a tetanized single motor axon by Bostock & Bergmans (1994), the early post-tetanic changes were compared with those produced by membrane depolarization, and the time course of supernormality was determined. The results indicate that the post-tetanic changes in excitability of the largest cutaneous afferents in the peripheral nerve do reflect changes in membrane potential, and provide no support for the view that there are differential effects on the nodal and internodal membrane. While increased extracellular $[K^+]$ remains the favoured explanation for the transient phase of post-tetanic hyperexcitability, as in motor axons (Bostock & Bergmans, 1994), the behaviour of human cutaneous afferents differs from that of motor axons, probably because human cutaneous afferents have greater inward rectification than motor axons (Bostock *et al.* 1994).

METHODS

Five series of experiments were conducted on six healthy subjects without clinical or neurophysiological evidence of a peripheral nerve disorder (age 29–51 years). No subject had a history of other medical conditions known to affect nerve function. All subjects gave informed consent to the experimental procedures, which had the approval of the Committee on Experimental Procedures Involving Human Subjects of the University of New South Wales.

The digital nerves of the index finger (digit II) were stimulated using ring electrodes around the proximal phalanx, and the evoked compound sensory action potential (CSAP) was recorded using bipolar self-adhesive surface electrodes ('Red-Dot', 3M Canada Inc., London, Ontario, Canada), 4 cm apart overlying the median nerve at the wrist. Skin temperature was measured at the second metacarpophalangeal joint and the wrist, and was kept above 32 °C at both sites by radiant heat and wrapping the limb in a blanket.

In all studies CSAP amplitude was measured from the negative peak to the following positive peak (i.e. the falling phase of the potential). The latency of the CSAP produced by the test stimulus was measured to peak. In the figures, data have been smoothed by averaging ten consecutive points (i.e. resulting in 1 sample every 30 s).

Post-tetanic changes in axonal excitability were studied following conditioning trains of stimuli delivered at 200 Hz for 0·5–10 min. Each stimulus in the train was 0·1 ms in duration, supramaximal for the CSAP. The test stimulus consisted of a single square-wave pulse of current, 0·1 ms duration, delivered at 1 s^{-1} prior to the onset of the tetanic stimulus train and continuing for up to 60 min after its end. Changes in excitability were measured on-line, with the computer altering stimulus intensity to keep the amplitude of the CSAP constant at 30–40% of maximum ('threshold tracking'; Bostock & Baker, 1988). In the initial studies, excitability was also followed using changes in the amplitude of the CSAP produced by a fixed submaximal stimulus adjusted to produce a CSAP of 30–40% prior to tetanization (the technique used by Applegate & Burke, 1989). In these studies, a number of tetanic trains were delivered in the same experiment (as in Fig. 1). In subsequent experiments only one tetanic stimulus train was delivered in each experiment, the changes in axonal excitability were followed using the threshold tracking protocol, and other measures of axonal function were also measured.

Supernormality was assessed using a supramaximal conditioning impulse delivered 7 ms before the submaximal test stimulus, the interval of 7 ms chosen because supernormality in cutaneous afferents is normally maximal at this interval (Kiernan *et al.* 1996). The threshold for the conditioned potential was compared with that for an unconditioned test potential. Changes in refractoriness were followed using a conditioning–test interval of 2 ms. The maximal potential produced by the supramaximal conditioning stimulus contaminated the test potential during refractoriness, and to a lesser extent during supernormality. To overcome this, the test potential was measured after subtraction of the response to the conditioning stimulus during both refractoriness and supernormality, the subtraction being performed on-line by computer. Excitability changes were assessed using test stimuli of 0·1 and 1 ms duration, allowing estimation of the strength–duration time constant (Bostock 1983; Bostock & Bergmans, 1994; Mogyoros *et al.* 1996). The strength–duration time constant (τ_{SD}) was calculated using Weiss's equation (Weiss, 1901), relating stimulus charge to stimulus duration (Bostock, 1983) as follows:

$$\tau_{SD} = 0{\cdot}1(I_{0{\cdot}1} - I_1)/(I_1 - 0{\cdot}1 \times I_{0{\cdot}1}), \quad (1)$$

where $I_{0{\cdot}1}$ and I_1 are the threshold currents for 0·1 and 1 ms stimuli. With these stimulus durations, the rheobase current (I_{rh}) can be estimated using the formula:

$$I_{rh} = (I_1 - 0{\cdot}1 \times I_{0{\cdot}1})/0{\cdot}9. \quad (2)$$

The time course of the supernormal excitability following a supramaximal conditioning stimulus was followed before and after tetanization for 10 min, by measuring the threshold current required to produce a conditioned test potential following a single supramaximal conditioning stimulus, for conditioning–test intervals of 10 (or 7), 20, 40 and 100 ms. (In two of the six subjects, the 7 ms conditioning–test interval was used instead of 10 ms because there was little or no supernormality at the 10 ms interval.)

The increase in excitability that follows prolonged (10 min) tetanization was compared with the excitability changes seen during ischaemia, and with membrane depolarization in six subjects. During ischaemia changes in a submaximal potential were recorded from the index finger using ring electrodes set 3 cm apart around the proximal phalanx, with stimulation of the median nerve at the wrist. Ischaemia was produced at wrist level (the site of stimulation) by inflation of a sphygmomanometer cuff 5 cm in width, to 200 mmHg for 10 min. For polarization, graded depolarizing currents of duration 15 ms were applied, beginning 10 ms before the test stimulus, in an attempt to generate a similar magnitude of threshold change to that seen with tetanization. The

strength of the depolarizing current was set at approximately 20% of the threshold for a CSAP of 30–40% of maximum in response to a 1 ms stimulus. During both ischaemia and polarization, changes in refractoriness and supernormality were assessed using conditioning–test intervals of 2 and 7 ms respectively, as described above.

Unless otherwise stated data are given as means ± S.E.M.

RESULTS

Prolonged trains of impulses cause activity-dependent changes in axonal excitability. In initial experiments these excitability changes were measured using two methods simultaneously: tracking the changes in amplitude of the CSAP produced by a constant submaximal stimulus (the method used by Applegate & Burke, 1989), and tracking the changes in stimulus intensity required to produce a CSAP of constant amplitude ('threshold tracking', see Weigl, Bostock, Franz, Martius, Müller & Grafe, 1989). Tetanic trains at 200 Hz for 30 s resulted in an increase in the current required to generate a CSAP of fixed amplitude (Fig. 1*A*), and a reduction in the amplitude of the CSAP produced by a fixed stimulus (Fig. 1*B*). Similar trains of 1 min duration resulted in a greater magnitude of change in threshold and amplitude, with a longer time course for recovery. This trend for greater change in threshold and amplitude did not continue with further increases in the duration of tetanic stimulation. Trains of 2, 5 and 10 min had little further effect on the magnitude of the induced hypoexcitability, but the time taken to recover continued to grow with train length. In addition, an inflection appeared on the recovery curves for both threshold and amplitude with trains of 2 min duration. With longer trains of 5 min, this inflection became more prominent, and with trains of 10 min duration the inflection produced overt hyperexcitability. During this period subjects experienced paraesthesiae (Ng *et al.* 1987; Applegate & Burke, 1989; Macefield & Burke, 1991*a*; see also Bostock & Bergmans, 1994).

Excitability changes

Conduction of a tetanic train at 200 Hz for 2 min resulted in an activity-dependent decrease in excitability of digital afferents in all subjects (Fig. 2*A*). The hypoexcitability was

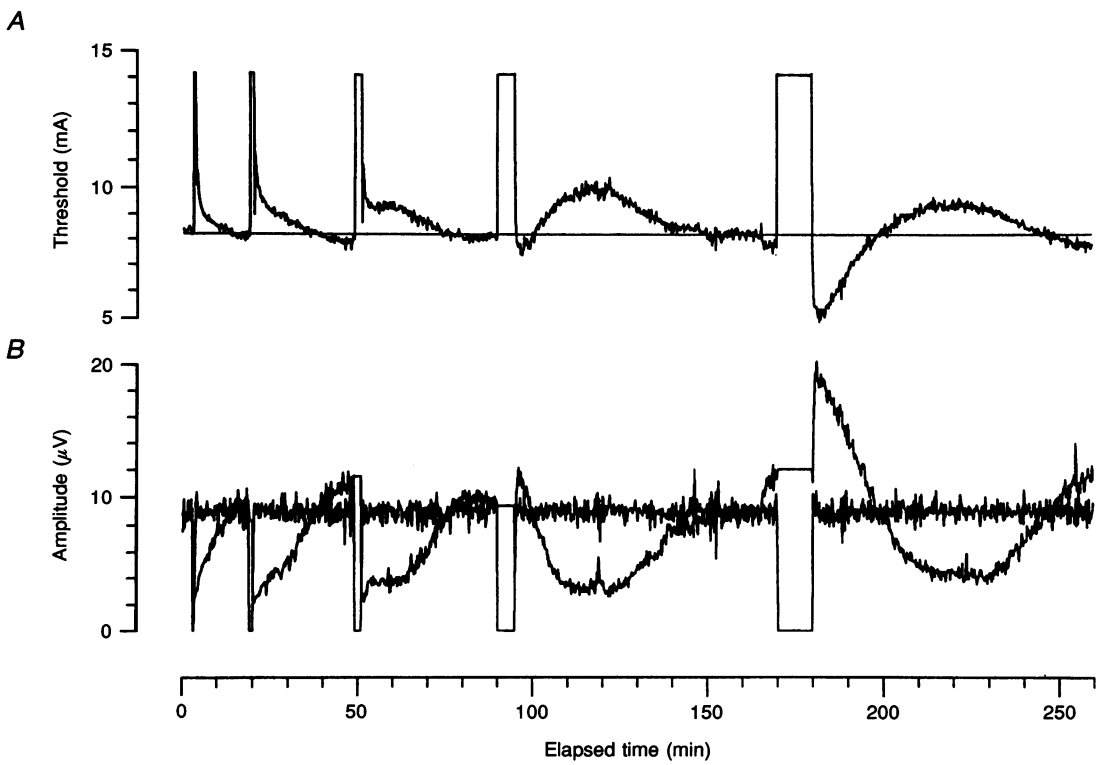

Figure 1. The excitability changes following prolonged tetanization

The changes in excitability of human cutaneous afferents following tetanization at 200 Hz for 30 s, 1 min, 2 min, 5 min and 10 min. Excitability changes were measured using a submaximal test pulse, of 0·1 ms duration, alternating between two different protocols, adjusting stimulus intensity to produce a CSAP of 40% maximum ('threshold tracking', *A*) and tracking the changes in amplitude of the CSAP produced by a constant stimulus intensity ('amplitude tracking', *B*). With a constant stimulus intensity (horizontal line, *A*), the activity-dependent excitability changes were manifest as a change in amplitude of the test potential, as shown in *B* (amplitude tracking). These excitability changes were the reciprocal of those obtained with threshold tracking (*A*), in which stimulus intensity was altered to maintain the test CSAP at a fixed amplitude (noisy horizontal trace, *B*). The efficacy of the computerized threshold tracking is reflected by the extent of the noise and the stability of the amplitude plot.

maximal on cessation of the conditioning train, with the current required to generate the target submaximal potential increasing to 128·5 ± 3·5% of the control level. (The threshold plots of Fig. 2*A* suggests that threshold increased over some seconds on cessation of tetanic stimulation, but this merely reflects the lag time of computerized tracking.) There then followed a gradual reduction in threshold current, with return to control levels after 30 min. These changes in threshold were accompanied by a small increase in latency of 0·1 ± 0·03 ms, with a similar time course for recovery (Fig. 2*B*). This long-lasting increase in threshold was termed H_2 by Bergmans (1970) by analogy with the P_2 positive after-potential described by Gasser (1935), and is presumed to be due to activation of the electrogenic sodium pump.

Increasing the duration of the conditioning train to 10 min resulted in a radically different change in axonal excitability (Fig. 3*A*). Immediately following cessation of the train, a phase of increased excitability developed, with a reduction in the current required to generate the target potential to 76·3 ± 7·2% of the control level. This transient phase of hyperexcitability lasted for 18 min, and was associated with reports of paraesthesiae by all subjects, usually beginning 20–30 s after cessation of the tetanus. The paraesthesiae increased in intensity over the following 3–4 min, and then gradually subsided, disappearing by 15 min in five subjects, lasting for a maximum of 20 min in one subject. This time course of paraesthesiae corresponded closely to the period of lowered threshold. The transient hyperexcitability gradually gave way to a long-lasting period of hypoexcitability, which reached a maximum 35–40 min after cessation of the tetanus. Here, threshold current reached a peak level of 117 ± 9·1% of control, and the return to baseline levels occurred gradually over > 60 min, at which time recordings were terminated. The pattern and extent of these excitability changes were identical in four series of experiments performed over a 12 month period in the same subjects (Fig. 5*A*).

These excitability changes associated with supramaximal tetanization for 10 min were accompanied by changes in latency (Fig. 3*B*). Immediately following cessation of the tetanic train, when cutaneous axons were hyperexcitable, there was prolongation of latency. The latency prolongation of 0·25 ± 0·04 ms exceeded the maximal latency prolongation of 0·1 ± 0·03 ms seen with tetanization for 2 min. Latency remained prolonged throughout the long-lasting period of subexcitability, gradually returning to control levels over more than 60 min.

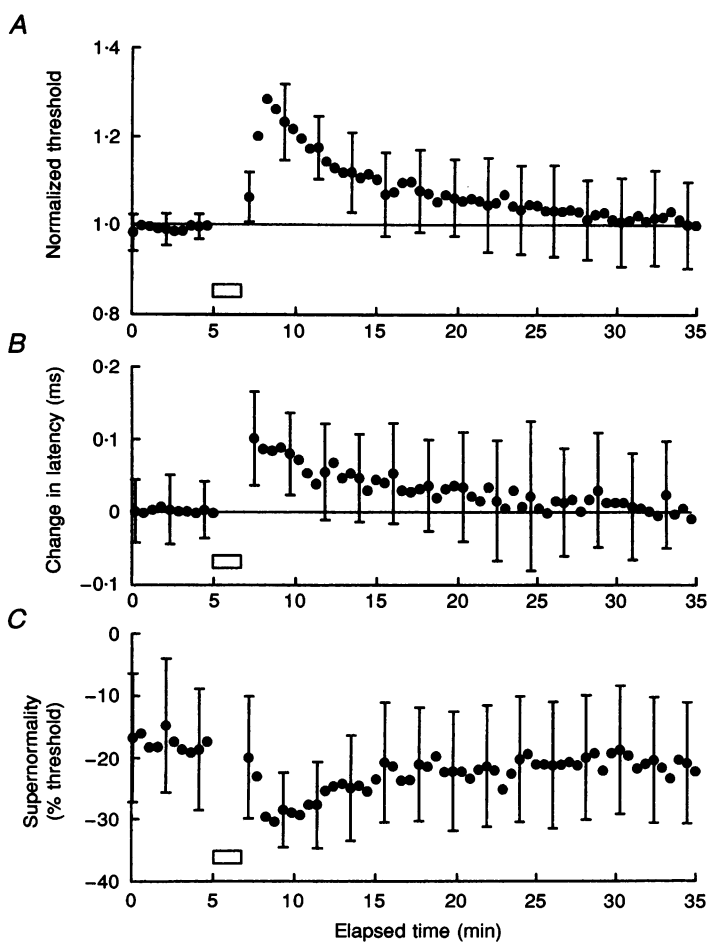

Figure 2. The excitability changes following tetanization for 2 min

Recovery of a submaximal test potential following tetanization of cutaneous afferents at 200 Hz for 2 min (indicated by the open boxes in this and other figures). The test stimulus was altered to keep the amplitude of the test CSAP constant (i.e. threshold tracking). Changes in normalized threshold current (*A*), latency (*B*) and supernormality (expressed as a percentage of threshold, *C*) are shown for 6 subjects. Results are expressed as means ± S.D.

Post-tetanic changes in supernormality and refractoriness

Following conduction of a single action potential, there is a stereotyped sequence of changes in excitability as axons are initially refractory, then superexcitable and finally subexcitable. The period of superexcitability normally lasts from 5 to 15 ms and is referred to as the supernormal period (see Kiernan *et al.* 1996). Barrett & Barrett (1982) showed that supernormality was due to passive discharge of current stored on internodal membrane, resulting in depolarization of nodal membrane, and measures of supernormality therefore provide a different measure of axonal excitability, sensitive to changes in membrane potential and internodal resistance. In the following studies changes in supernormality were followed at the conditioning–test interval of 7 ms, chosen to reflect the period of maximal supernormality (Kiernan *et al.* 1996).

Following tetanic trains of 2 min duration, supernormality increased from its resting level of 17·6 ± 3·7% to 30·4 ± 2·5% (Fig. 2*C*). The increase in supernormality corresponded to the development of greatest subexcitability (Fig. 2*A*). Supernormality then gradually returned to resting levels with a time course of recovery similar to that of the activity-dependent hypoexcitability. With longer tetanic trains of 10 min duration, there was again an inverse relationship between excitability and supernormality (Fig. 4*C*). That is, when excitability increased in the immediate post-tetanic period, the extent of supernormality as measured at the 7 ms conditioning–test interval decreased, and when excitability subsequently decreased, supernormality increased. (Close correlation of the plots in Fig. 4*A* and *C* reveal that, in general, the relationship was reciprocal but this is not so on a moment-to-moment basis, perhaps indicating that the change in excitability was determined by a number of factors.) The transient phase of hyperexcitability was associated with a reduction in supernormality from its resting level of 13·7 ± 2·7% to 9·6 ± 3·3%. The subsequent long-lasting phase of axonal hypoexcitability was accompanied by an increase in supernormality to 30·6 ± 3·8%. Accordingly, it is likely that the post-tetanic changes in excitability resulted from a change in membrane potential, i.e. axonal hyperpolarization with 2 min trains, and transient axonal depolarization superimposed on long-lasting hyperpolarization with the 10 min trains.

This view was supported by measuring the changes in refractoriness following tetanization for 10 min (Fig. 3*C*). Refractoriness was measured as the increased current required to produce the test potential when the conditioning–test interval was 2 ms, an interval that sampled the relatively refractory period. With the transient hyperexcitability immediately following cessation of the

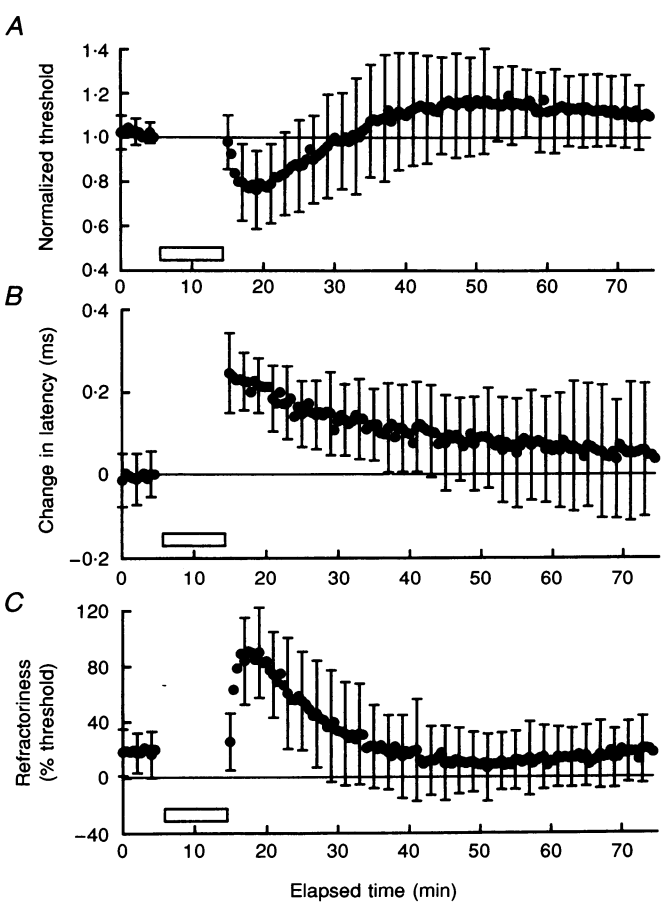

Figure 3. The excitability changes following tetanization for 10 min

Recovery of excitability after conditioning by a tetanic train of stimuli delivered at 200 Hz for 10 min. Changes in normalized threshold current (*A*), peak latency (*B*) and the refractory period (expressed as a percentage of threshold, *C*) are shown for 6 subjects. Results are expressed as means ± S.D.

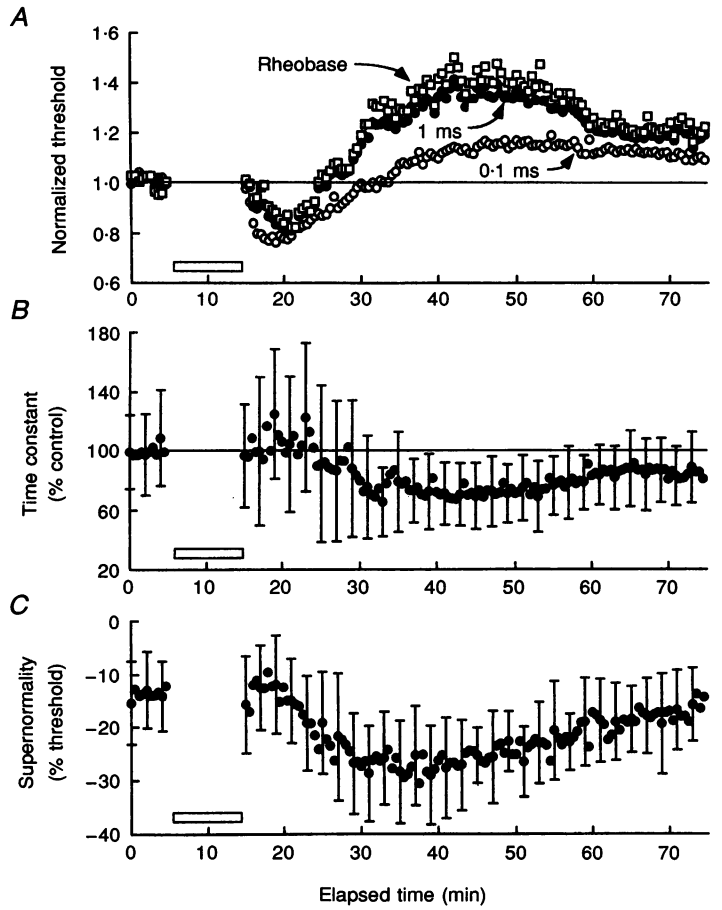

Figure 4. Changes in rheobase, strength–duration time constant and supernormality

Changes in excitability following conditioning by a tetanic train of supramaximal stimuli delivered at 200 Hz for 10 min. *A*, thresholds were measured with 0·1 ms (○) and 1 ms (●) test pulses and show greater changes with the 1 ms test pulse. The plot of changes in rheobase (□) was calculated from the two measures of threshold (see Methods). Corresponding changes in strength–duration time constant (*B*) and supernormality (*C*). Results are expressed as means ± S.D. for 6 subjects.

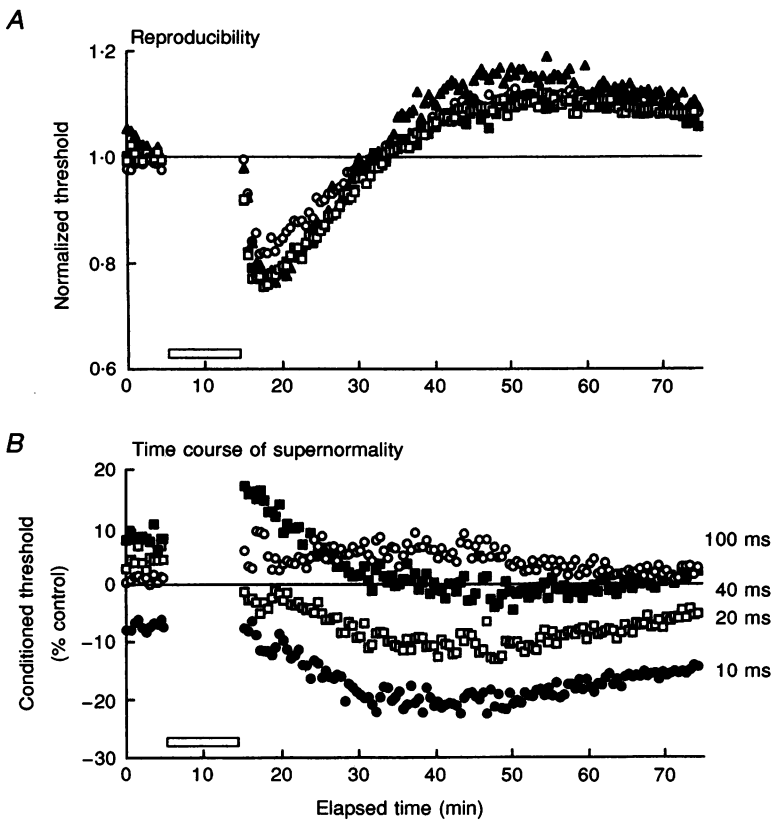

Figure 5. Excitability changes and the time course of supernormality

A, reproducibility of the changes in excitability of human cutaneous afferents following supramaximal tetanization at 200 Hz for 10 min. Mean data from 4 separate series of experiments on the same 6 subjects. *B*, the time course of supernormality in sensory fibres following tetanization for 10 min, expressed as mean data for 6 subjects. Changes in the threshold of a submaximal test potential conditioned by a single supramaximal stimulus were tracked, using conditioning–test intervals of 10 (●), 20 (□), 40 (■) and 100 ms (○).

tetanic train there was a large increase in refractoriness from its pre-tetanic level of 18·8 ± 6·9% to 90·7 ± 12·8%. The increase in refractoriness was probably due to inactivation of Na^+ channels (see later), and this was presumably responsible for the apparently paradoxical prolongation in latency of the test potential by 0·25 ms (Fig. 3*B*), occurring in the face of increased axonal excitability (Fig. 3*A*). The subsequent period of long-lasting hypoexcitability was associated with a reduction in refractoriness to a nadir of 8·8 ± 8·8%, followed by gradual return to baseline over more than 60 min. The changes in refractoriness presumably reflect changes in Na^+ channel inactivation at the node, the chief determinant of the refractory period, and are those expected for a sequence of axonal depolarization followed by hyperpolarization. That both refractoriness and supernormality underwent changes appropriate for the change in excitability suggests that nodal Na^+ channels and internodal (largely K^+) channels were seeing similar changes in membrane potential (cf. Bostock & Bergmans, 1994).

Changes in strength–duration time constant and rheobase current

During the period of long-lasting hypoexcitability that followed tetanization for 10 min, the changes in threshold current were, when normalized to pre-tetanic levels, greater using a test potential of 1 ms duration than 0·1 ms (Fig. 4*A*). However, this was not the case during the transient phase of hyperexcitability. These differences resulted in changes in the strength–duration time constant, calculated using eqn (1) (see Methods). During the post-tetanic increase in excitability, the time constant increased by 25·0 ± 17·9%, while during the subsequent decrease in excitability the time constant was reduced by 34·4 ± 9·5%. These variations in the strength–duration time constant support the view that the changes in axonal excitability reflect a change in membrane potential. In addition, measurement of the excitability changes with test potentials of short and longer duration allowed estimation of changes in rheobase (Bostock 1983; Bostock & Bergmans, 1994; see Methods, eqn (2)), which underwent similar but proportionally slightly greater changes (Fig. 4*A*).

Time course of the supernormal period

The supernormal period normally begins at 3–4 ms, peaks at 5–10 ms and subsides at 15–20 ms into a phase of late subnormality. Periaxonal accumulation of K^+ in the extracellular space below the myelin, as may occur with prolonged tetanization, can result in a prolonged negative potential (David *et al.* 1993), and this has been invoked to explain the gross prolongation of the supernormal period (to > 4 s) seen in a single human motor axon as a result of prolonged tetanic stimulation (Bostock & Bergmans, 1994).

To look for a distortion in the time course of supernormality in sensory fibres following tetanization for 10 min, changes in the threshold of a submaximal test potential conditioned by a single supramaximal stimulus were followed, using conditioning–test intervals of 10, 20, 40 and 100 ms. Prior to tetanization, supernormality was present at the 10 ms interval (●) but not at the other intervals (Fig. 5*B*). Subnormality was just present at 20 ms (□), was maximal at 40 ms (■) and had subsided at 100 ms (○). Following tetanization supernormality extended into the 20 and 40 ms intervals, reaching peak levels of 13·1 and 4·6%,

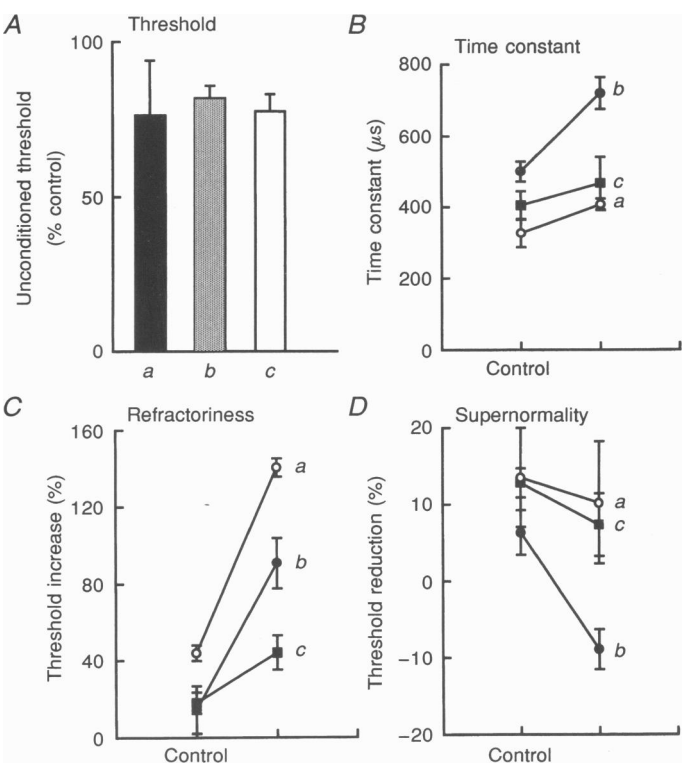

Figure 6. Comparison of the effects of tetanization, ischaemia and membrane polarization

A, the reduction in threshold of cutaneous afferents associated with three separate manoeuvres: supramaximal tetanization for 10 min (■), ischaemia for 10 min (▩) and the application of a depolarizing current (□). The corresponding changes in strength–duration time constant (*B*), refractoriness (*C*) and supernormality (*D*) are shown for tetanization (○), ischaemia (●) and for a depolarizing current (■). Results are expressed as means ± S.E.M. for 6 subjects, with control data (Control) on the left. The post-tetanic values of threshold, strength–duration time constant, refractoriness and supernormality were measured at the time of the maximal increase in excitability using conditioning–test intervals of 2 ms for *C* and 7 ms for *D*.

respectively, but this occurred when axons were least excitable (see Fig. 5*A*) and merely reflects the normal reciprocity between excitability and supernormality. In the 60 min following the end of the tetanic train, the supernormal period did not extend to the 100 ms interval in any subject.

In the data of Fig. 5*B* the shortest conditioning–test interval was 10 ms and sampled supernormality after its peak at 7 ms. The plot (●) therefore does not reveal the brief decrease in supernormality present in Fig. 4*C*. Nevertheless, the changes in supernormality and in its time course were largely those expected from the changes in excitability. There was no gross distortion of the time course of the supernormal period of sensory axons and specifically no evidence for this during the transient phase of increased excitability. This finding differs from that of Bostock & Bergmans (1994) who demonstrated prolonged supernormality lasting up to 4 s in a hyperstimulated single motor axon when that axon was hyperexcitable and ectopically active.

Comparison with ischaemia and polarization

To determine whether the transient post-tetanic increase in excitability had similar effects on cutaneous axons as ischaemia and a depolarizing current, the changes in strength–duration time constant, supernormality and refractoriness were compared for threshold changes of similar magnitudes (Fig. 6*A*). Tetanization for 10 min, ischaemia and the depolarizing current reduced threshold by 23·7 ± 7·2, 18·3 ± 4·1 and 22·5 ± 5·5%, respectively. There were qualitatively similar but quantitatively different changes in these measures of axonal excitability with each manoeuvre (Fig. 6*B*, *C* and *D*). For example, refractoriness increased by 96·5% following tetanization, compared with an increase of 71·9% during ischaemia and 26·2% with polarization (Fig. 6*C*). There was a corresponding reduction in the level of supernormality (Fig. 6*D*): from a resting level of 13·5 ± 6·6% to 10·2 ± 8·0% with tetanization, and from 12·8 ± 1·9% to 7·3 ± 4·1% with polarization. The magnitude of change was greater during ischaemia, with cutaneous afferents becoming refractory at the 7 ms interval.

DISCUSSION

The present study has documented the excitability changes in human cutaneous afferents following prolonged tetanization. These excitability changes were reproducible, and were accompanied by changes in other biophysical properties that are sensitive to membrane potential (refractoriness, supernormality and strength–duration time constant), suggesting that they resulted from changes in membrane potential. However, contrary to the findings of Bostock & Bergmans (1994) for a single human motor axon, there was no evidence that internodal membrane was behaving differently from nodal membrane. In addition, following tetanization sufficient to produce ectopic activity (and thereby paraesthesiae – see Burke & Applegate, 1989), there was no evidence for the grossly prolonged supernormal period documented by Bostock & Bergmans (1994). This finding suggests that the biophysical mechanisms underlying ectopic impulse activity are likely to be different in sensory and motor axons.

Time course of supernormality

Bostock & Bergmans (1994) showed that supernormality could last as long as 4 s in a single human motor axon following tetanization for more than 10 min, such that excitability would inevitably be enhanced when test stimuli were delivered at 1 s^{-1}. In contrast, the present study found no such prolongation of the supernormal period in human cutaneous afferents.

There are two possible reasons for the differences between the present findings and those of Bostock & Bergmans (1994). First, Bostock & Bergmans studied the behaviour of a single axon, under conditions designed to make it ectopically active. By contrast a large population of axons was studied here and, during the phase of transient hyperexcitability, only a small proportion of this population would have been ectopically active at any moment, from which only 30–40% were sampled to produce the test potential. Secondly, our techniques for studying supernormality were different. Bostock & Bergmans studied the changes in threshold for a test potential using a spontaneous discharge as the conditioning discharge. In the present study, data were sampled at regular intervals throughout recovery regardless of the ectopic activity associated with paraesthesiae (see below). However, using a similar method, Bostock & Bergmans found a prolongation in supernormality following tetanization (though to only 240 ms rather than 4 s).

Could the present studies have failed to detect a prolongation in the supernormal period that was responsible for the hyperexcitability and thereby the sensation of paraesthesiae experienced by subjects following tetanization for 10 min? This seems unlikely. While all subjects experienced paraesthesiae (and this implies that some axons were ectopically active, see Burke & Applegate, 1989), the paraesthesiae did not usually last the full extent of the period of hyperexcitability. In all but one subject paraesthesiae were first detected after the onset of the depolarizing notch and disappeared before it had subsided. There were no corresponding discontinuities in the pattern of supernormality. It is therefore likely that ectopic activity did not mask the true extent of the supernormality. More importantly, in the present studies, the changes in supernormality were intuitively appropriate, qualitatively (though perhaps not quantitatively) those expected for axonal depolarization.

Mechanisms of excitability changes

The primary change following prolonged tetanization of cutaneous afferents is the development of a long-lasting activity-dependent depression in axonal excitability. Presumably this results from hyperpolarization due to

activation of the electrogenic Na^+–K^+ pump (Ritchie & Straub, 1957; Bergmans, 1970, 1982; Schoepfle & Katholi, 1973; Raymond, 1979; Barrett & Barrett, 1982; Bostock & Grafe, 1985; Gordon *et al.* 1990; Morita *et al.* 1993; Bostock & Bergmans, 1994). In addition, activation of Na^+-dependent K^+ channels may have contributed to this hypoexcitability (Koh *et al.* 1994). The present study provides convincing evidence that the post-tetanic hypoexcitability of human cutaneous afferents is also due to axonal hyperpolarization.

Following tetanization for 10 min, this long-lasting hyperpolarization is preceded by a period of transient hyperexcitability, during which sensory axons may become ectopically active, and subjects may report paraesthesiae (Ng *et al.* 1987; Applegate & Burke, 1989; Burke & Applegate, 1989; Macefield & Burke, 1991*a*). The accompanying changes in refractoriness and supernormality suggest that the post-tetanic decrease in threshold results from axonal depolarization. What are the mechanisms responsible for this depolarization?

Reversed action of the Na^+–Ca^{2+} exchanger with each action potential in the tetanic train (Tatsumi & Katayama, 1995) could have dissipated surface charge by replacing surface-bound Ca^{2+} ions by unbound freely diffusible Na^+ ions. Neglecting the stoichiometry of the exchanger, the decrease in the concentration of membrane-bound Ca^{2+} would theoretically contribute to the post-tetanic hyperexcitability, but it is unlikely to be a major factor given that there is no significant change in refractoriness or supernormality during hyperventilation-induced hyperexcitability of cutaneous axons (Mogyoros, Kiernan, Burke & Bostock, 1997; see also Macefield & Burke, 1991*b*), a phenomenon that is usually attributed to reduction in surface-bound Ca^{2+} ions.

By analogy with the behaviour of post-ischaemic motor axons and computer simulations (Bostock, Baker, Grafe & Reid, 1991; Bostock, Baker & Reid, 1991), Bostock & Bergmans (1994) suggested that the ectopic activity of a hyperstimulated human motor axon resulted from high extracellular $[K^+]$ in the presence of profound pump-induced hyperpolarization. The high extracellular $[K^+]$ results from the diffusion barrier created by the myelin sheath, with K^+ accumulating outside the internodal membrane, gradually taken up into the myelin and slowly diffusing back into the submyelinic extra-axonal space. This creates conditions analogous to the effects of submyelinic injection of K^+ ions, as studied by David *et al.* (1993) and Kapoor *et al.* (1993*a*, *b*).

Presumably similar mechanisms operate in post-tetanic sensory axons. If so, the differences in behaviour of post-tetanic sensory and motor axons need to be explained: in the sensory studies, there was no evidence for a prolonged supernormal period when axons were hyperexcitable, as was the case in the motor studies. Human sensory axons differ from human motor axons in a number of respects: greater inward rectification (Bostock *et al.* 1994); greater expression of a non-inactivating 'threshold' Na^+ conductance in sensory axons (Bostock & Rothwell, 1997); longer strength–duration time constants (Panizza *et al.* 1994, Mogyoros *et al.* 1996); and lesser supernormality and late subnormality (Kiernan *et al.* 1996). Activation of inwardly rectifying channels would limit the post-tetanic hyperpolarization, dissipate some of the extracellular K^+ accumulation and decrease the resistance of the internodal membrane, factors which would allow the depolarizing effect of raised extracellular K^+ concentration to become apparent without distortion of the supernormal period. It is possible that the presence of threshold channels would bias sensory axons even further towards an increase in excitability. However, in as much as changes in strength–duration time constant result from changes in this conductance (Bostock & Rothwell, 1997; Mogyoros *et al.* 1997), threshold channels probably make a relatively minor contribution to the transient post-tetanic depolarization (see Fig. 4).

Applegate, C. & Burke, D. (1989). Changes in excitability of human cutaneous afferents following prolonged high-frequency stimulation. *Brain* **112**, 147–164.

Barrett, E. F. & Barrett, J. N. (1982). Intracellular recording from vertebrate myelinated axons: mechanism of the depolarizing afterpotential. *Journal of Physiology* **323**, 117–144.

Bergmans, J. (1970). *The Physiology of Single Human Nerve Fibres.* Vander, Louvain, Belgium.

Bergmans, J. (1982). Repetitive activity induced in single motor axons: a model for pathological repetitive activity. In *Abnormal Nerves and Muscles as Impulse Generators*, ed. Culp, W. J. & Ochoa, J., pp. 393–419. Oxford University Press, New York.

Bostock, H. (1983). The strength–duration relationship for excitation of myelinated nerve: computed dependence on membrane parameters. *Journal of Physiology* **341**, 59–74.

Bostock, H. & Baker, M. (1988). Evidence for two types of potassium channel in human motor nerve *in vivo*. *Brain Research* **462**, 354–358.

Bostock, H., Baker, M., Grafe, P. & Reid, G. (1991). Changes in excitability and accommodation of human motor axons following brief periods of ischaemia. *Journal of Physiology* **441**, 513–535.

Bostock, H., Baker, M. & Reid, G. (1991). Changes in excitability of human motor axons underlying post-ischaemic fasciculations: evidence for two stable states. *Journal of Physiology* **441**, 537–557.

Bostock, H. & Bergmans, J. (1994). Post-tetanic excitability changes and ectopic discharges in a human motor axon. *Brain* **117**, 913–928.

Bostock, H., Burke, D. & Hales, J. P. (1994). Differences in behaviour of sensory and motor axons following release of ischaemia. *Brain* **117**, 225–234.

Bostock, H. & Grafe, P. (1985). Activity-dependent excitability changes in normal and demyelinated rat spinal root axons. *Journal of Physiology* **365**, 239–257.

Bostock, H. & Rothwell, J. C. (1997). Latent addition in motor and sensory fibres of human peripheral nerve. *Journal of Physiology* **498**, 277–294.

Burke, D. & Applegate, C. (1989). Paraesthesiae and hypaesthesia following prolonged high-frequency stimulation of cutaneous afferents. *Brain* **112**, 913–929.

David, G., Barrett, J. N. & Barrett, E. F. (1993). Activation of internodal potassium conductance in rat myelinated axons. *Journal of Physiology* **472**, 177–202.

Gasser, H. S. (1935). Changes in nerve-potentials produced by rapidly repeated stimuli and their relation to the responsiveness of nerve to stimulation. *American Journal of Physiology* **111**, 35–50.

Gordon, T. R., Kocsis, J. D. & Waxman, S. G. (1990). Electrogenic pump activity in rat optic nerve. *Neuroscience* **37**, 829–837.

Kapoor, R., Smith, K. J., Felts, P. A. & Davies, M. (1993*a*). Potential oscillations recorded from rat central and peripheral axons under anaesthesia: a mechanism for ectopic impulse generation. *Journal of Physiology* **459**, 163*P*.

Kapoor, R., Smith, K. J., Felts, P. A. & Davies, M. (1993*b*). Internodal potassium currents can generate ectopic impulses in mammalian myelinated axons. *Brain Research* **611**, 165–169.

Kiernan, M. C., Mogyoros, I. & Burke, D. (1996). Differences in the recovery of excitability in sensory and motor axons of human median nerve. *Brain* **119**, 1099–1105.

Koh, D. S., Jonas, P. & Vogel, W. (1994). Na^+-activated K^+ channels localized in the nodal region of myelinated axons of *Xenopus*. *Journal of Physiology* **479**, 183–197.

Macefield, G. & Burke, D. (1991*a*). Long-lasting depression of central synaptic transmission following high-frequency stimulation of cutaneous afferents: a mechanism for post-vibratory hypaesthesia. *Electroencephalography and Clinical Neurophysiology* **78**, 150–158.

Macefield, G. & Burke, D. (1991*b*). Paraesthesiae and tetany induced by voluntary hyperventilation. *Brain* **114**, 527–540.

Mogyoros, I., Kiernan, M. C. & Burke, D. (1996). Strength-duration properties of human peripheral nerve. *Brain* **119**, 439–447.

Mogyoros, I., Kiernan, M. C., Burke, D. & Bostock, H. (1997). Excitability changes in human sensory and motor axons during hyperventilation and ischaemia. *Brain* (in the Press).

Morita, K., David, G., Barrett, J. N. & Barrett, E. F. (1993). Posttetanic hyperpolarisation produced by electrogenic Na^+-K^+ pump in lizard axons impaled near their motor terminals. *Journal of Neurophysiology* **70**, 1874–1884.

Ng, A., Burke, D. & Al-Shehab, A. (1987). Hyperexcitability of cutaneous afferents during the supernormal period: relevance to paraesthesiae. *Brain* **110**, 1015–1031.

Ochoa, J. L. & Torebjörk, H. E. (1980). Paraesthesiae from ectopic impulse generation in human sensory nerves. *Brain* **103**, 835–853.

Panizza, M., Nilsson, J., Bradley, J. R., Rothwell, J. & Hallett, M. (1994). The time constants of motor and sensory peripheral nerve fibres measured with the method of latent addition. *Electroencephalography and Clinical Neurophysiology* **93**, 147–154.

Raymond, S. A. (1979). Effects of nerve impulses on threshold of frog sciatic nerve fibres. *Journal of Physiology* **290**, 273–303.

Ritchie, J. M. & Straub, R. W. (1957). The hyperpolarisation which follows activity in mammalian non-medullated fibres. *Journal of Physiology* **136**, 80–97.

Schoepfle, G. M. & Katholi, C. R. (1973). Posttetanic changes in membrane potential of single medullated nerve fibres. *American Journal of Physiology* **225**, 1501–1507.

Tatsumi, H. & Katayama, Y. (1995). Na^+ dependent Ca^{2+} influx induced by depolarisation in neurons dissociated from rat nucleus basalis. *Neuroscience Letters* **196**, 9–12.

Weigl, P., Bostock, H., Franz, P., Martius, P., Müller, W. & Grafe, P. (1989). Threshold tracking provides a rapid indication of ischaemic resistance in motor axons of diabetic subjects. *Electroencephalography and Clinical Neurophysiology* **73**, 369–371.

Weiss, G. (1901). Sur la possibilité de rendre comparables entre eux les appareils servant l'excitation électrique. *Archives italiennes de Biologie* **35**, 413–446.

Acknowledgements

The work was supported by Glaxo-Wellcome Australia Ltd and the National Health and Medical Research Council of Australia. J.-M.G. was in receipt of a fellowship from the Fondation Simone et Cino del Duca, Paris.

Author's email address

M. C. Kiernan: Matthew.Kiernan@unsw.edu.au

Received 15 August 1996; accepted 19 December 1996.