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ACTIVITY-DEPENDENT EXCITABILITY CHANGES IN NORMAL AND DEMYELINATED RAT SPINAL ROOT AXONS

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SUMMARY

1. Myelinated nerve fibres with a reduced safety factor for conduction due to demyelination are easily blocked by trains of impulses. To find out why, *in vivo* recordings from rat ventral root fibres demyelinated with diphtheria toxin have been supplemented with *in vivo* and *in vitro* recordings from normal fibres.

2. Despite a small rise in extracellular potassium activity, normal fibres were invariably hyperpolarized by intermittent trains of impulses. This hyperpolarization resulted in an increase in threshold and also in an enhancement of the depolarizing after-potential and the superexcitable period.

3. Replacement of NaCl in the extracellular solution by LiCl completely blocked both the membrane hyperpolarization and the threshold increase which were normally observed during intermittent trains of impulses.

4. At demyelinated nodes which were blocked by trains of impulses (10–50 Hz), conduction block was preceded by a rise in threshold current and an increase in internodal conduction time, but by no detectable reduction in the outward current generated by the preceding node.

5. It was found possible to prevent the threshold from changing during a train by automatic adjustment of a d.c. polarizing current. This 'threshold clamp' prevented the conduction failure and virtually abolished the changes in internodal conduction time.

6. The threshold changes were attributed to hyperpolarization, as in normal fibres, since (a) the polarizing current required to prevent them was always a depolarizing current, and (b) they were accompanied by an increase in superexcitability.

7. The post-tetanic depression that can follow continuous trains of impulses was attributed to the combination of increased threshold and enhanced superexcitable period due to hyperpolarization.

8. It is concluded that the susceptibility of these demyelinated fibres to impulse trains is not due to a membrane depolarization induced by extracellular potassium accumulation but to a membrane hyperpolarization as a consequence of electrogenic sodium pumping.

INTRODUCTION

One of the characteristics of demyelinated axons is a poor ability to transmit trains of impulses. This phenomenon has been described in experimentally induced demyelination of peripheral nerves (Cragg & Thomas, 1964; Davis, 1972; Lehmann, Lehmann & Tackmann, 1971; Low & McLeod, 1977), spinal roots (Rasminsky & Sears, 1972) and spinal cord (McDonald & Sears, 1970), and is thought to underlie some of the symptoms of human demyelinating diseases (Waxman, 1981). Three quite different mechanisms have been put forward to account for the conduction block produced by impulse activity. Rasminsky & Sears (1972) suggested that the intracellular sodium accumulation at the 'driving node' (i.e. the node preceding the 'blocking node' at which action potentials started to fail) would reduce the sodium equilibrium potential and hence limit the driving current. Brismar (1981), on the other hand, found that voltage-clamped demyelinated nodes had a normal sodium equilibrium potential but increased delayed rectification. He suggested that depolarization due to extracellular potassium accumulation would activate more potassium channels and reduce the excitability of the widened node. A third possibility was mentioned briefly by Rasminsky (1978), that the conduction failure might be related to the changes in excitability seen in normal fibres during repetitive activity, as described by Raymond (1979).

To distinguish between these possibilities we have first determined what changes in membrane potential and electrical excitability occur in normal rat ventral roots subjected to repetitive stimulation, and tested for the contribution of changes in extracellular potassium and calcium activity, a^o_K and a^o_{Ca}, respectively and pH to these changes. Secondly, we have examined sites of activity-dependent conduction block in demyelinated axons *in vivo*, and looked for changes in membrane current, membrane potential and electrical excitability. We have found that the conduction failure is due to a decrease in excitability of the blocking node, rather than to a fall in current from the driving node. The decrease in excitability is due to hyperpolarization, and if this hyperpolarization is counteracted by artificial depolarization the fibres can conduct long trains of impulses without change. A brief report of this result has been published (Bostock & Grafe, 1984).

METHODS

A combination of *in vivo* and *in vitro* techniques were used. Demyelinated fibres were studied *in vivo*, by a technique which enabled recording at a site of impulse-dependent conduction block. To gain insight into the mechanisms involved, the demyelinated fibres were compared with normal ones, which were studied both *in vivo* and *in vitro*. The *in vitro* preparation provided better mechanical stability for intra-axonal recording, and also allowed control over the extracellular ionic environment of the fibres.

Animals

Female Wistar rats weighing 180–250 g were used. Focal demyelinating lesions were produced in the cauda equina by micro-injection of diphtheria toxin (40 Fu./ml, 5–10 μl) between Vaseline seals as previously described (Bostock & Sears, 1978), except that Thiogenal (Merck, 120 mg/kg) proved a much better short-lasting anaesthetic than pentobarbitone. Unoperated controls, or animals injected with diphtheria toxin 7–13 days previously, were anaesthetized with urethane (1·25 g/kg

I.P., supplemented as required) for a laminectomy to expose the cauda equina. Ventral roots were either removed for *in vitro* or left intact for *in vivo* recording.

In vitro methods

The excised ventral root was held in position in a Perspex chamber by two suction electrodes, and strapped to the Sylgard-covered surface by two additional lengths of spinal root (Fig. 1). Attention was paid so that at least 8 mm of both cut ends of the nerve were within the suction electrodes. This procedure reliably resulted in a length of the spinal root between the suction electrodes which was supernormal 10 ms after a control spike. The supernormality of the compound action potential was routinely tested before intracellular recordings were started.

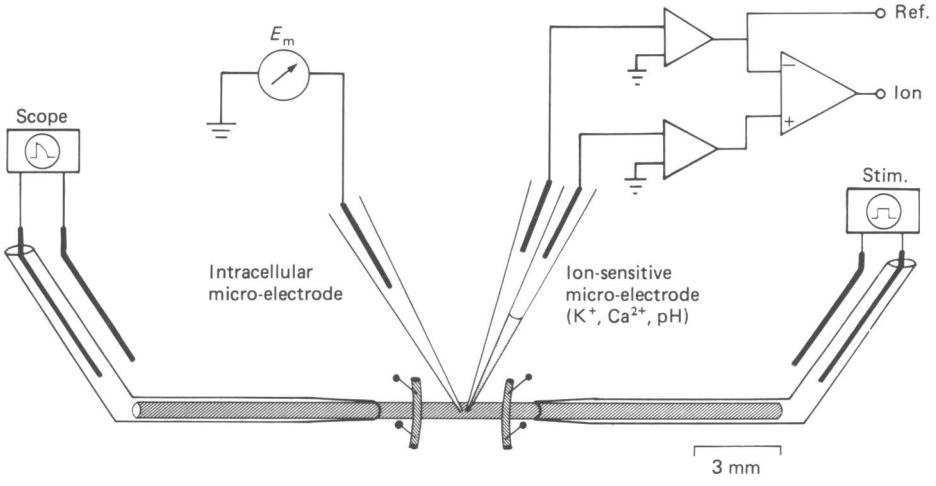

Fig. 1. Scheme of the experimental set-up for *in vitro* recordings, described in the text.

The chamber was continuously perfused with a solution containing (mmol/l): NaCl, 118; KCl, 3·0; $CaCl_2$, 1·5; $NaHCO_3$, 25; NaH_2PO_4, 1·2; $MgCl_2$, 1·0; glucose, 10 (gassed with 95 % O_2–5 % CO_2, pH = 7·4) or test solution at 30 °C. Glass micropipettes, drawn with a Brown–Flaming micropipette puller (Sutter Instrument Co.) and filled with 4 M-potassium acetate were inserted into the root at an oblique angle by a piezo-driven micromanipulator (built by M. Frankenberger). Satisfactory micropipettes had resistances in the range 40–70 MΩ. Intracellular recordings were made by a Dagan 8100 Single Electrode System. In some experiments extracellular recordings with ion-sensitive micro-electrodes were also performed.

Our usual criterion for a good impalement was that the fibre should have a depolarizing after-potential (d.a.p.) of 0·5 mV or more at 10 ms. On withdrawing the electrode such fibres always had resting potentials close to −80 mV. They were also measurably superexcitable at 10 ms; i.e. the threshold for excitation by an applied current pulse was reduced 10 ms after a spike, and the latency of an impulse conducted from one of the suction electrodes was reduced. This criterion of d.a.p. size or the related superexcitability was chosen since normal ventral root fibres *in vivo* are superexcitable, and the lack of a d.a.p. or superexcitability is a sign of depolarization (cf. Eccles & Krnjević, 1959; Barrett & Barrett, 1982). Also, since the lack of a d.a.p. is associated with low input resistance (Barrett & Barrett, 1982), such depolarized fibres could not be expected to show normal electrogenic hyperpolarization.

To measure the latency of an intracellularly or extracellularly recorded action potential and compare it with the latency of a second spike, starting 10 ms later, an analogue latency monitor was constructed. The same 10 V/ms ramp was started by each stimulus trigger, and stopped when the membrane potential reached half-peak amplitude. The potential reached by the ramp was then transferred to one of two storage capacitors for separate potentiometric chart recording of the

latencies of the first and second spike (see Figs. 3, 4 and 5). The threshold to an intracellularly applied current pulse was monitored by another new analogue circuit, which decreased or increased the pulse amplitude depending on whether or not the last pulse elicited a spike.

In vivo methods

The technique for recording from a single node *in vivo* is illustrated in Fig. 2. The methods of membrane current recording and threshold tracking are described in detail in a previous paper (Bostock, Sears & Sherratt, 1983). After opening the dura, the roots were rinsed in a solution containing (mmol/l): NaCl, 147; KCl, 3; $CaCl_2$, 2; $MgCl_2$, 1; glucose, 10, Trizma (Sigma) 5 (gassed with 100% O_2, pH = 7·4) and left immersed in a small volume. A layer of oxygenated liquid paraffin

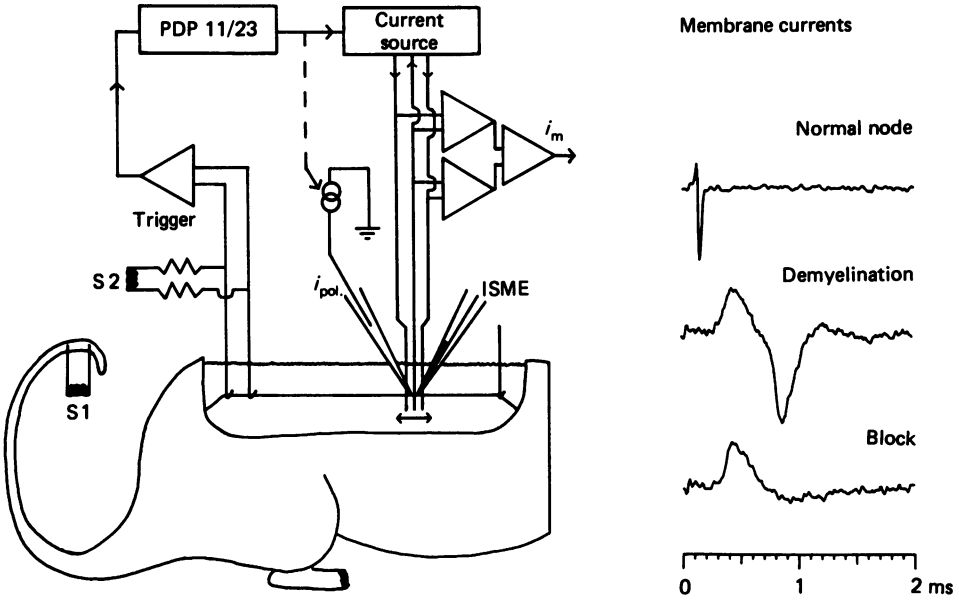

Fig. 2. Simplified diagram of *in vivo* recording arrangement and typical membrane current wave forms used to locate a blocking node. (Circuits for recording membrane current and threshold current are detailed separately in Fig. 1*A* and *B* of Bostock *et al.* 1983.)

was then floated on top, and one of the sacral ventral roots supported in this layer on electrodes. (The root was kept intact, since cutting a root impaired its circulation and led to a slow build up of extracellular potassium and loss of superexcitability.) A single fibre in the root was excited selectively by adjusting a stimulus S1 to needle electrodes in the tail, and looking for an all-or-none response at the electrodes connected to the 'trigger' amplifier. This signal was used to trigger a signal averager, used for recording membrane currents (i_m) from the tripole. The membrane current signal was used to locate the tripole over a normal or demyelinated node, the latter identified by a long delay between outward and inward current phases (see Fig. 2 and Rasminsky & Sears, 1972). To test if conduction would fail at the demyelinated node, the root was tetanized by an isolated stimulator (S2) connected to the 'trigger' electrodes via 1 MΩ resistors. Conduction failure at the node under the tripole was signalled by failure of the inward current, while the outward current due to activity at the preceding node persisted (Fig. 2). Internodal conduction time was measured by an analogue circuit as the time between outward and inward current phases. Failure of the inward current did not alter the conduction time recorded, but was registered separately by the LSI 11/23 computer used as a multichannel digital recorder. Each channel was normally filtered with a 0·5 s time constant and sampled at 1 Hz.

The excitability of the node under the tripole was tested as previously described (Bostock *et al.* 1983) by computer control of a brief (10–50 μs) tripolar current stimulus, which was decreased or increased depending on whether or not it caused an impulse to collide with the one excited by S1. The facility to alternate two different threshold measurements was used in some experiments to monitor threshold current 10 ms after a spike, as well as at the normal interval of at least 125 ms.

Polarizing currents were applied via a Ringer-filled glass micropipette (labelled 'i_{pol}' in Fig. 2) touching the root opposite the centre of the tripole. For 'threshold-clamp' operation the tripolar stimulus amplitude was kept constant and a d.c. polarizing current incremented or decremented instead. In some experiments an ion-sensitive micro-electrode (ISME) was inserted into the root opposite the centre of the tripole.

Ion-sensitive micro-electrodes

The method used for the construction of the double-barrelled ion-sensitive micro-electrodes has been described elsewhere (Grafe, Rimpel, Reddy & ten Bruggencate, 1982). For the present study, theta-capillaries with tip-diameters of 1·5–2 μm were constructed. The Corning ion-exchanger resin 477317 and the Ca^{2+}- and proton-cocktails from Fluka (21048, 82500) were used to measure the extracellular potassium and calcium activities and the extracellular pH, respectively. An activity coefficient of 0·74 was used to calculate the extracellular potassium activity (a^{o}_{K}) (Meier, Ammann, Morf & Simon, 1980).

RESULTS

A. Effects of impulse trains on normal fibres

Impulse activity is known to affect the excitability of a normal fibre in two conflicting ways. Following the refractory period after each impulse there is a period of increased excitability and conduction velocity, the supernormal period (Adrian & Lucas, 1912), associated with a negative (depolarizing) after-potential (Gasser & Erlanger, 1930). Following a large number of impulses, on the other hand, there is a much longer-lasting period of reduced excitability and conduction velocity, associated with a positive (hyperpolarizing) after-potential (Gasser, 1935). The development of the hyperpolarization can be followed without interference from the supernormal period by regularly interrupting the impulse train for periods longer than the supernormal period. Using such intermittent stimulation, Connelly (1959) obtained evidence that the hyperpolarization is due to an electrogenic sodium/potassium pump, responding mainly to the rise in intracellular sodium concentration close to the nodes. In most experiments we have also used intermittent trains, but where possible we have in addition measured excitability or membrane potential 10 ms after an impulse, to follow changes in the supernormal period or underlying d.a.p. respectively. The changes in supernormal period will be used to make inferences about the membrane potential (see Discussion), and they help explain the effects of continuous impulse trains described in section *B*(iii).

(*i*) *In vitro experiments.* Some effects of tetanizing all the A fibres in a rat ventral root are illustrated in Fig. 3. The extracellularly recorded potassium activity (Fig. 3*A*) increased a small amount during the tetanus, but despite this the fibre impaled with a micro-electrode hyperpolarized (Fig. 3*B*). The hyperpolarization outlasted the tetanus by several minutes, and its decay paralleled an undershoot in the potassium activity, consistent with increased activity of an electrogenic sodium/potassium pump caused by elevated intracellular sodium (cf. Ballanyi, Grafe & ten Bruggencate, 1983).

Hyperpolarization reduced the excitability of the fibre as shown by the parallel

increase in latency to the first of the regular test stimuli (Fig. 3*C*: control). The d.a.p. increased in amplitude and duration during the tetanus (Fig. 3*E*), and its amplitude at 10 ms also followed the hyperpolarization (Fig. 3*D*, see also Fig. 8 in Eccles & Krnjević, 1959). As in the frog and lizard fibres described by Barrett & Barrett (1982), the d.a.p. showed no dependence on extracellular potassium, since it increased both

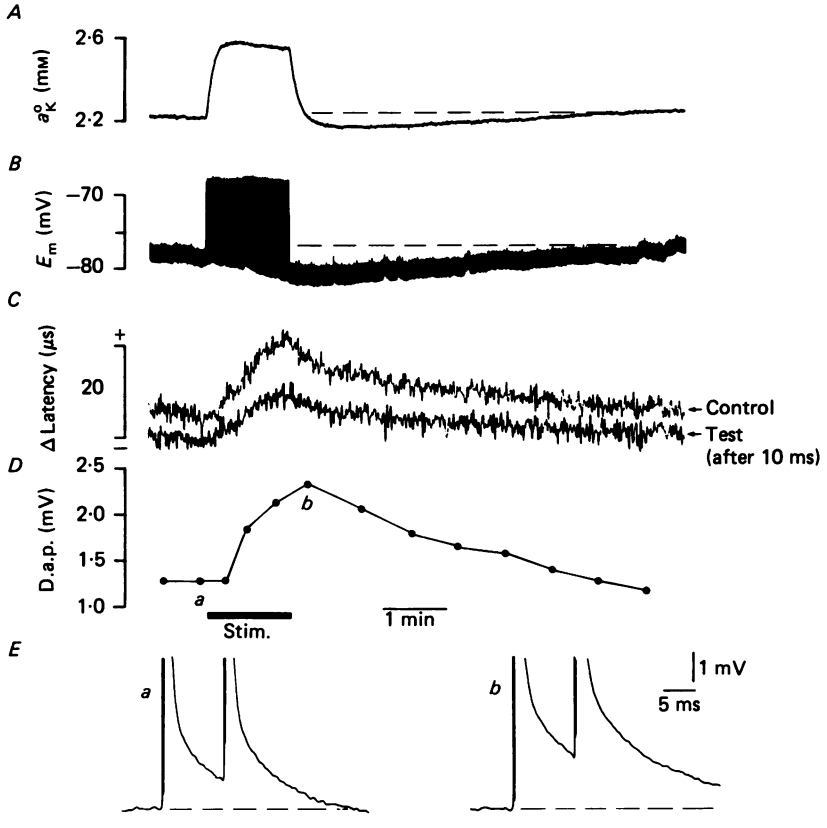

Fig. 3. Effects of repetitive stimulation on normal rat ventral root axon *in vitro*. *A*, a^o_K measured close to intracellular micro-electrode used to record membrane potential (E_m; *B*). *C*, times between stimulus and intracellularly recorded spikes, recorded with latency monitor. Control spikes elicited every second, test spikes 10 ms later. *D*, amplitude of d.a.p. 10 ms after control spike (mean of ten). *E*, after-potential wave forms (mean of ten sweeps), before and after stimulation. During period indicated by bar, each test stimulus was followed by 400 ms, 200 Hz tetanus (mean rate 80 Hz).

in high and low potassium. Supernormality at the same time was indicated by the slightly shorter latency to the second of the regular test stimuli (Fig. 3), and this latency difference increased in parallel with the d.a.p. and the hyperpolarization.

In addition to the measurements of a^o_K, we have also looked for changes in a^o_{Ca} and pH during tetanization, since these ions also have marked effects on electrical excitability. No changes could be detected in a^o_{Ca} (three roots) or pH (three roots) during stimulus trains of 300 Hz for up to 2 min (e.g. Fig. 4). Furthermore, to estimate

the functional importance of possible changes of a^{o}_{Ca} and pH_o, which might occur in regions not accessible for the ion-sensitive micro-electrodes, we have explored passive activity changes of these ions. Such experiments revealed that an increase of the calcium concentration in the bathing solution by 0·5 mmol/l or a decrease of pH_o from 7·5 to 7·0 could increase the conduction time of the fibres similar to a train of about 100 Hz for 30–60 s. However, these threshold changes were not accompanied by an

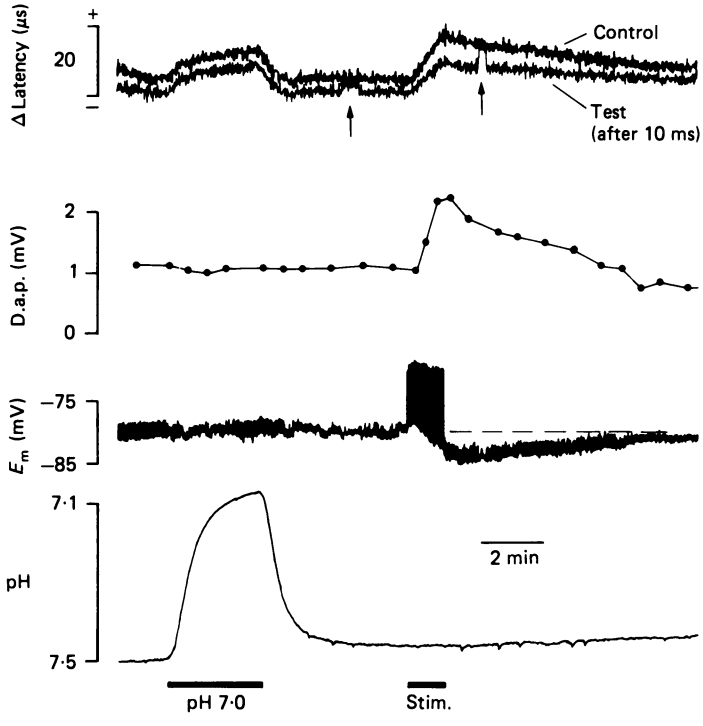

Fig. 4. Effects of changes in extracellular pH on normal ventral root axon *in vitro*. Traces labelled as in Fig. 3, but pH recorded with pH-sensitive micro-electrode. Effect of pH change in bathing solution (by addition of HCl) compared with an intermittent tetanus (mean stimulation rate 80 Hz). In the top trace, arrows indicate times when the interval between control and test stimuli was increased to 100 ms.

effect on membrane potential, supernormality or the d.a.p. (as an example see Fig. 4). These observations are consistent with the close association of the supernormality with membrane potential (Barrett & Barrett, 1982) and with the effect of a^{o}_{Ca} on the excitability of cerebellar parallel fibres as measured by Malenka, Kocsis & Waxman (1983).

In another series of experiments we have attempted to distinguish between the membrane hyperpolarization and an increase of intracellular Na^+ as the mechanism underlying the threshold increase during train of impulses. In such experiments extracellular NaCl was completely replaced by LiCl. Li^+ is known to be able to substitute for Na^+ as the charge carrier for the inward current during the action potential. On the other hand, in contrast to Na^+, an increase of intracellular

lithium activity (a^{i}_{Li}) does not stimulate the electrogenic sodium pump (Thomas, 1972). Consequently, Li^+ solutions should be able to separate between effects due to a decrease of the driving force for the inward current (intracellular Li^+ accumulation) and effects due to the pump activation (Schoepfle & Katholi, 1973). A short application time of Li^+ (*ca.* 4 min) was used to prevent indirect effects of Li^+, such as an intracellular K^+ depletion and a membrane depolarization which occur after longer exposures to Li^+ solutions (Grafe, Reddy, Emmert & ten Bruggencate, 1983). A typical example is illustrated in Fig. 5. In the normal extracellular solution an

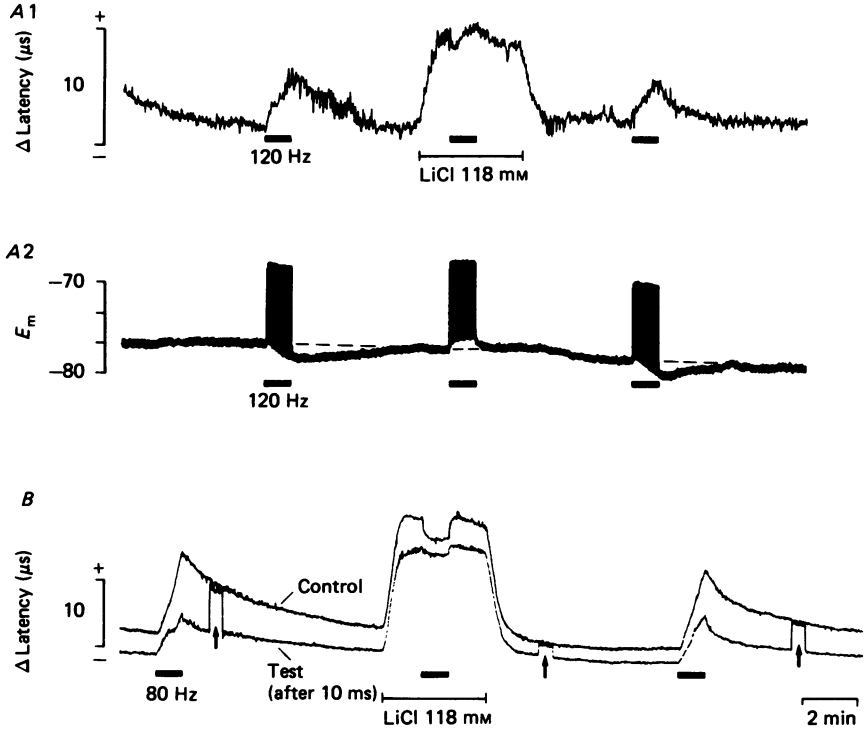

Fig. 5. Effects of Li^+ on response of normal fibres to repetitive stimulation *in vitro*. *A*, intracellularly recorded latency changes (*A*1) and membrane potential changes (*A*2) during intermittent tetanization (300 Hz for 400 ms/s), with and without complete replacement of extracellular NaCl by LiCl. *B*, latency measurements on compound action potential during a similar experiment to show the effects on supernormality. (Arrows indicate 100 ms interstimulus intervals, as in Fig. 4.)

increase in the latency of the intracellularly recorded action potential correlated well with the membrane hyperpolarization during and after an intermittent train of impulses (Fig. 5*A*). In the presence of Li^+, however, a complete block of the membrane hyperpolarization occurred. Instead a small *depolarization* and a *decrease* of the threshold were seen during the stimulation period. The shift in the base line of the latency recording in the presence of Li^+ is probably due to the permeability ratio for Na^+ and Li^+ in the sodium channel (in frog node P_{Li}/P_{Na} 0·9 : 1; Hille, 1972).

Extracellular compound action potentials were used to measure the changes in the

supernormality during trains in Na^+ and Li^+ solutions (Fig. 5*B*). We observed that the prolonged increase of the supernormality seen during an intermittent train of impulses in normal solutions reversed to a transient *diminution* of supernormality in Li^+ solutions. This corresponds well to the small membrane depolarization recorded intracellularly (Fig. 5*A*2). (The cause of this transient depolarization in LiCl is not known, but its time course is similar to that of extracellular potassium accumulation (Fig. 3*A*). It may be present in NaCl, but masked by the electrogenic hyperpolarization.) The experiments with Li^+ solutions show that the threshold increases caused by train of impulses are related to a rise in intracellular Na^+, not because the driving force for inward current is reduced, but because of the pump-induced hyperpolarization.

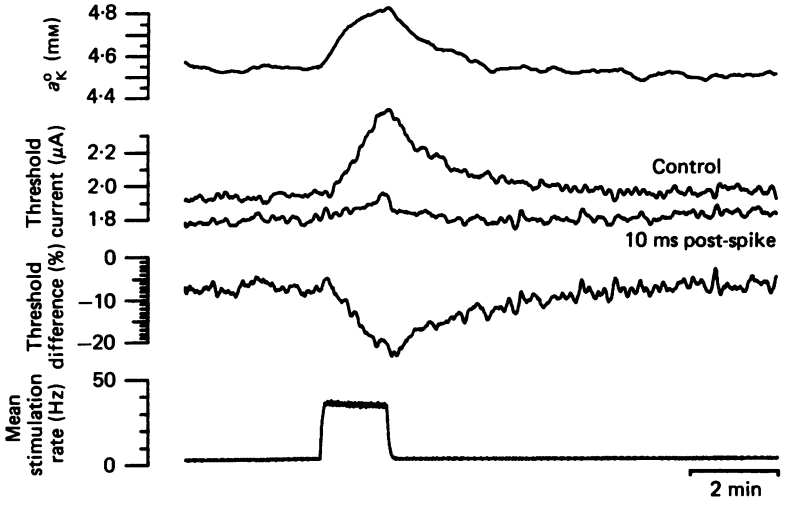

Fig. 6. Effect of repetitive stimulation on node in normal ventral root axon *in vivo*. a_K^o recorded with ISME in root close to node. Threshold current tested every 900 ms, alternately with and without conditioning impulse 10 ms before. Stimulus train was 80 Hz tetanus of all Aα fibres, applied for 20 ms every 450 ms.

(*ii*) *In vivo experiments.* Fig. 6 resembles Fig. 3, except that only extracellular measurements were made from the *in vivo* preparation. The threshold current required to excite a particular node was used as a measure of excitability, and the supernormal period was indicated by the percentage change in threshold current 10 ms after an impulse (i.e. the superexcitability). Extracellular potassium activity recovered more slowly than it did *in vitro*, and did not always show an undershoot, since lower frequencies of stimulation were usually used in the *in vivo* experiments. a_K^o undershoots *in vitro* or *in vivo* are most prominent after long trains of impulses and high frequencies. Despite the rise in potassium, the threshold current and the superexcitability increased, consistent with the hyperpolarization recorded directly *in vitro*.

B. Recordings from demyelinated fibres in vivo

(*i*) *Effects of impulse trains on excitability and superexcitability.* Changes in threshold current and superexcitability were recorded at eight nodes which were blocked by trains of impulses with a mean frequency of 50 Hz or less. In each case there was a substantial rise in threshold current and latency before conduction failed. In seven cases there was also a clear increase in superexcitability (e.g. Fig. 7). (In the exceptional fibre, superexcitability was high at rest and did not change significantly with the 10 Hz train that was sufficient to block conduction.) In Fig. 7 the

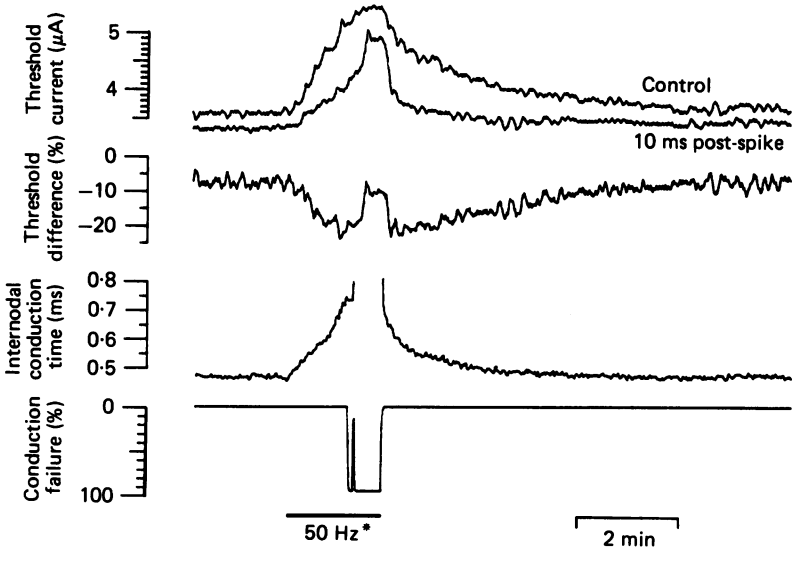

Fig. 7. Effects of repetitive stimulation on demyelinated node, showing threshold changes preceding conduction failure. Demyelination caused by injection of diphtheria toxin, 13 days previously. Internodal conduction time measured between outward and inward current peaks at test node. Failure of inward current and impulse transmission indicated by break in conduction time record, and separate conduction failure trace. (Asterisk after stimulation rate signifies mean rate of an intermittent tetanus in this and the next Figure.)

superexcitability increased rapidly from about 7% at rest to 22% with the 50 Hz intermittent tetanus, consistent with hyperpolarization of the fibre as in Fig. 6. When conduction (of the control and conditioning impulses) failed, superexcitability was abruptly reduced. It was not reduced to zero, probably because of electrotonic spread of the d.a.p. from the preceding node, which would still have been excited by the conditioning impulse.

(*ii*) *Polarizing currents and 'threshold clamp'.* The increase in superexcitability occurring at demyelinated nodes before they block, provides good indirect evidence that they are hyperpolarizing (see Discussion). A more direct approach to this crucial question was opened up by the use of polarizing currents. According to Brismar's (1981) hypothesis concerning the role of potassium channels in the conduction failure, depolarization of these demyelinated fibres should have increased the threshold

current for spike initiation. This was never found, and an example of the contrary is shown in Fig. 8*A*. Application of 50 nA hyperpolarizing current to the root opposite the affected node caused an increase in threshold that blocked conduction, whereas 50 nA depolarizing current reduced the threshold current and internodal conduction time. Block of this fibre by a 20 Hz tetanus is shown in Fig. 8*B*.

To see how much the changes in conduction could be accounted for by the reduction in excitability of the blocking node, we then switched the ouput of the threshold hunter to the polarizing circuit. The threshold current was thus clamped to its resting value by depolarizing or hyperpolarizing the node. Under this 'threshold clamp' condition, the effects of the 20 Hz tetanus were offset by a depolarizing current with

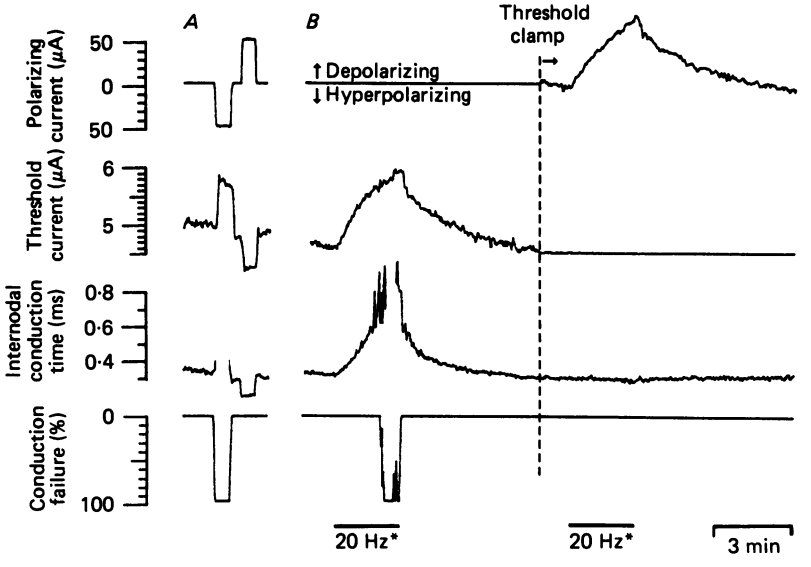

Fig. 8. Polarizing current and conduction failure (10 day diphtheria toxin lesion). *A*, effect of small polarizing currents on demyelinated node. Hyperpolarization causes instant conduction block. *B*, effect of 20 Hz intermittent tetanus on same node. After control tetanus without polarization, polarizing current was set by threshold hunter in 'threshold-clamp' configuration (see text).

a time course similar to that of the unclamped threshold changes (Fig. 8*B*). As a result, changes in internodal conduction time and the conduction failure were effectively suppressed, demonstrating that they were primarily due to the changes in excitability of the blocking node. We have clamped six other nodes which were otherwise blocked by stimulation at between 10 and 50 Hz, with similar results (e.g. Fig. 1 in Bostock & Grafe, 1984).

(*iii*) *Continuous impulse trains and post-tetanic conduction block.* So far the results have been obtained with intermittent impulse trains to permit separate recording of the slow development of depression and of superexcitability. With continuous trains more complicated threshold trajectories are seen, because of the interaction between these factors. A series of such curves from a normal frog fibre has been presented by

Raymond (1979). A consistent feature is a rapid rise in threshold at the end of the tetanus when the interspike interval suddenly becomes much longer than the supernormal period. In demyelinated fibres a further complication arises if conduction block occurs during the train, since that reduces the superexcitability for the next impulse (see *B*(i) above). Intermittent conduction failure may result, as has been reported at other regions of low safety factor for conduction (see discussion by Raymond & Lettvin, 1978). Unfortunately, it is not possible to monitor threshold

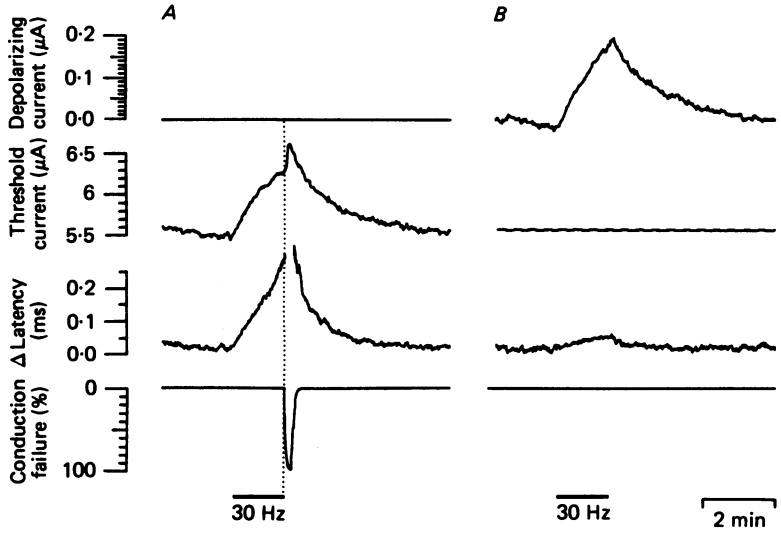

Fig. 9. Post-tetanic conduction block after continuous impulse train (8 day diphtheria toxin lesion). *A*, effect of 30 Hz continuous stimulation on demyelinated node. Dotted line marks end of tetanus to show clearly the timing of the post-tetanic threshold increase and conduction failure. *B*, same tetanus applied under 'threshold clamp'.

changes faithfully during uniform impulse trains, since the threshold measurements inevitably interfere with the timing of the impulses. An experiment in which the threshold measurements interfered minimally is shown in Fig. 9*A*. Stimulation was at a rate just insufficient to cause conduction failure during the train. Release of superexcitability at the end of the train caused a sharp rise in threshold, which resulted in a short-lived but complete post-tetanic conduction block. In Fig. 9*B* the same continuous tetanus was applied under threshold clamp, which prevented the hyperpolarization and enhanced supernormal period. As a result the clamp showed the same simple time course as with intermittent stimulation.

(*iv*) *Driving current and conduction failure.* Rasminsky & Sears (1972) described a fibre in which the longitudinal current driving the node at which conduction block eventually occurred decreased progressively during a tetanus, in parallel with an increase in internodal conduction time. We have entirely failed to reproduce this result. At none of the eleven blocking nodes examined in detail was there a noticeable reduction in the driving (outward) current from the preceding node. In four experiments we made recordings of the membrane currents at the blocking node and

one of these is illustrated in Fig. 10. On the right are plotted examples of the membrane current records, each an average of eight traces to improve the signal-to-noise ratio. The early outward current peak due to the activity of the preceding node remained constant, while the inward current appeared later and later and then disappeared. The time courses of these events are plotted on the left, with the outward current peaks, measured on each average, at the top.

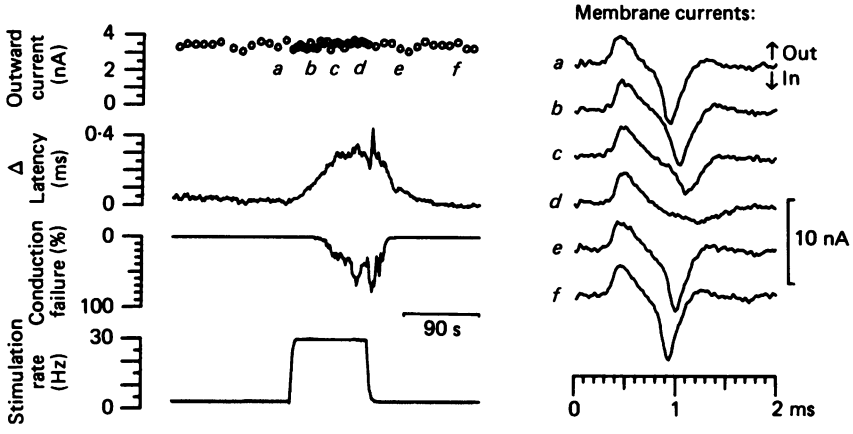

Fig. 10. Driving current at a blocking node. Right-hand side: average membrane current wave forms ($n = 8$) recorded at a demyelinated node before, during and after stimulation to the point of intermittent conduction failure. Amplitudes of the early outward current, corresponding to driving current from preceding node, are plotted at top left. No relation to stimulation or conduction failure is evident.

DISCUSSION

The term 'safety factor' was applied to conduction in myelinated nerve by Tasaki (1959), who defined it as the ratio between the current available to excite a node (i.e. the driving current), and the threshold current required to excite it. Demyelination often reduces the safety factor to a value close to unity, so that some fibres are blocked and some fibres conduct very slowly, and treatments that produce small changes in safety factor can block or unblock large numbers of fibres (Schauf & Davis, 1974). One treatment known to reduce safety factor is the conduction of a long train of impulses, and this is thought to underlie some symptoms of demyelinating disease in humans such as the 'fading out' of vision that some patients experience after fixating on a target continuously for a period of seconds (Waxman, 1981).

In principle this further reduction of an already reduced safety factor could arise in two ways, by a fall in the driving current and/or by a rise in the threshold current. We have described two types of experiments which indicate that only a rise in threshold is important. First, preventing the change in excitability at a blocking node by the 'threshold-clamp' technique also prevents both conduction failure and the increase in internodal conduction time that normally precedes conduction failure (e.g.

Figs. 8 and 9). Secondly, the early outward current recorded at a blocking node, which corresponds to the driving current, does not change significantly during a tetanus that blocks conduction (e.g. Fig. 10). Since we never recorded a drop in driving current, as was described in a demyelinated fibre by Rasminsky & Sears (1972). We cannot be sure of the mechanism in that case. It is perhaps significant that although their fibre had an internodal conduction time of 700 μs at rest, it was not blocked by stimulation at 60 Hz for over 8 min. In our experience a fibre with such a conduction delay should be blocked more quickly and by lower frequencies. We have found that cutting the spinal roots, as done by Rasminsky & Sears (1972) and in some preliminary experiments, led to potassium accumulation (recorded with an ion-sensitive micro-electode) and depolarization of the fibre (as indicated by a loss of superexcitability, see below). When this occurred, the fibres were much more resistant to impulse trains.

Several possible mechanisms might underlie the increase in threshold current at the blocking node. These factors are (a) a membrane depolarization, (b) a membrane hyperpolarization and (c) an accumulation of intracellular sodium. Brismar (1981) suggested that a potassium-induced depolarization might be important. Also Kocsis, Malenka & Waxman (1983) recorded a reduction in excitability (which could lead to conduction block) of cerebellar parallel fibres stimulated at 50 Hz, which they attributed to a rise in extracellular potassium. This could not have been the case for our fibres, since depolarization overcame the conduction block (e.g. Figs 8 and 9).

For an alternative cause of the threshold increase a much earlier model is available. Gasser (1935) observed that repetitive stimulation of normal frog nerve produced a long-lasting hyperpolarization, which was accompanied by a decrease in excitability. For normal fibres we could confirm this observation by direct intra-axonal recordings (Fig. 3). For the demyelinated fibres, on the other hand, it was not practicable to obtain evidence by use of intracellular recordings that the threshold increases responsible for conduction failure were also due to hyperpolarization. However, indirect evidence that hyperpolarization was important was obtained from measurements of superexcitability. The relation between threshold current and membrane potential is U shaped (Fig. 11*A*), strong enough hyperpolarizations as well as depolarizations can result in a conduction block. However, the relation between superexcitability and membrane potential is monotonic (Fig. 11*B* and *C*), only hyperpolarization resulting in an increase in superexcitability (see also Bergmans (1982) for single human fibres; according to Barrett & Barrett's (1982) model, the relation arises from the potential dependence of the input resistance of the fibres). We therefore consider that the increase in superexcitability seen at demyelinated nodes before block (e.g. Fig. 7) is good evidence that they were hyperpolarizing. This conclusion is also confirmed by the fact that depolarization during 'threshold clamp' overcame the conduction block.

According to Connelly (1959), who used intermittent stimulation to follow the potential of an isolated frog nerve during a train, the hyperpolarization is due to an electrogenic sodium/potassium pump. This conclusion was later confirmed by Schoepfle & Katholi (1973), who also used Li^+ solutions to block the membrane hyperpolarization. However, this view has been challenged recently by Bergman, Dubois & Bergman (1980) who claimed that the post-tetanic hyperpolarization,

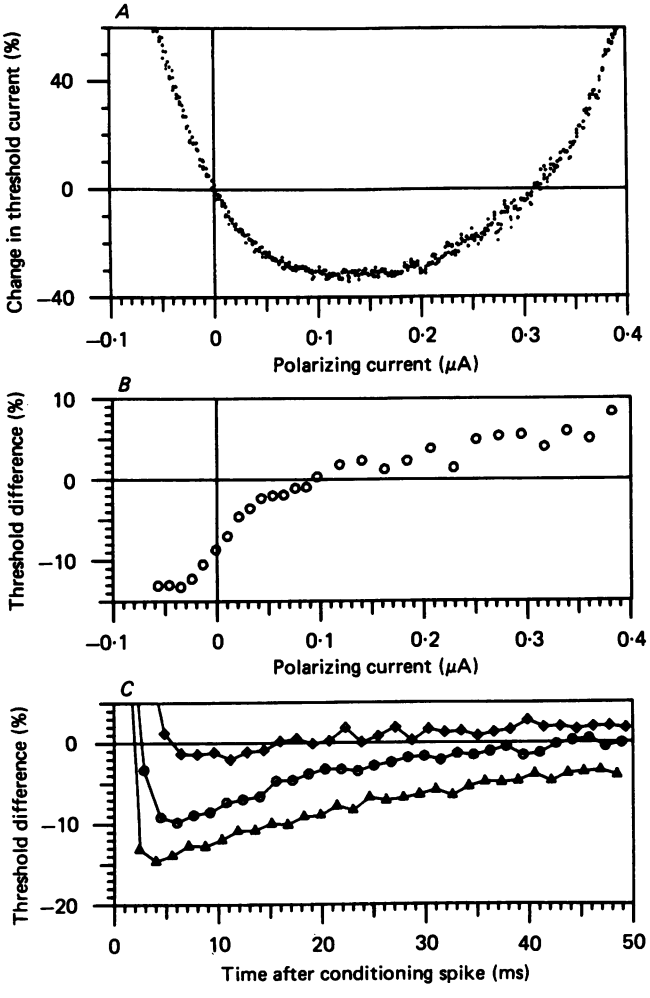

Fig. 11. Effects of polarization on excitability and superexcitability of normal node *in vivo*. *A*, percentage change in threshold current as a function of polarizing current (depolarizing current positive). (NB: to avoid potassium accumulation, threshold was measured near end of 100 ms polarizing pulse, and depolarizing pulses alternated with hyperpolarizing pulses of same amplitude.) *B*, superexcitability as a function of polarizing current. Percentage difference between threshold current with and without conditioning spike 10 ms previously. *C*, effect of small polarizing currents on recovery curve of same node. ○, percentage change in threshold current at different times after conditioning spike. △, 0·05 μA hyperpolarization. ◇, 0·05 μA depolarization.

though related to the sodium pump, is not due to the pump being electrogenic but due to extracellular potassium depletion. We do not think that their interpretation is correct, at least for rat fibres, since intermittent stimuli clearly show the onset of the hyperpolarization at a time of elevated extracellular potassium activity (Fig. 3).

A third factor contributing to the threshold increase might be a reduction in the sodium equilibrium potential due to the rise in intracellular sodium. Koles &

Rasminsky (1972) used a computer model to show that a small increase in intracellular sodium could block conduction in a fibre with its safety factor critically reduced by demyelination. Their model did not, however, take account of the hyperpolarization that the sodium increase would produce by stimulation of the electrogenic sodium pump. If reduction in the sodium equilibrium potential was the principal cause of the threshold increase, then the reduction in sodium current would be expected to reduce the d.a.p. (Barrett & Barrett, 1982) and hence the superexcitability, but this never happened. Furthermore, our experiments in Li^+ solutions argue, at least for normal fibres, against a significant contribution of a fall in sodium equilibrium potential to the threshold increase (see Fig. 5).

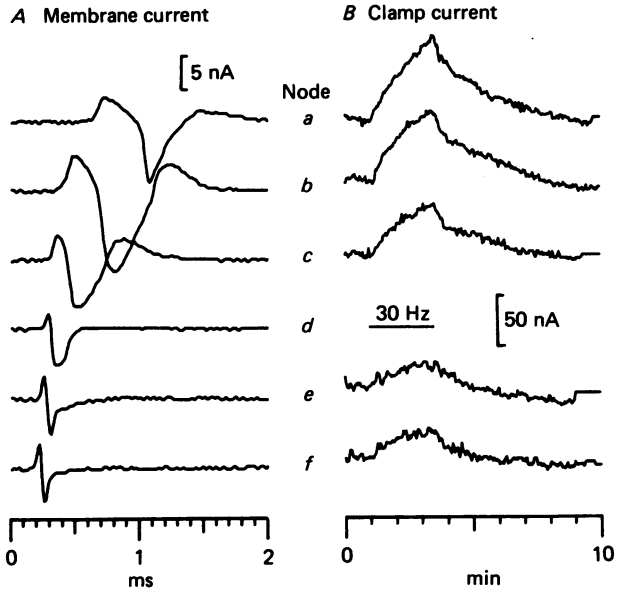

Fig. 12. Comparison between membrane currents and 'threshold-clamp' currents at different nodes in the same fibre. *A*, averaged membrane current ($n = 16$) at six adjacent nodes in a fibre exposed to diphtheria toxin (10 day lesion). *B*, depolarizing current required to prevent threshold changes at five of these nodes when subjected to similar 30 Hz tetani.

The 'threshold-clamp' technique described in this paper not only provides evidence for the importance of electrogenic hyperpolarization in these fibres, it also provides an extracellular means of quantifying pump activity. If, as we have argued, the pump current is the main factor causing the threshold increase during and after an intermittent tetanus, then the threshold clamp current should be related to the pump current simply by the short-circuit factor. Our general impression that the electrogenic pump was functioning at least as well in the demyelinated as in the normal fibres was strengthened by one fibre in which we were able to clamp five nodes (Fig. 12). Similar 30 Hz tetani required more clamp current to prevent threshold changes at the three nodes showing slow saltatory conduction and enhanced inward current generation, than at the two relatively normal nodes. These observations, together

with the inference from superexcitability measurements that resting potentials were normal in the demyelinated fibres, indicate that the damage to the paranodal apparatus occurring with demyelination does not impair sodium transport. This conclusion was also reached by Brismar (1981) on the basis of the normal sodium equilibrium potentials found in voltage-clamped demyelinated fibres.

In conclusion, our data reveal that demyelinated rat spinal root fibres fail to transmit long-lasting trains of impulses because of membrane hyperpolarization. We think it likely that the same mechanism is involved in demyelinating lesions in man, whether in the peripheral or central nervous systems, where conduction failure follows impulse activity. Only in ischaemic conditions, or where sodium pump activity may be compromised for other reasons, would the opposite mechanism of a depolarizing block be likely. Such a situation might be revealed by the absence of a superexcitable or supernormal period in the conducting fibres. Our results also have implications for the search for a symptomatic treatment of demyelinating disease by modifying nerve conduction (Schauf & Davis, 1974; Bostock, Sherratt & Sears, 1978; Davis & Schauf, 1981). It was shown, for example that prolonging action potential duration by altering sodium inactivation could improve the safety factor and overcome conduction block due to demyelination (Bostock *et al.* 1978). The extra sodium influx and electrogenic pump activity incurred may, however, produce a net reduction in safety factor at physiologically useful impulse rates. Interference with the sodium pump may provide temporary relief from impulse-dependent conduction failure, but is unlikely to provide a satisfactory basis for treatment.

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