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CHANGES IN EXCITABILITY AND ACCOMMODATION OF HUMAN MOTOR AXONS FOLLOWING BRIEF PERIODS OF ISCHAEMIA

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SUMMARY

1. The mechanism of post-ischaemic ectopic impulse generation in nerve is not known, and previous measurements of excitability changes in human motor axons have appeared to conflict. We have used automatic threshold tracking and different stimulus–response combinations to follow the effects on excitability of brief (5–10 min) periods of ischaemia, too short to induce motor fasciculations. Excitability changes have been compared at different sites in axons innervating hand, arm and foot muscles.

2. Threshold was determined as the percutaneous stimulus current required to excite a single motor unit, or to evoke a constant multiunit response, after rectifying and integrating the electromyogram (EMG). Three different waveforms of stimulus current were compared: short ($\leqslant$ 2 ms) pulses, long (100–200 ms) pulses to measure rheobase, and 100 ms current ramps. We also measured accommodation by recording the effects of subthreshold depolarizing currents on excitability.

3. Ischaemic and post-ischaemic excitability changes were greatest in the proximal parts of the longest motor axons, and greater if the sphygmomanometer cuff was inflated over, rather than proximal to, the stimulating site.

4. Using integrated EMG responses from abductor digiti minimi, the ulnar nerve stimulated above the elbow became rapidly much less excitable after ischaemia when tested with short pulses, but more excitable when tested with current ramps. The rheobase rose briefly, but then fell, often below resting level, always staying below the pulse and ramp thresholds.

5. The latency of the response to a rheobasic stimulus altered in parallel with the threshold to short current pulses, and increased dramatically after ischaemia. This latency increase was associated with a prolonged phase of 'negative accommodation', i.e. the continued increase in excitability to a maintained subthreshold depolarizing current.

6. Changes in excitability and accommodation similar to those occurring after ischaemia were recorded following high frequency trains of stimuli. They were attributed primarily to hyperpolarization by the electrogenic sodium pump, since comparable changes could be induced by passing a steady hyperpolarizing current through the stimulating electrode.

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7. Threshold and latency recordings from single motor units during and after ischaemia resembled in most respects the multiunit responses, but single unit rheobase did not show a post-ischaemic fall below the resting level. Repetitive firing contributed to the low multiunit thresholds recorded with long current pulses during the post-ischaemic period.

8. We conclude that human motor nerves become simultaneously both more and less excitable than normal after 10 min of ischaemia, depending on the choice of stimulus and response. The changes in accommodation and excitability recorded do not, however, provide an explanation for the spontaneous activity occurring after longer periods of ischaemia.

INTRODUCTION

The excitability changes occurring in human motor axons during and after a period of ischaemia were first documented by Kugelberg (1944, 1946*a*, *b*). He found that rheobase, determined as the smallest current required to elicit a visible motor response in 1st dorsal interosseus, fell transiently both during and after a pressure cuff was applied to the upper arm for 10 min (Kugelberg, 1946*a*). The periods of reduced rheobase corresponded to the times when spontaneous motor fasciculations could be most readily induced by hyperventilation, and Kugelberg considered that this hyperexcitability was closely related to the concurrent ischaemic and post-ischaemic paraesthesiae. The ischaemic and post-ischaemic periods of hyperexcitability were differentiated by the accompanying changes in accommodation. Nerves accommodated more quickly during ischaemia, but there was a post-ischaemic 'breakdown of accommodation', resulting in repetitive firing (Kugelberg, 1946*b*).

Bergmans (1982*b*), using the all-or-none appearance of a motor unit potential to estimate the voltage threshold of a single ulnar motor fibre, obtained an apparently contradictory result: while the threshold did fall during ischaemia, it rose post-ischaemically, and was high during most of the period of paraesthesiae. Bergmans (1982*a*) also found that a state similar to the post-ischaemic one could be induced by prolonged high frequency stimulation (e.g. at 300 Hz for 10 min). He showed that the two states, post-ischaemic and post-tetanic, were characterized by a high threshold to electrical stimulation, reduced accommodation to long current pulses, and an increased superexcitable period, and he argued that they were both caused by hyperpolarization by the sodium pump (Bergmans, 1982*b*). He was left with an unresolved paradox, that a process (electrogenic hyperpolarization) which increased threshold, and therefore reduced excitability, also apparently made the fibres hyperexcitable and spontaneously active.

We have now applied automatic threshold tracking techniques to this problem, using combinations of different stimulus parameters to follow the concurrent changes in accommodation and excitability. 'Excitability' and 'accommodation' are useful concepts, but vague and therefore potentially confusing. 'Excitability' denotes the ease with which a preparation can be excited, and therefore implies a stimulus and a response, the excitability being higher when a given stimulus evokes a greater response (cf. Seneviratne & Peiris, 1968; Applegate & Burke, 1989), or when a smaller stimulus evokes the same response. We prefer the second method, the only

one applicable to single-unit preparations, when excitability is measured by the threshold for an all-or-none response. In this paper the stimulation is always by electric current, so a nerve is more excitable if the same response is evoked by a smaller current, i.e. the threshold current is lower. This leaves open the time course of the stimulus current and the measure of response. We will show that these are crucial variables, and that not only the extent, but also the direction of changes in excitability can depend on the choice of stimulus waveform and response measure. The most generally used and useful type of stimulus is a short ($\leqslant 2$ ms) current pulse, and when we use 'threshold' without qualification a short current pulse can be assumed. The short pulse threshold provides a good indication of membrane potential (Bergmans, 1970). This is because the current–voltage curves of myelinated axons are almost linear for the initial, fast component of electrotonus (Baker, Bostock, Grafe & Martius, 1987), and the current threshold for short pulses is therefore directly proportional to the voltage threshold of the axon(s). Short current pulses are not the only valid way of testing electrical excitability, however, and we have already mentioned that Kugelberg (1944) used long current pulses to measure rheobase. For this paper we also used triangular current pulses, not to mimic 'critical slope' measurements of accommodation (see below), since human axons only exhibit a critical slope during ischaemia, but to help relate the short pulse and long pulse thresholds.

What type of stimulus current is most effective depends on 'accommodation', the process(es) whereby slow or sustained subthreshold depolarization reduces excitability, or increases it less than expected. Different measures of accommodation in axons have been used in the past, and considered equivalent on the assumption that accommodation can be described by a single, exponential time constant (Hill, 1936). These include the 'critical slope' or minimum current gradient for excitation (Vallbo, 1964), and, for human nerves during and after ischaemia, the 'relative slope of accommodation', derived from the threshold for excitation with an exponentially rising current (Kugelberg, 1944), and the 'accommodative threshold ratio' of the threshold to single to that for double activation with 10 ms pulses (Bergmans, 1982*b*). We previously showed that Hill's theory is quite inappropriate as a description of accommodation in mammalian myelinated fibres (Baker & Bostock, 1989). Direct tracking of the threshold changes induced by subthreshold depolarizing currents demonstrated the complexity of accommodation in these fibres, and that there is usually a close parallel between the threshold changes and the underlying electrotonic changes in membrane potential (Bostock & Banker, 1988; Baker & Bostock, 1989). This method of 'threshold electrotonus' provided evidence about the mechanisms of accommodation in human motor axons during ischaemia, and we have now adapted it to study the post-ischaemic state.

Following limited periods of ischaemia (up to about 10 min in our subjects), the changes in threshold to a short current pulse were essentially monophasic, and no motor fasciculations were seen. Following longer periods of ischaemia (15 min or more), the time course of the threshold changes became more complex, and we had difficulty tracking single motor units because of the occurrence of involuntary motor discharges. The simpler excitability changes following brief periods of ischaemia are considered in this paper, and the more complicated changes associated with spontaneous activity in the following paper (Bostock, Baker & Reid, 1991). Some of

the observations on threshold changes have been reported in preliminary communications by Franz, Weigl, Grafe, Baker & Bostock (1988) and by Weigl, Bostock, Franz, Martius, Muller & Grafe (1989).

METHODS

The methods used in Munich for recording thresholds and averaging threshold changes in ischaemia from different subjects (e.g. Fig. 1*A*) were described by Weigl *et al.* (1989). Surface temperature was maintained close to 32 °C by radiant heat. The methods used in London for recording thresholds and threshold electrotonus have mostly been described previously by Bostock & Baker (1988) and Baker & Bostock (1989). Stimuli from a current source were delivered every second through surface electrodes (either saline pads or 3M Red Dot monitoring electrodes), with the cathode over the nerve of interest and the anode at least 10 cm away, over a convenient muscle well away from the course of the nerve. In most experiments the ulnar nerve was stimulated just above the elbow, with the anode over brachioradialis. The polarizing currents used in some experiments were added to the stimulating current electronically and delivered through the same current source and surface electrodes. The total current passed was monitored to ensure linear addition. Both single and multiunit electromyograms (EMG) were usually recorded via chlorided silver cup electrodes filled with electrode jelly and taped over the central and tendon regions of the muscle. In some experiments a single fibre EMG (SFEMG) needle electrode (cf. Ekstedt, Haggqvist & Stalberg, 1969; but with three 25 μm nickel leading-off wires within a 25 gauge needle) was inserted into first dorsal interosseus (FDI) or abductor digiti minimi (ADM) to obtain single units which could not be discriminated by surface recording. Stimulus alternation and concurrent tracking of up to four thresholds was achieved in some experiments by computer, and in others by a purpose-built 4-channel threshold tracker.

Ischaemia was induced by a sphygmomanometer cuff, normally 12 cm wide, inflated to 200 mmHg, and applied proximal to the stimulation site. Exceptions to this arrangement are specified in Results, e.g. a narrow cuff (6 cm wide), or other inflation pressures, or the cuff applied over the stimulating cathode. In the latter case a 3M Red Dot monitoring electrode was used for stimulation.

For experiments of the type illustrated in Figs 2, 3, 4 and 7, we used four stimulus conditions in sequence: 2 ms pulse, 200 ms rectangular pulse, control (no stimulus), and 200 ms triangular pulse. After amplification (80–10000 Hz bandwidth), the EMG signal was full-wave rectified and integrated over 200 ms, and the last control integral subtracted before comparing with a preset level to determine whether the stimulus should be increased or reduced. Each stimulus current waveform (monitored from a resistor in series with the stimulating electrode) and integrated EMG response was digitized (Gould OS4020 digital oscilloscope, 2048 points, 204·8 ms) and compressed and averaged by the PDP 11/23 computer. Average waveforms (512 points, $n = 6$) for the four stimulus conditions and two channels were stored on disk every 30 s. To plot the data, thresholds were obtained from the peak of each current average, and latencies, defined as times to half integral, from the averaged integrals after subtraction of the control integral.

In experiments of the type illustrated in Figs 1*B*, 5 and 7–11, values of threshold current were sampled at 1 Hz from the output of the threshold tracker or computer-driven D–A converter, before conversion to pulses and currents. This provided better time resolution without introducing detectable errors. For the single-unit recordings (e.g. Figs 5 and 10), latencies were recorded by an analog circuit, a 4-channel version of the latency monitor described by Bostock & Grafe (1985).

To maintain a constant nerve temperature in the face of varying thermal input from the blood supply, the temperature of the whole arm was maintained near 37 °C in some experiments. This was achieved by surrounding it with a flexible tube (25 cm diameter) through which air was blown from a fan heater. The air temperature was held at 37–38 °C by electronic control of the heater element. This strategy was found necessary to keep temperature changes recorded from the skin surface under a saline pad electrode within 1 °C. It had the disadvantages that it took at least 30 min for the arm to equilibrate to the higher temperature, and that the higher temperature enhanced the post-ischaemic threshold changes. When we switched to Red Dot electrodes to record threshold changes under a cuff, we found that surface temperature under the cuff fell by no more than 0·2 °C during 5 min ischaemia, and no temperature control was used.

RESULTS

Post-ischaemic depression at different sites

When nerve excitability is tested with brief ($\leqslant$ 2 ms) current pulses, then periods of ischaemia up to 10 min are invariably followed by post-ischaemic depression. We have previously shown that in normal subjects post-ischaemic depression of the peroneal nerve is greater and lasts longer after 10 min than after 5 or 3 min of

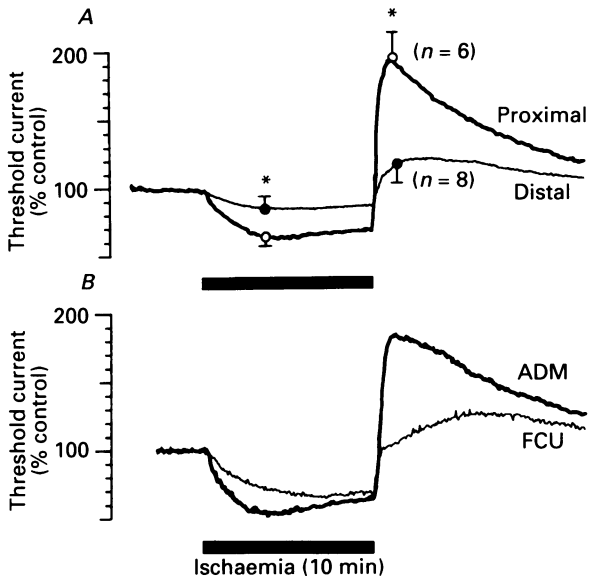

Fig. 1. Effects of ischaemia on axonal excitability at different sites. *A*, comparison between threshold changes induced by 10 min of ischaemia at the knee (○, thick trace) and ankle (●, thin trace) in peroneal nerve innervating extensor digitorum brevis. Recordings from different subjects scaled to the same pre-ischaemic level and averaged, S.D. indicated at times of maximal threshold changes, which were significantly greater at the proximal site (unpaired *t* test; $*P < 0{\cdot}001$). Brief (0·1 ms) current pulses were automatically adjusted to maintain CMAP at one third maximal. Temperature 32 °C. *B*, comparison between threshold changes in abductor digiti minimi (ADM, thick trace) and flexor carpi ulnaris (FCU, thin trace) stimulated alternately at same site (elbow) on ulnar nerve of single subject (H.B.). Black bars indicate 10 min periods of cuff inflation.

ischaemia (Strupp, Bostock, Weigl, Piwernetz, Renner & Grafe, 1990). In that study, the axons were stimulated at the knee (fibular head), and compound muscle action potentials (CMAP) recorded from extensor digitorum brevis (EDB). Similar averaged recordings are shown in Fig. 1*A* (thick trace, proximal), and compared with the much smaller threshold changes recorded at the ankle (thin trace, distal). This confirms the earlier finding by Kugelberg (1944) that ischaemic and post-ischaemic threshold changes are greater in proximal than distal parts of a nerve. We have found similar gradients of ischaemic hyperexcitability and post-ischaemic depression along the median and ulnar nerves, e.g. by comparing stimulation at the elbow with stimulation at the wrist (see also Fig. 11 below). The proximal excitability changes in median nerve fibres innervating abductor pollicis brevis, or ulnar nerve fibres

innervating abductor digiti minimi (ADM) are similar to those in the peroneal nerve (e.g. Fig. 1*B*).

In addition to the proximo-distal gradient, Kugelberg (1944) noted that when axons innervating two different muscles were stimulated at the same site, ischaemia induced greater excitability changes in longer than shorter axons. This is confirmed in Fig. 1*B*, where fibres innervating ADM and flexor carpi ulnaris (FCU) were stimulated at the same site and the two thresholds tested alternately. A similar difference between short and long axons was recorded in peroneal axons, comparing excitation of EDB with excitation of peroneus longus.

Rapid post-ischaemic threshold increases as seen in Fig. 1 were recorded by Bergmans (1982*b*) from single human motor fibres and presumed to reflect the time course of hyperpolarization by the electrogenic sodium pump, similar to that recorded in post-anoxic cat spinal roots by Lundberg & Oscarsson (1954). Possible reasons for the variation with stimulation and recording site will be considered in the Discussion. The direct effect of pressure on ischaemic susceptibility will be dealt with after describing some different types of excitability measurement.

Although our threshold measurements are in agreement with Kugelberg (1944) in finding the most pronounced changes in the proximal parts of the longest axons, they are in apparent conflict in that Kugelberg recorded a post-ischaemic *increase* in excitability in ulnar fibres after 10 min of ischaemia. Furthermore, the ulnar motor fibres were reported to become hyperexcitable and discharge spontaneously after more prolonged ischaemia (Kugelberg & Cobb, 1951; see also the following paper). We considered that this failure to detect any evidence of an increase in excitability developing after 10 min of ischaemia might be due (a) to the choice of stimulus waveform (brief pulse) and response measure (peak CMAP), (b) to our choice of stimulation site, or (c) to an insufficient duration of ischaemia. We have explored each of these possibilities and found all three to be important. The first two are considered in this paper, and the third in the following paper.

Excitability changes and stimulus waveform

To explore the importance of stimulus waveform, we have compared threshold measurements made with a 2 ms current pulse, a 200 ms current pulse to estimate rheobase, and a 200 ms triangular current pulse, peaking at 100 ms. The triangular stimulus is also referred to as a current ramp, since under normal conditions excitation at threshold only occurred during the depolarizing phase. (In some hyperpolarized fibres, however, the repolarizing phase of the triangle also made a significant contribution to excitation at threshold). Excitability was tested with each shape of stimulus in turn, and the rectified and integrated EMG used to measure the response. Figure 2 shows the time courses of the three different measures of threshold, separately and superimposed, in an ulnar nerve (elbow-ADM) subjected to 10 min of ischaemia. In this experiment, the stimulus current and EMG response waveforms were averaged every 30 s, and the peak stimulus currents measured from the averaged waveforms (see Methods).

The 2 ms stimulus duration was chosen to be long compared with fast electrotonus (the initial rapid phase, due to depolarization of the node and myelin sheath only), and short compared with slow electrotonus (the slower membrane potential changes

due to depolarization of the internodal axon and activation of slow channels) (Baker *et al.* 1987). As in previous studies (Bostock & Baker, 1988; Baker & Bostock, 1989) threshold current for a 1–2 ms pulse is denoted I_{th}. For short pulses, the use of rectified and integrated EMG, rather than peak CMAP, as the response measure

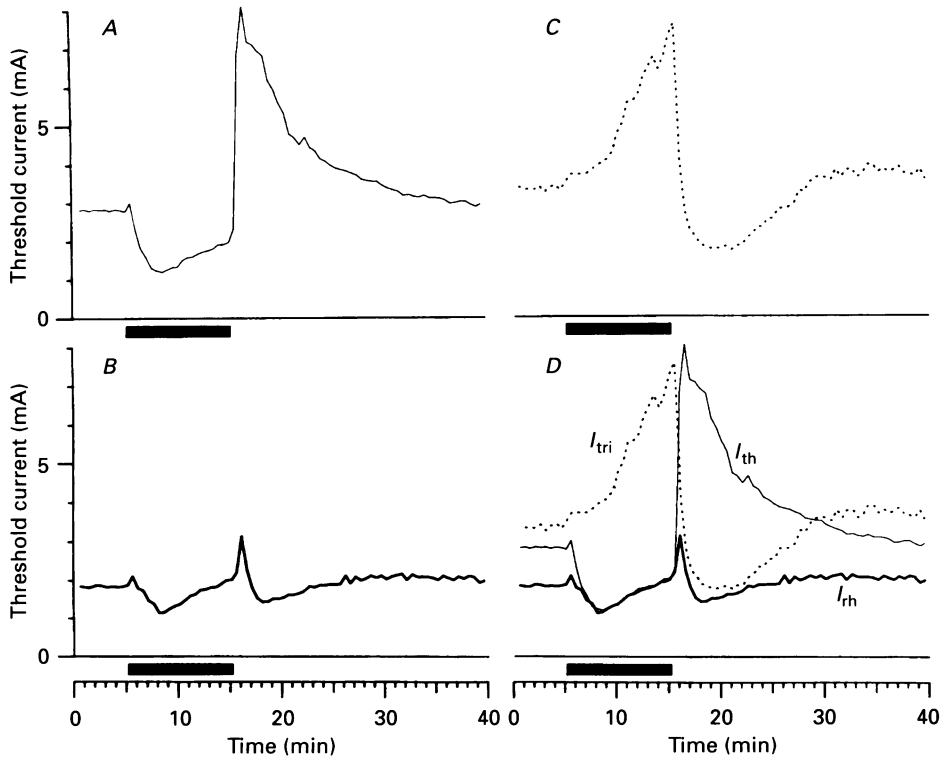

Fig. 2. Dependence of excitability changes induced by ischaemia on stimulus waveform. Threshold currents were those required to evoke constant submaximal rectified and integrated EMG response from ADM; ulnar nerve stimulated at elbow. Stimuli: *A*, 2 ms rectangular pulse (I_{th}); *B*, 200 ms rectangular pulse (I_{rh}); *C*, 200 ms triangular pulse (I_{tri}); *D*, I_{th}, I_{rh} and I_{tri}, showing complementary behaviour of I_{th} and I_{tri}. Black bars indicate period of cuff inflation (10 min). 37 °C.

made little difference, as shown in another experiment by using the two measures alternately.

Rheobase can be defined as the threshold current measured with an infinitely long pulse. We found that to obtain accurate estimates of rheobase, the stimulus duration had to exceed 100 ms in the post-ischaemic period, and we settled on a duration of 200 ms for the rheobasic stimulus (I_{rh}) and for the window during which we integrated the rectified EMG. To prevent noise or voluntary or spontaneous activity interfering with the thresholds, the integrated activity was always compared with the integral in the absence of stimulation (see Methods). The changes in I_{rh}, shown by the thick line in Fig. 2*B* and *D*, resembled the changes in rheobase recorded by Kugelberg (1946*a*). (Kugelberg did not specify the stimulus duration he used, but from a figure in Kugelberg (1944) a duration of at least 200 ms seems likely.)

Although I_{th} and I_{rh} converged during ischaemia, there was always a striking separation in the post-ischaemic period, when I_{th} rose abruptly, but I_{rh}, after only a brief rise, fell and often, as in Fig. 2, went below its resting value. The post-ischaemic nerve in Fig. 2 was therefore in the interesting state of being both less excitable, as tested with brief pulses (cf. Bergmans, 1982*b*) and more excitable, as tested with long current pulses (cf. Kugelberg, 1946*a*).

We found that the behaviour of the threshold to a triangular or ramp stimulus, shown as the dashed line I_{tri} in Fig. 2*C* and *D*, helped to explain the time course of the changes in I_{rh}. During ischaemia I_{tri} rose, reflecting the increase in accommodative power of the axons. (We have previously shown that this involves a change in the mechanism of accommodation, from activation of slow potassium channels to inactivation of sodium channels (Bostock & Baker, 1989).) After the end of ischaemia, the fibres no longer accommodated to the current ramp and I_{tri} fell well below its resting value. The lack of accommodation by post-ischaemic motor fibres was remarked upon and demonstrated in other ways by both Kugelberg (1944) and Bergmans (1982*b*) and is shown in terms of threshold electrotonus below. As I_{tri} fell, it appeared to 'push down' I_{rh}, which had been rising, since a 200 ms rectangular current is always a more effective stimulus than a triangle of the same peak amplitude. The 200 ms duration chosen for I_{tri} was arbitrary, but not critical. Use of 100 or 400 ms triangular stimuli gave similar values of I_{tri} during the post-ischaemic period. The peak depolarizing current, rather than the rate of rise of current, appeared to be the most important factor. This behaviour contrasts with that described in some other preparations, in which a 'critical slope' has been found necessary for excitation, and has been used as a measure of accommodation (Vallbo, 1964).

The experiment just described was performed with the arm maintained at 37 °C (see Methods), to ensure that none of the threshold changes recorded were caused by changes in temperature. Raising the temperature well above the normal level (30–32 °C at the skin) had the effect of exaggerating the post-ischaemic rise in I_{th}. This temperature effect was not evaluated systematically, but in nine recordings from the same nerve, the peak value of I_{th} after 10 min of ischaemia was consistently higher (190–250% of control, $n = 5$) at 37 °C than when temperature was uncontrolled (160–185%, $n = 4$). However, the behaviour of I_{rh} and the complementary changes in I_{th} and I_{tri} were not obviously affected by temperature (compare Figs 2 and 7).

Relationship between the three measures of threshold

Electrical excitability measured with a long current pulse (I_{rh}) clearly had two components, one due to excitation by the onset of the stimulus, and therefore related to I_{th}, and one due to delayed excitation by the later part of the stimulus, and therefore related to I_{tri}. Since excitability can be defined as the reciprocal of threshold current (Adrian & Lucas, 1912), this suggests the relationship:

$$1/I_{rh} \approx 1/I_{tri} + 1/I_{th} \quad (1)$$

or

$$I_{rh} \approx \frac{I_{tri} \times I_{th}}{I_{tri} + I_{th}}. \quad (2)$$

Thus during ischaemia fibres accommodate well, I_{tri} becomes high and I_{rh} tends to I_{th}; following ischaemia I_{th} can become very high and I_{rh} tends to I_{tri}; but for resting nerve, when I_{tri} and I_{th} are of similar magnitude, I_{rh} is much lower than either. Equations (1) and (2) have been found to hold approximately whenever all three

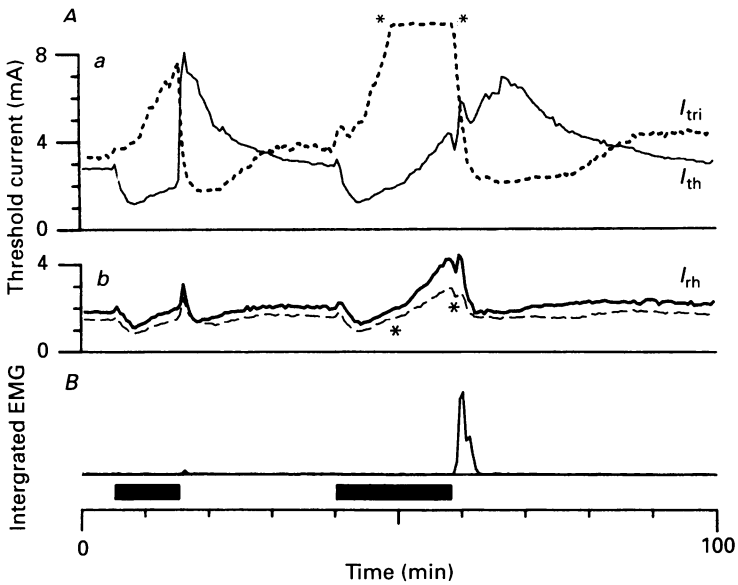

Fig. 3. Excitability changes following prolonged ischaemia. Extended plot of experiment of Fig. 2, showing behaviour of three thresholds with subsequent 18 min period of ischaemia. In *Aa* the triangular (I_{tri}) stimulus was limited to 9 mA to avoid tissue damage. Thin dashed trace in *Ab* is calculated from I_{th} and I_{tri} according to right-hand side of eqn (2); asterisks show period when I_{tri} was invalid. *B*, integrated EMG, recorded during intervals between stimuli, to show spontaneous motor activity (uncalibrated).

thresholds have been recorded. This is illustrated in Fig. 3, for the example of Fig. 2, and also in Fig. 7. Figure 3 shows an additional 18 min period of ischaemia, and illustrates what was stated in the Introduction, that after longer periods of ischaemia the changes in threshold (I_{th}) became more complicated and motor fasciculations occurred.

Delayed post-ischaemic responses

The combination of low I_{tri} with high I_{th} in the post-ischaemic period implies that the response to a rheobasic stimulus should be delayed. This phenomenon is demonstrated in Fig. 4. Figure 4*A* shows one in three of the rectified, integrated and averaged EMG responses to I_{rh} for the experiment of Fig. 2. During ischaemia the responses started earlier, while post-ischaemically they started later and became less synchronous. The latencies of these responses, defined as the times to half-maximal integrated response, are plotted in Fig. 4*B* as L_{rh}, and compared with the relatively constant latencies to the 1 ms stimuli (L_{th}). There is a striking parallel between the

latency changes to a long current pulse (L_{rh}) and the threshold changes to a short pulse (I_{th}), presumably because both reflect the changes in membrane potential. L_{rh} is plotted for many of the recordings that follow, because it provides an independent measure of the membrane changes occurring, unaffected by the proportion of the

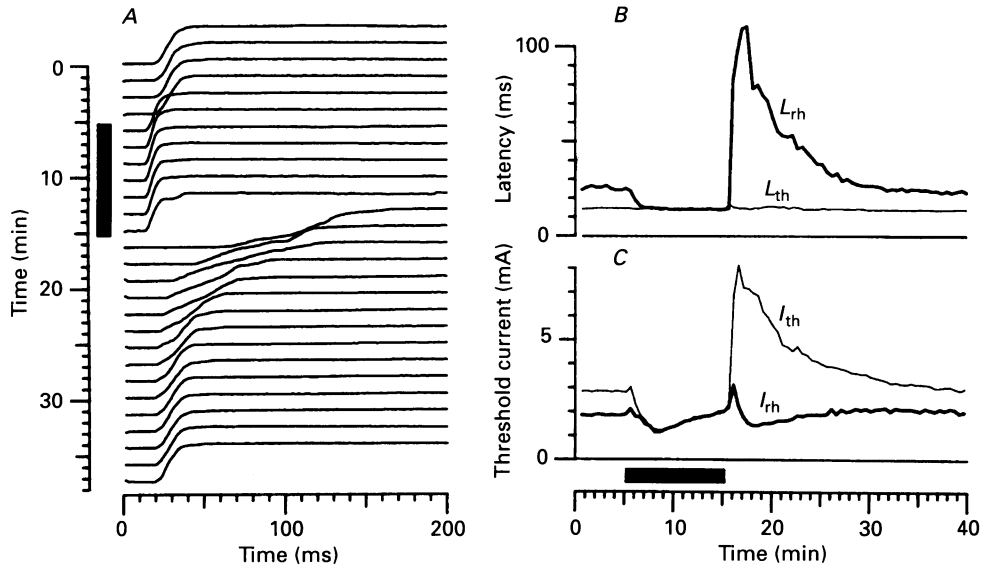

Fig. 4. Delayed responses during post-ischaemic period. *A*, time course of rectified and integrated EMG responses to 200 ms rheobasic stimuli during experiment of Fig. 2. Each trace is mean of eight responses, after subtraction of control responses and scaling to same peak height. Elapsed time and bar to indicate ischaemia shown on the left. (For clarity, only one average in three recorded is plotted.) *B*, L_{rh} (thick line) is latency of half-maximal response to I_{rh}, as shown in *A*. L_{th} (thin line) is latency of half-maximal response to I_{th}. *C*, thresholds as in Fig. 2, replotted for comparison with latencies.

applied current reaching the axons. These large changes in L_{rh}, associated with changes in the electrotonic properties of the axons (see below), should not be confused with the much smaller changes in latency due to changes in conduction velocity, which affect L_{th} as well as L_{rh}. The conduction velocity changes were described by Nielsen & Kardel (1974). The changes in L_{rh} are related to the changes in chronaxie described by Bourguignon & Laugier (1923).

Single fibre thresholds

Both Kugelberg (1946*b*) and Bergmans (1970) observed that post-ischaemic nerves tend to fire repetitively. The question therefore arises as to what extent the post-ischaemic hyperexcitability shown by I_{rh} and I_{tri} in Fig. 2, and to what extent the increase in latency (L_{rh}) described in the last section, may have been due to repetitive firing. To obtain responses that were unaffected by repetitive firing, we also recorded from some single fibres, using the all-or-none presence of a motor unit potential (MUP) to control the stimulus current, and the time to the first MUP (when present) as the measure of latency. Figure 5 shows recordings from two single units,

one isolated with surface stimulation and recording simply by virtue of its low threshold (cf. Bergmans, 1970), and one with a SFEMG electrode inserted into first dorsal interosseus. (Another single-unit recording is illustrated in Fig. 10 below.) In most respects the latency and threshold recordings closely resembled the multiunit

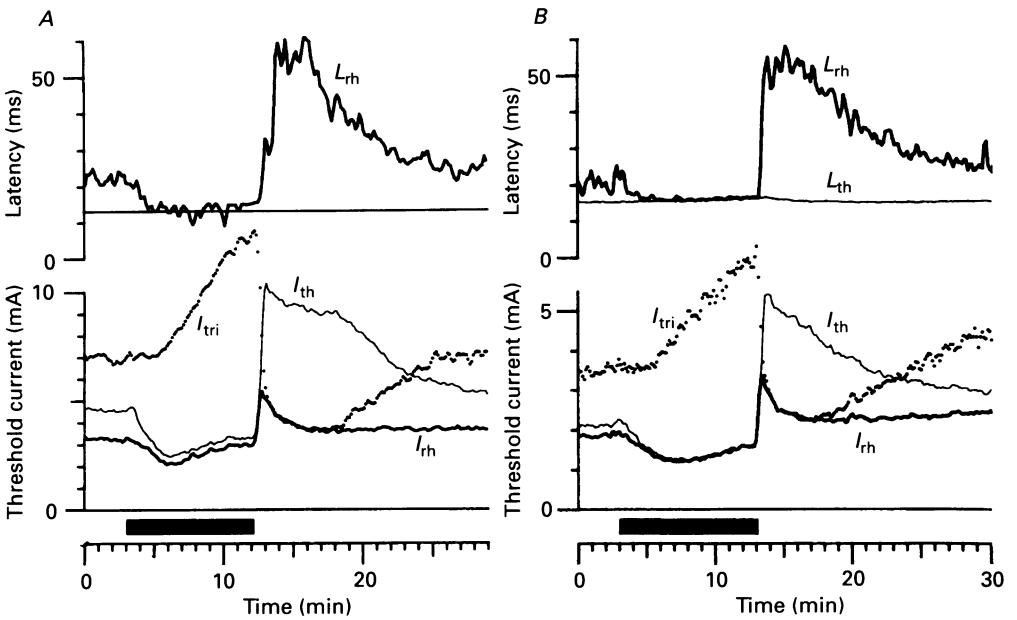

Fig. 5. Excitability and latency changes of single motor units. Recordings from 2 units in subject G.R. *A*, median nerve unit in abductor pollicis brevis, isolated non-invasively (cf. Bergmans, 1970). *B*, ulnar unit in first dorsal interosseus, isolated with intramuscular needle electrode. Top panel: L_{rh}, latency from start of rheobasic stimulus to first EMG spike; L_{th} in *B*, latency from start of I_{th} pulse to first EMG spike; horizontal line in *A* indicates control latency to I_{th} pulse. Bottom panel: thresholds of single units to different stimuli as in Fig. 2 (but I_{th} pulse duration 1 ms in *A*, 2 ms in *B*).

recordings using rectified and integrated EMG, but in the post-ischaemic period the single-unit rheobase did not fall below its pre-ischaemic value. The same has been true of five other single-units following ischaemia of up to 10 min. (A fall in single-unit rheobase has, however, been observed after 15 minutes of ischaemia.) On the other hand, repetitive firing to the rheobasic stimuli was observed during the period of reduced I_{tri}.

This result suggests that the post-ischaemic fall in multiunit rheobase was due to repetitive firing, rather than to a fall in rheobase of the individual fibres. Supporting evidence came from an experiment (not illustrated) in which the rectified, but not integrated EMG was used as response measure for threshold tracking, while the rectified and integrated EMG was recorded. I_{rh} did not show the usual fall, but the integrated EMG increased considerably during the early post-ischaemic period.

In the light of these results, the post-ischaemic fall in multiunit 'rheobase' shown in Fig. 2 might be regarded as artifactual. However, repetitive firing contributes to the physiological response to the stimuli (muscle contraction) as well as to the

rectified and integrated EMG. We therefore prefer to regard multiunit I_{rh} as a valid measure of excitability (see Discussion), but one that depends on repetitive firing as well as on the rheobases of the individual fibres.

Comparison with excitability changes induced by tetanic stimulation and hyperpolarization

Bergmans (1982*b*) noted the similarity between post-ischaemic and post-tetanic changes in excitability and accommodation, and attributed both to hyper-

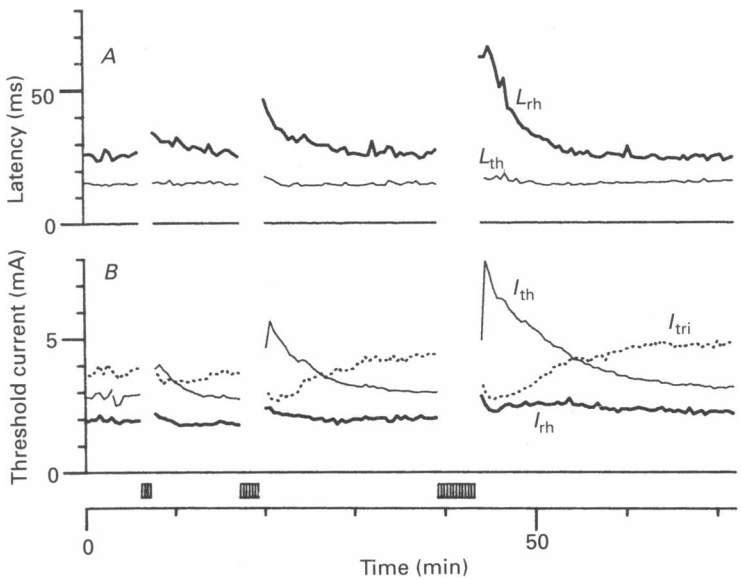

Fig. 6. Post-tetanic excitability and latency changes. Changes in multiunit latencies (*A*) and thresholds (*B*) following supramaximal stimulation of ulnar nerve at 300 Hz for 1, 2 and 4 min. Temperature, 37 °C. (Symbols and methods as in Figs 2 and 4.)

polarization by the electrogenic sodium pump. Figure 6 shows the effects of impulse trains on the three thresholds and two latencies of the same nerve as in Figs 2–4. The after effects of stimulating at 300 Hz for 4 min clearly resembled the recovery from ischaemia. Unfortunately, longer duration trains of supramaximal stimuli were not tolerated by our subjects. We were therefore unable to determine the changes in excitability leading to the post-tetanic repetitive activity described by Bergmans (1982*a*).

The effects of percutaneous hyperpolarizing currents on the three thresholds are compared with ischaemia in Fig. 7. The threshold changes from 9–11 min and 70–72 min were remarkably similar. Both hyperpolarization and release of the pressure cuff (a) immediately increased I_{th} well above its control level, (b) reduced I_{tri} more slowly below its control level, and (c) first increased and then reduced I_{rh}. When the hyperpolarizing current was switched off, the nerve was left slightly hyper-excitable to all three stimuli, and recovered over 3–4 min. (Complementary slow changes in excitability were recorded in response to sustained depolarizing currents, suggesting that 'ultra-slow sodium inactivation' (Fox, 1976) may be important in these axons.)

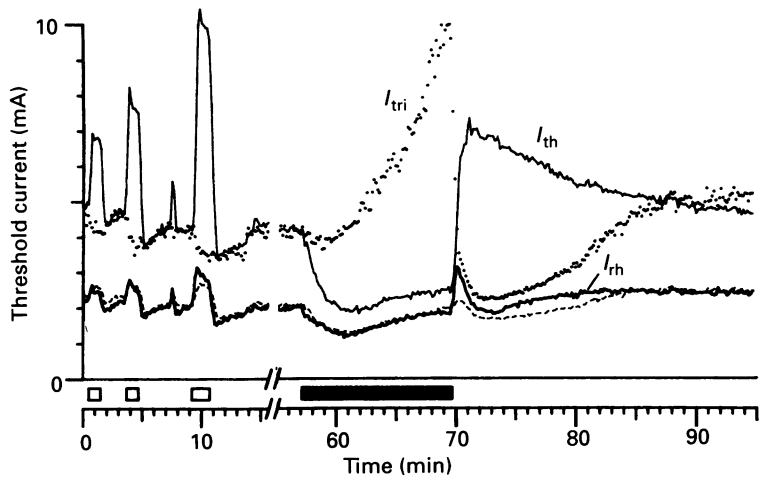

Fig. 7. Hyperpolarization and ischaemia on nerve excitability. Multiunit threshold changes on passing DC hyperpolarizing currents of 1, 2 and 4 mA, compared with the effects of ischaemia on the same nerve (see text). The thin, dashed trace was calculated from I_{th} and I_{tri} by eqn (2), as in Fig. 3.

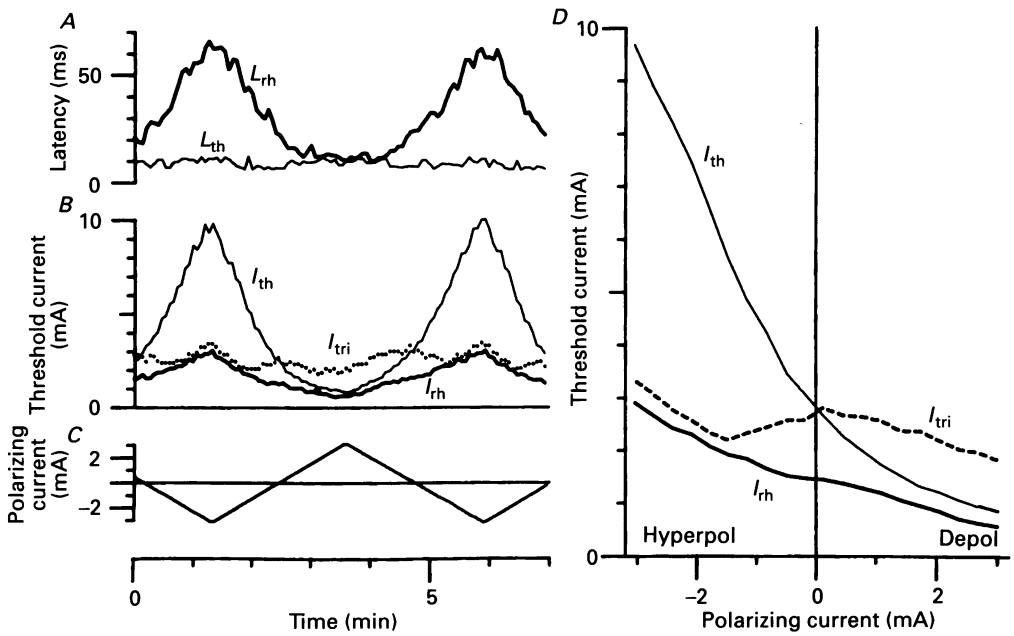

Fig. 8. Polarizing currents and nerve excitability. Changes in multiunit latencies (*A*) and thresholds (*B*) on passing a slowly varying polarizing current (*C*) through the stimulating electrode. *D*, average thresholds plotted against polarizing current. (Symbols as in Figs 2 and 4.)

We were at first very impressed with the reduction in I_{tri} induced by hyperpolarizing current, and thought that perhaps if one could pass enough hyperpolarizing current for long enough, I_{tri} might fall below the control value for I_{rh}, 'pushing it down' to induce hyperexcitability and even ectopic discharges. We found, however, that there were strict limits to the excitatory effects of hyperpolarization on I_{tri}, even if the current was maintained for several minutes. In

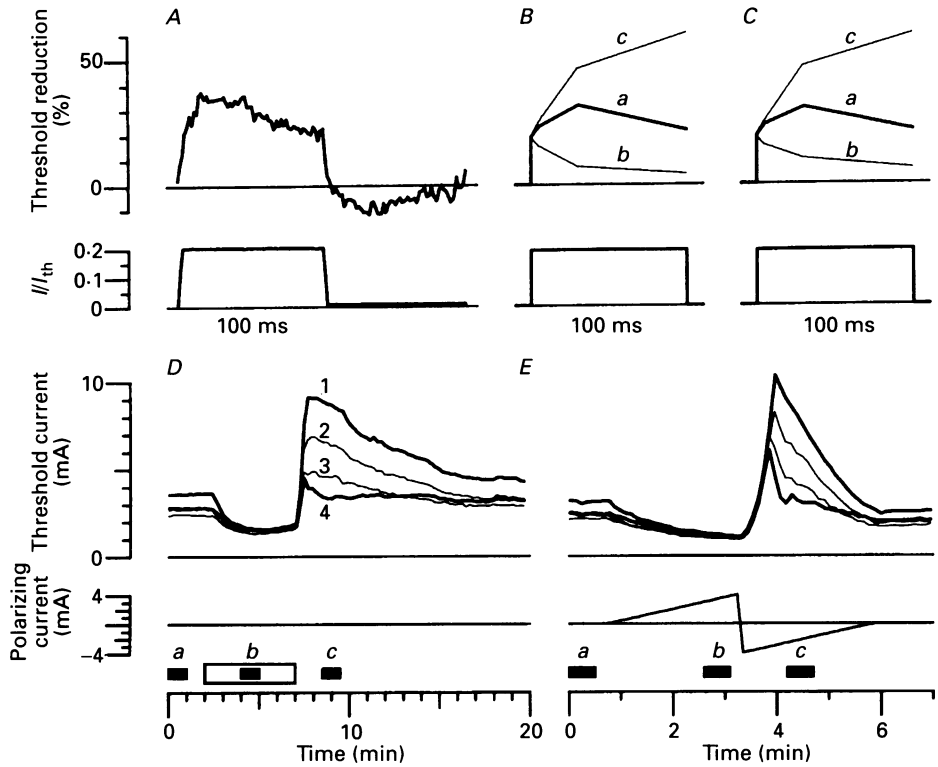

Fig. 9. Changes in threshold electrotonus induced by ischaemia and polarizing currents. *A*, accommodation in normal nerve, indicated by recording of threshold electrotonus, i.e. the threshold changes induced by a subthreshold current pulse, in this case a depolarization, 20% of I_{th} for 100 ms (see text). *Ba*, 4-point plot of resting threshold electrotonus, calculated from measurements in *D* at time *a*. Similarly, *Bb* indicates threshold electrotonus during ischaemia, *Bc* after ischaemia. *C*, 4-point plots of threshold electrotonus, *a* at rest, *b* during applied depolarizing current, *c* during applied hyperpolarizing current, calculated from measurements in *E*. *D*, threshold currents (I_{th}) during and after 5 min of ischaemia, produced by pressure cuff over stimulating electrode and indicated by wide, open bar below. Trace 1, control threshold (I_{th}). Traces 2–4, thresholds recorded 5, 30 and 100 ms respectively after start of 100 ms current pulses, 20% of I_{th}. Narrow filled bars *a–c* indicate periods over which traces 1–4 were averaged to calculate the threshold reductions in *B*. (The 4 points of each curve in *B* were calculated from traces *D*1–4 as follows: 1st point, 20%, since current pulse, 20% of threshold, must initially reduce threshold by 20%; 2nd point, $100 \times (D1 - D2)/D1$; 3rd point, $100 \times (D1 - D3)/D1$, etc.) *E*, thresholds as in *D*, showing effects of maintained depolarizing and hyperpolarizing currents that produced excitability changes comparable to the ischaemia. Periods *a–c* correspond to traces *Ca–Cc*.

Fig. 8 a slowly varying polarizing current was used to generate a plot of threshold current *vs* polarizing current for the three stimulus waveforms. Hyperpolarizing currents only reduced I_{tri} a small amount, beyond which they increased it, and the rheobase was never reduced during hyperpolarization.

Effects of ischaemia and polarization on threshold electrotonus

We have previously measured accommodation in rat and human motor axons by plotting the time course of the threshold changes induced by subthreshold polarizing currents (Bostock & Baker, 1988; Baker & Bostock, 1989). We have referred to this technique as threshold electrotonus, since the threshold changes can provide an accurate reflection of the electrotonic potentials in the axons. In depolarized axons, however, an additional fast component of accommodation appears, unrelated to membrane potential and probably due to sodium channel inactivation (Baker & Bostock, 1989). As stated in the Introduction, we wished to use the method of threshold electrotonus to investigate the post-ischaemic changes in accommodation, but the usual method could not be applied, since it required the nerve to be in a stable state for 5–10 min. Instead, we selected three points on the threshold electrotonus curve and tracked them continuously. Figure 9*A* shows a conventional multiunit recording of threshold electrotonus. In response to depolarizing current pulse, 20% of I_{th}, the threshold was initially reduced by 20%. The threshold reduction then increased for about 25 ms (a phase of 'negative accommodation') before accommodation set in and brought the threshold reduction back to about 20%. For Fig. 9*D*, I_{th} was tracked through 10 min of ischaemia (trace 1), and also thresholds 5, 30 and 100 ms after the start of a polarizing current 20% of I_{th} (traces 2–4 respectively). Reconstructed plots of threshold electrotonus before, during after ischaemia are shown in Fig. 9*B*, traces *a–c* respectively. As in the previous paper (Baker & Bostock, 1989), ischaemia not only abolished the negative accommodation, but caused a phase of rapid accommodation. Trace *c* shows that during the early post-ischaemic period there was no accommodation to the depolarizing current; instead there was a prolonged phase of negative accommodation, with excitability increasing throughout the 100 ms period of applied current. Figure 9*C* and *E* shows that these dramatic changes in the accommodative properties of the axons can be mimicked very well, simply by passing polarizing currents.

Excitability changes under the pressure cuff

We earlier suggested that one possible reason for the failure to detect hyperexcitability after 10 min of ischaemia was our choice of stimulation site. Although we confirmed that the most proximal parts of a nerve show the greatest changes in excitability both during and after ischaemia, and the most proximal ischaemic parts of a nerve are inevitably those under a cuff, we had not attempted to record the excitability changes actually occurring under the cuff. This was partly because Merrington & Nathan (1949) had concluded that post-ischaemic paraesthesiae depended only on ischaemia, and not on pressure, and partly because we had expected that any threshold measurements made under a pressure cuff would be unreliable, since the pressure would be likely to affect the proportion of applied current reaching the nerve. An interesting observation made us reconsider the need

to look under the cuff. An upper arm cuff was inflated to 200 mmHg for 10 min and then let down to 100 mmHg, low enough to allow arterial blood distal to the cuff, but not low enough to fill the capillaries under the cuff or to allow venous return. No paraesthesiae were experienced, but from an electrode just below the cuff we

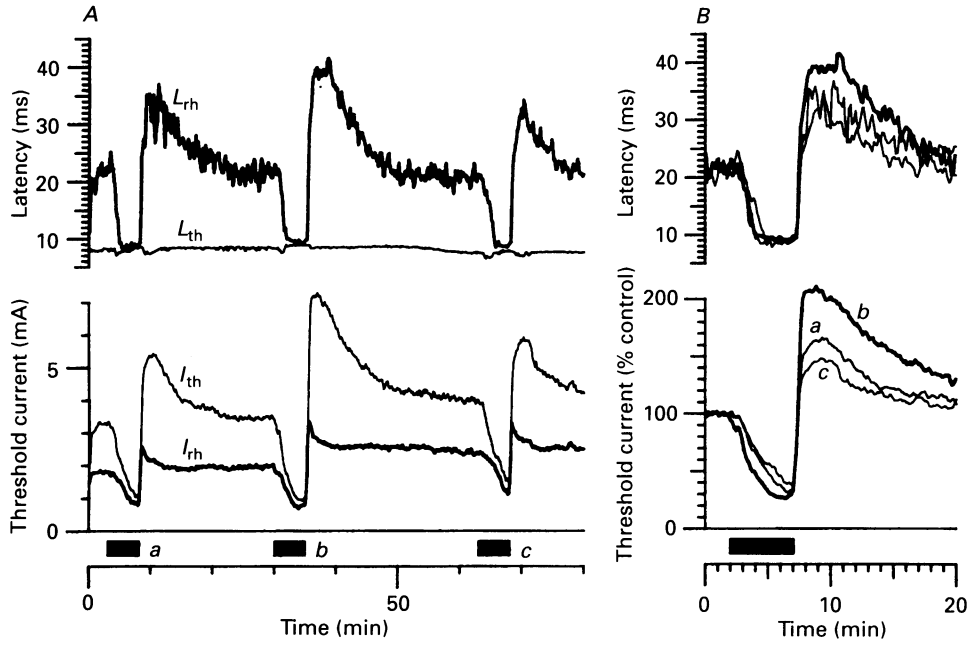

Fig. 10. Effect of cuff position on sensitivity to ischaemia. Latencies and thresholds recorded for single unit in first dorsal interosseus, stimulated in ulnar nerve of H.B. just above elbow. *A*, 5 min of ischaemia was induced at *a* and *c* by inflating proximal cuff, at *b*, by inflating cuff over stimulating electrode. Thresholds and latencies as in Fig. 5. *B*, L_{rh} and I_{th} data replotted from *A*, with threshold changes expressed as percentage of pre-ischaemic value, and times of cuff inflation superimposed. Thick traces, cuff over electrode (*b*); thin traces, cuff proximal to electrode (*a* and *c*).

recorded a normal post-ischaemic threshold increase. Five minutes later, with the veins in the distal limb painfully distended, the cuff was released completely. Strong paraesthesiae were experienced, but no further threshold change. Since post-ischaemic paraesthesiae were normally experienced after 10 min of ischaemia in this subject (M.B.), the observation pointed to the nerve under the cuff as the sole source of these paraesthesiae. Repeating this experiment with an electrode under the cuff showed that the ischaemic threshold changes were faster and greater under the cuff than below it (cf. Fig. 11 below), and that the ischaemic changes under the cuff were delayed until the pressure was released completely.

The experiment illustrated in Fig. 10 was undertaken to test whether the greater susceptibility of the axons under the cuff to that below the cuff was simply due to the nerve under the cuff being more proximal, or whether the cuff pressure itself was

important. Two cuffs were applied to the forearm, with a stimulating electrode under the narrow, distal cuff. At *a* and *c* the proximal cuff was inflated for 5 min, and at *b* the distal cuff. The changes in I_{th} for the single unit were faster and greater at *b* than *a* and *c*. This difference could not be attributed to the cuff affecting the path of

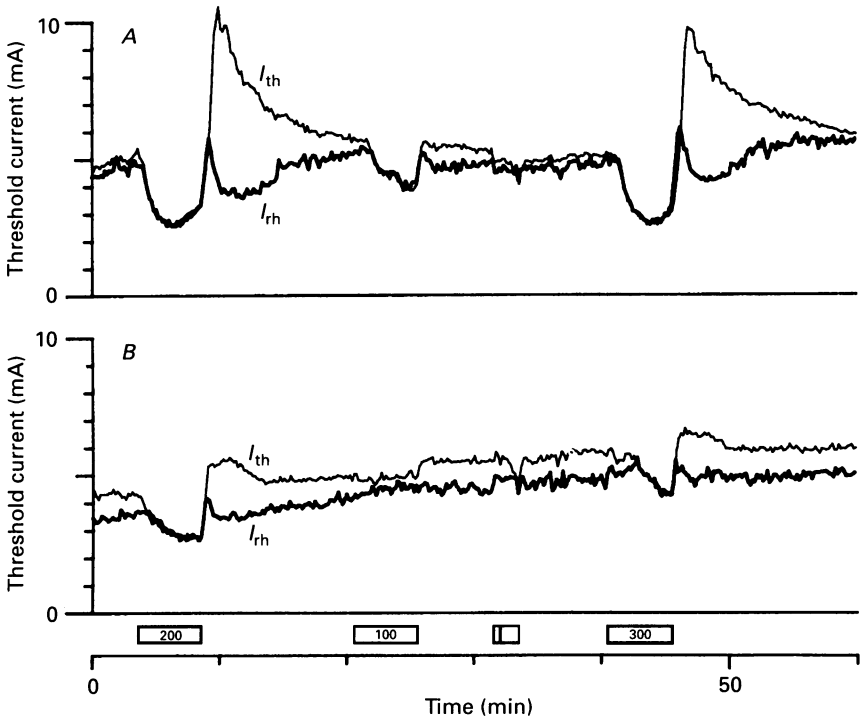

Fig. 11. Effect of cuff pressure on sensitivity to ischaemia. *A*, multiunit pulse threshold (I_{th}) and rheobase (I_{rh}) recorded under pressure cuff over ulnar nerve above elbow, for 5 min inflations to different pressures as indicated by the numbers within the bars (mmHg). *B*, thresholds as in *A*, recorded concurrently, but stimulating just distal to the pressure cuff.

current flow, since the post-ischaemic increase in L_{rh} was also greatest when the cuff over the electrode was inflated. It could not be attributed to a reduced fall in temperature during ischaemia due to thermal insulation by the cuff, since (i) the cuff was always present over the stimulating electrode and inflated to 20 mmHg, except at *b*, and (ii) repeating the cuff protocol with a thermistor in place of the stimulating electrode, we recorded temperature drops of no more than 0·2 °C, which were not detectably different between *a*, *b* and *c*. In a similar experiment we found that ischaemic and post-ischaemic multiunit threshold changes at the wrist were greater if a cuff over the stimulating electrode was inflated than one on the upper arm. We also tested the possibility that proximity to non-ischaemic limb was important by inflating both cuffs at once. This was no different to inflating the distal cuff alone.

Recently, Fern & Harrison (1990) have suggested that pressures in the range 70–250 mmHg may affect the excitability of mammalian axons by mechanical deformation. To test whether mechanical factors could be involved in the greater susceptibility of the nerve under the cuff, we compared the effects of different pressures (Fig. 11). Under the cuff (*A*) as well as distal to the cuff (*B*), the effects of inflation to 300 mmHg were no greater than inflation to 200 mmHg. This and another, similar experiment indicated that the excitability changes were related to the arrest of circulation, and not to deformation of the axons. (The pressure effects recorded by Fern and Harrison may have been caused by longitudinal pressure gradients at the junctions between normal and compressed parts of the nerve. Such gradients can block conduction by displacing myelin to cover the nodes of Ranvier (Ochoa, Fowler & Gilliatt, 1972).) In Fig. 11, inflation to 100 mmHg produced only partial ischaemia under the cuff, although in other experiments release of pressure from 200 to 100 mmHg did not even partially relieve the ischaemia under the cuff. This suggests some hysteresis in the blood vessels.

DISCUSSION

In this and the following paper, we have applied threshold tracking techniques to post-ischaemic human nerve, to resolve the apparent contradiction in the literature about the nature of the excitability changes, and to try to understand the biophysical basis of the ectopic discharges. As with others who have investigated post-ischaemic or the related post-tetanic discharges (Bergmans, 1983; Applegate & Burke, 1989), we thought they might prove to be useful models for the axonal hyperexcitability occurring in some pathological conditions (Diamond, Ochoa & Culp, 1982). We chose to restrict this study to motor axons, although they are less prone to discharge ectopically than sensory axons, because they present a more homogeneous population for multiunit studies, and for the ease of recording single-unit and asynchronous activity. Motor unit potentials are unaffected by ischaemia of the durations used in this paper, so that changes in CMAPs can be interpreted more simply than changes in sensory nerve action potentials.

Site of maximal changes in excitability

Our first measurements (e.g. Fig. 1) confirmed by automatic threshold tracking that susceptibility to ischaemia is greatest in the most proximal part of the longest axons, as reported by Kugelberg (1944). A proximo-distal gradient in ischaemic susceptibility has also been documented for human sensory nerve fibres (Lutschg & Ludin, 1982). Our interest in these variations in ischaemic and post-ischaemic excitability changes stemmed primarily from the need to identify sites in the nerve that were most likely to act as ectopic foci. The reason for the gradients in ischaemic susceptibility is not clear. Gradients in internodal length were invoked by Lutschg & Ludin (1982), but they could not account for the observations on thresholds. Both intrinsic factors (e.g. related to the metabolism of the axon) or extrinsic factors (e.g. related to dispersal of potassium ions from the axonal environment) may be involved, but the latter could not readily explain the very different behaviour of axons to different muscles stimulated at the same site in the nerve (Fig. 1*B*). Groat

& Koenig (1946*a*, *b*) found a strikingly linear relationship between time to ischaemic block and position (fraction of length) in cat nerves, which extended from the nucleus along the entire length of the motor axon. Such a relationship could arise from the metabolic requirement to transport material to and from the distal parts of the axon, since the further from the neuromuscular junction the more the material requiring transport, and the sodium pump and axoplasmic transport are thought to compete for the same supply of ATP (Ochs, 1975). On the other hand, the relative insensitivity to ischaemia of the distal, compared with the proximal parts of an axon (Fig. 1*A*) is reminiscent of the relative insensitivity of diabetic nerves, which appears to be determined by the substrate levels for anaerobic metabolism (Strupp *et al.* 1990). Groat & Koenig proposed a proximo-distal gradient of substrates (1946*a*), but also considered a relationship to the newly discovered phenomenon of axoplasmic flow (1946*b*).

We also found that sensitivity to ischaemia was greatest underneath a pressure cuff (Fig. 10), and that this sensitivity appeared to depend on exclusion of blood from the nerve rather than mechanical deformation (Fig. 11). The expulsion of blood should reduce the oxygen and glucose content of the nerve, and also reduce the extra-axonal volume available for diluting potassium ions released during ischaemia. In rat nerve, blood vessels account for 2·5% of the intra-fascicular nerve volume (Bell & Weddell, 1984). Taking the mean oxygen capacity of the blood as half way between that for an arterial P_{O_2} of 90 mmHg (0·2 ml ml^{-1}) and a venous P_{O_2} of at least 26 mmHg (0·1 ml mg^{-1}) (Lagerlund & Low, 1987), the intra-fascicular blood oxygen content should be 0·375 ml O_2 (100 ml nerve)$^{-1}$. This could sustain the nerves of 2-month-old rats for 22 s at the consumption rate of $1{\cdot}7 \times 10^{-4}$ ml O_2 ml^{-1} s^{-1} (Low, Schmelzer & Ward, 1986), or the nerves of 21-month-old rats for almost twice as long. Comparable figures for human nerves, and for the volume of extrafascicular vessels are not available, but it seems likely that the reduction in this vascular component of nerve oxygen capacity accounts for at least some of the extra sensitivity to ischaemia of nerve underneath a pressure cuff. Another factor of possible importance is that the nerve under the cuff is inaccessible to arterial blood that reaches the distal limb via nutrient arteries in the humerus and anastomotic vessels round the elbow joints (cf. Bier's spots, Lewis, 1927). Whatever the reason, we conclude that it is advisable to stimulate under the cuff to detect the excitability changes leading to post-ischaemic ectopic discharges. This approach was therefore adopted in the following paper, despite the risk of mechanical artifacts affecting the threshold measurements.

Rheobase and threshold current: a paradox resolved

Our most important finding was that the post-ischaemic changes in excitability were quite different, depending on how excitability was measured. When the threshold of a single motor axon was tracked, using a short ($\leqslant$ 2 ms) stimulus, there was a large, post-ischaemic rise in threshold, as described by Bergmans (1982*b*) (see Fig. 5). Very similar results were obtained with multiunit recording, whether the peak CMAP (Fig. 1) or the rectified and integrated EMG (Fig. 2) was used as the response measure. According to classical models of myelinated axons (Frankenhaeuser & Huxley, 1964; Goldman & Albus, 1968) and their strength–duration

behaviour (Bostock, 1983), use of stimulus currents longer than 2 ms should have made little difference. In fact, however, with much longer stimuli to record rheobase, single-unit excitability returned quickly to its pre-ischaemic level, while the latency to the first spike increased by 40 ms or more (Fig. 5). Kugelberg (1944) had used the smallest current to produce a visible movement as a measure of rheobase, a measure probably closer to our use of the rectified and integrated EMG, as in Fig. 2. With this measure we also found an absolute increase in excitability in the post-ischaemic period, though not in all subjects.

Each of these thresholds (I_{th}, single and multiunit I_{rh}) provides a valid measure of excitability, appropriate to particular circumstances. Thus the safety factor for conduction depends on nodal excitability, and it has been shown previously that activation of the electrogenic sodium pump by trains of impulses can block conduction in demyelinated fibres by reducing excitability (Bostock & Grafe, 1985). In this case, the relevant threshold measure is I_{th}, since the propagating impulse provides only a brief (< 1 ms) depolarizing current to the critical node. On the other hand, Kugelberg (1946*a*) found a low value of rheobase (I_{rh}) to be a good indication of hyperexcitability, in the sense of a tendency to discharge ectopically. The single and multiunit rheobases behave somewhat differently, because the single-unit measure is concerned simply with the question of whether the axon discharges or not, while the multiunit measure takes account of repetitive discharges and relates better to the functional consequences of the ectopic firing. The ramp threshold (I_{tri}), which responded to ischaemia in almost exactly the opposite way to I_{th}, may be an appropriate measure of excitability for excitation of nerve by slowly rising generator potentials.

The non-classical divergence between I_{rh} and I_{th} during the post-ischaemic period can be understood on the basis of the revised electrical model of myelinated nerve proposed by Barrett & Barrett (1982) and confirmed by observations on electrotonus in rat nerves (Baker *et al.* 1987). A formulation of this model for human motor axons is presented in the following paper, where computed responses to short and long current pulses at different membrane potentials are illustrated (Fig. 10 in Bostock *et al.* 1991). There is a slow component of electrotonus, due to polarization of the internodal axon membrane by current passing under or through the myelin sheath. The slow electrotonus is enhanced by hyperpolarization, presumably due to the closure of potassium channels in the internodal axolemma (e.g. Fig. 2 in Barrett & Barrett, 1982). Having a high resistance, the hyperpolarized internode can integrate applied depolarizing currents for 100 ms or more, so that a small maintained current can generate impulses at long latency. This interpretation is supported by our observations on the latency changes to rheobasic stimuli (Figs 4 and 5) and the measurements of threshold electrotonus in Fig. 9. The evidence in the following paper (Bostock *et al.* 1991) that raised extracellular potassium levels are important after 15 min of ischaemia suggests that an additional factor may be involved in the negative accommodation. When an axon becomes hyperpolarized relative to the potassium equilibrium potential, activation of slow potassium channels by depolarization should generate an inward current and further depolarization.

Comparison between ischaemia/post-ischaemia and depolarization/hyperpolarization

Some of our observations on excitability and accommodation, particularly those on threshold electrotonus in Fig. 9, appeared to indicate that the effects of ischaemia are indistinguishable from those of applied depolarizing currents, and the after effects of ischaemia indistinguishable from applied hyperpolarizing currents. There were, however, important differences. The most striking was that in ischaemia the threshold to a ramp stimulus (I_{tri}) progressively increased (Figs 2*C*, 5 and 7), whereas it fell during applied depolarizing currents (Fig. 8*D*). Conversely, the post-ischaemic state was better at reducing I_{tri} than applied hyperpolarizing currents. These differences imply that ischaemia does not simply reduce electrogenic pumping at the node, and that following ischaemia there is not simply an increase in nodal pump activity. According to Barrett & Barrett's (1982) electrical model, ischaemia could increase accommodation (and therefore I_{tri} relative to I_{th}) more than applied currents by having a predominantly internodal action: either through blocking a mainly internodal resting pump current, or by causing periaxonal K^+ accumulation under the myelin sheath. Similarly, the observation that release from ischaemia reduces accommodation more than applied currents could be due to stimulation of a predominantly internodal sodium pump. In the model in the following paper the pump is located in the internodal axon (Bostock *et al.* 1991).

The mechanism of post-ischaemic fasciculations

Although we have found striking changes in excitability and accommodation in post-ischaemic nerve, they have not provided an answer to the question: why are longer periods of ischaemia followed by spontaneous fasciculations? After ischaemia limited to 10 min, we have found evidence only of hyperpolarization and, despite the changes in accommodation, no reason to suppose that a greater amount of hyperpolarization should lead to impulse generation. On the other hand, we have found that it is precisely that part of a nerve which shows the greatest evidence of hyperpolarization after 10 min that appears to be the primary source of ectopic impulses after a longer period of ischaemia. A solution to this problem is proposed in the following paper, based on recordings from nerve beneath a pressure cuff, made under conditions found to precipitate post-ischaemic fasciculations.

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