Excitability changes in human sensory and motor axons during hyperventilation and ischaemia

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**Summary**

This study was undertaken to compare the excitability changes of sensory and motor axons during hyperventilation and ischaemia, and to determine why ectopic impulse activity develops more readily during hyperventilation, and in sensory fibres. During hyperventilation for 20 min, all six subjects reported paraesthesiae in the hand and face, and four out of the six developed muscle twitching and cramps, associated with significant decreases of 20–30% in the threshold current required to produce sensory and motor potentials of constant size. During ischaemia four out of the six subjects reported paraesthesiae, but none reported muscle twitching. There were significant decreases of 15–20% in threshold for sensory and motor fibres. Ischaemia produced a marked decrease in supernormality, an increase in refractoriness and an increase in latency of the test compound sensory or motor potential, changes that were not seen with hyperventilation. The decrease in threshold during these manoeuvres was associated with a significant increase in strength–duration time constant (τ_{SD}), indicating a relatively greater decrease in rheobase current. Using the technique of latent addition, we found that the changes in τ_{SD} were consistent with a recently proposed model in which non-inactivating, voltage-dependent ‘threshold channels’ (presumably persistent Na\(^+\) channels) develop more readily during hyperventilation, and in sensory fibres. During hyperventilation for 20 min, all six subjects reported paraesthesiae in the hand and face, and four out of the six developed muscle twitching and cramps, associated with significant decreases of 20–30% in the threshold current required to produce sensory and motor potentials of constant size. During ischaemia four out of the six subjects reported paraesthesiae, but none reported muscle twitching. There were significant decreases of 15–20% in threshold for sensory and motor fibres. Ischaemia produced a marked decrease in supernormality, an increase in refractoriness and an increase in latency of the test compound sensory or motor potential, changes that were not seen with hyperventilation. The decrease in threshold during these manoeuvres was associated with a significant increase in strength–duration time constant (τ_{SD}), indicating a relatively greater decrease in rheobase current. Using the technique of latent addition, we found that the changes in τ_{SD} were consistent with a recently proposed model in which non-inactivating, voltage-dependent ‘threshold channels’ (presumably persistent Na\(^+\) channels) are active at resting potential. The failure of hyperventilation to alter conduction velocity, refractoriness or supernormality appreciably indicates that, unlike ischaemic depolarization, hyperventilation does not increase inactivation of conventional Na\(^+\) channels or activation of K\(^+\) channels, and this implies that the hyperventilation-induced increase in excitability is not the result of conventional depolarization, as seems to occur during ischaemia. These results suggest that hyperventilation has a rather selective action on the threshold channels, and they help to explain its greater effectiveness compared with ischaemia in provoking ectopic discharges. The greater expression of threshold channels in sensory than in motor fibres can explain why hyperventilation induces paraesthesiae before fasciculation and why only paraesthesiae occur during ischaemia.

**Keywords**: excitability; sodium channels; refractoriness; supernormality; hyperventilation; ischaemia

**Abbreviations**: CMAP = compound muscle action potential; CSAP = compound sensory action potential; \(I_{th}\) = rheobase; \(\tau_{SD}\) = strength–duration time constant

**Introduction**

When human peripheral nerves are made ischaemic, e.g. by inflating a pressure cuff around the upper arm, most normal subjects experience paraesthesiae (Poole, 1956), due to ectopic discharges in the affected cutaneous afferents (Ochoa and Torebjörk, 1980), but fasciculation occurs only rarely. In a previous study it was found that the changes in excitability of motor and sensory fibres (as tested with 0.1-ms current pulses) were different after ischaemia for ≥5 min, but the changes during ischaemia were very similar (Bostock et al., 1994). Moreover, their accommodation to subthreshold depolarizing currents, thought to reflect the action of axonal K\(^+\) channels, was similar, so that the biophysical basis for the greater tendency for sensory fibres to discharge ectopically during ischaemia could not be explained. Since then, human sensory and motor axons have been shown to differ in excitability in other ways. Sensory fibres have significantly
longer strength–duration time constants than motor fibres (Panizza et al., 1994; Bostock and Rothwell, 1997; Mogyoros et al., 1996) and their rheobase ($I_{rh}$) is estimated to be substantially lower (Mogyoros et al., 1996), even though the diameters and conduction velocities of the most excitable motor and sensory fibres are similar.

An explanation for these differences in excitability between sensory and motor axons was recently proposed on the basis of measurements of ‘latent addition’, i.e. the changes in excitability produced by brief (60 µs) depolarizing and hyperpolarizing current pulses (Bostock and Rothwell, 1997). A slow voltage-dependent component in the recovery from hyperpolarizing pulses was attributed to the presence of ‘threshold channels’, analogous to the persistent Na⁺ channels described in some other excitable cells (Alzheimer et al., 1993; de Schutter and Bower, 1994). These non-classical ion channels were inferred to be active at the resting potential and expressed more in sensory than motor fibres. Computer simulation provided evidence that differences in the expression of threshold channels could account satisfactorily for the sensory/motor differences in strength–duration time constant ($\tau_{SD}$) and $I_{rh}$, as well as those in latent addition. It was further suggested that they might be responsible for the greater tendency of depolarized sensory fibres to discharge repetitively.

Paraesthesiae are common in many neuropathies but fasciculation is rare. Conversely, in multifocal motor neuropathy with or without conduction block, involvement of sensory fibres is unusual. A consequence of the difference in ion channel expression discussed above would be that sensory axons might be less susceptible to conduction block than motor axons, but more likely to become ectopically active. This study was undertaken to compare the excitability changes in sensory and motor axons during ischaemia and during hyperventilation. The results provide evidence that threshold channels are activated similarly in both forms of axonal hyperexcitability but that, with hyperventilation, this is achieved without membrane depolarization or significant effects on other ion channels. Given this, the differential distribution of threshold channels can explain why paraesthesiae occur without fasciculation during ischaemia and before fasciculation during hyperventilation.

**Methods**

Four sets of experiments were conducted on six normal subjects (three male, three female, aged 20–51 years) with no clinical or neurophysiological evidence of a peripheral nerve disorder. All subjects gave informed consent to the experimental procedures which had the approval of the Committee of Experimental Procedures Involving Human Subjects, University of South Wales.

In two sets of experiments the changes in excitability, supernormality, $\tau_{SD}$ and $I_{rh}$ were followed during hyperventilation for 20 min or during ischaemia for 10 min. In the hyperventilation experiments, end-tidal pCO₂ was recorded continuously via a nasal catheter, and was displayed on the computer using an analogue channel. Ischaemia was produced by inflation of a sphygmomanometer cuff (5 cm diameter) to 200 mmHg. The cuff was just proximal to the wrist so that the compression was applied at the site of nerve stimulation (see below), and excitability was tested at the site of expected maximal change (Bostock et al., 1991).

The excitability changes were determined using the technique of threshold tracking, as described by Bostock et al. (1994). The median nerve was stimulated at the wrist by square-wave current pulses, usually of 0.1 or 1.0 ms duration, delivered at one per second using surface electrodes of 1 cm diameter taped to the skin 4 cm apart, orientated longitudinally along the course of the nerve. The antidromic compound sensory action potential (CSAP) was recorded from the index finger by ring electrodes set 3 cm apart around the proximal phalanx. In motor studies, the orthodromic compound muscle action potential (CMAP) was recorded using surface electrodes over the abductor pollicis brevis, with the active electrode at the motor point and the reference on the proximal phalanx. The intensity of the test stimulus was controlled and adjusted automatically in steps of 2% with a computer to produce compound sensory and muscle action potentials of 30–40% of maximum alternating between the stimulus for sensory axons and that for motor axons. To follow changes in excitability, a 0.1-ms test stimulus was used. To follow changes in $\tau_{SD}$ and $I_{rh}$, an additional test stimulus of 1.0 ms duration was used, and calculations based on the threshold currents to 0.1 ms ($I_{0.1}$) and 1.0 ms ($I_{1.0}$) stimuli were performed off-line using formulae derived from Weiss’ Law (Bostock and Bergmans, 1994):

$$\tau_{SD} = 0.1 \frac{(I_{0.1} - I_{1.0})}{[I_{1.0} - (0.1 \times I_{0.1})]}$$

$$I_{rh} = [I_{1.0} - (0.1 \times I_{0.1})] / 0.9$$

To follow changes in supernormality, the test stimulus was delivered alternately in isolation or after a supramaximal conditioning current pulse of 0.1 ms duration, using a conditioning–test interval of 10 ms so that the test stimulus sampled the supernormal period following the response to the conditioning stimulus. Because the maximal potential produced by the supramaximal conditioning stimulus contaminated the conditioned potential, the conditioned potential was measured on-line after subtraction of the response to the conditioning stimulus (as in Kiernan et al., 1996). Accordingly, in the first two series of experiments, the computer alternated at one per second between four different stimulus combinations for sensory axons and four for motor axons, namely unconditioned test stimulus of 0.1 ms duration, unconditioned test stimulus of 1.0 ms duration, conditioned and test stimuli of 0.1 ms duration, and conditioning stimulus alone. In the third series of experiments, changes in refractoriness and supernormality of sensory axons were followed during ischaemia (six experiments) or hyperventilation (three experiments) using conditioning–test intervals of 2, 4, 6 and 10 ms (ischaemia) and 2 ms (hyperventilation).
Excitability, hyperventilation, ischaemia

Fig. 1 The threshold changes during hyperventilation in two subjects. The threshold of sensory and motor axons began to decrease immediately with the decline in alveolar pCO₂ (arbitrary units) and this continued even when pCO₂ reached a plateau. After hyperventilation, threshold increased rapidly and returned to the control level as the pCO₂ level recovered.

In the fourth set of experiments membrane time constants were studied using the technique of latent addition. Excitability was tested with 60-µs current pulses adjusted in amplitude to keep the compound sensory or muscle action potential constant at 30–40% of maximum. The study was conducted at two per second, with the test pulse delivered by itself (control), or preceded by a 60-µs conditioning hyperpolarizing pulse set to −90% of the amplitude of the last control test pulse. In four experiments, latent-addition time constants were calculated from data obtained by varying the conditioning–test interval from 0 to 500 µs, at rest, during hyperventilation or ischaemia and while depolarizing or hyperpolarizing current pulses were passed through the stimulating electrodes. In seven experiments, the threshold change produced by a −90% (hyperpolarizing) conditioning stimulus delivered 0.2 ms before the test pulse was measured during hyperventilation or ischaemia and compared with simultaneously measured thresholds.

The amplitude of the CSAP or CMAP was measured from negative peak to positive peak. Latency was measured to negative peak in all studies. In all experiments, skin temperature was monitored continuously at the first metacarpophalangeal joint and wrist, and was kept above 32°C by wrapping the limb in a blanket and, if necessary, by radiant heat.

Results

Changes in excitability of cutaneous and motor axons during hyperventilation and ischaemia

All subjects were instructed to hyperventilate steadily for 20 min, the maximal decrease in end-tidal pCO₂ being 55 ± 2.7% (mean ± SEM) of the control level. Each subject reported paraesthesiae of the hand and face, and the intensity of paraesthesiae increased as pCO₂ declined further. In four out of the six subjects, muscle twitching and cramps developed in the hand and facial muscles a few minutes after paraesthesiae were first reported. The fall in end-tidal pCO₂ usually began abruptly but the decreases in the thresholds for sensory and motor axons were sometimes abrupt (Fig. 1A), sometimes more gradual (Fig. 1B) and usually continued during hyperventilation even after pCO₂ reached a steady state. When the subjects stopped hyperventilating the thresholds increased rapidly and returned to the control levels as the pCO₂ level recovered. Hyperventilation produced significant decreases in the thresholds for sensory and motor axons. The maximal threshold decrease was 26 ± 4.6% for sensory and 24 ± 3.8% for motor axons (mean ± SEM; \( P = 0.005 \) for sensory, \( P = 0.001 \) for motor axons).

The threshold current required to produce a CSAP or CMAP of 30–40% of maximum decreased during ischaemia, the changes beginning within 30 s and usually reaching a maximum within 3–5 min (Figs 2 and 3). Four out of the six subjects reported ischaemic paraesthesiae. These paraesthesiae were much less intense than those experienced after deflation of the cuff at the end of the 10-min ischaemic episode. None of the subjects reported muscle twitching, either during ischaemia or after its release. The maximal decrease in threshold current was significant for both sensory and motor axons (17 ± 4.2% \( P = 0.006 \) for sensory, 21 ± 6.1% \( P = 0.0155 \) for motor axons; Figs 2–4).

Supernormality and refractoriness during hyperventilation and ischaemia

Supernormality of sensory and motor axons was expressed as a percentage threshold reduction. Prior to hyperventilation, supernormality at the 10-ms conditioning–test interval was greater in motor than sensory axons, as has been found previously (Kiernan et al., 1996), 7.8 ± 1.07% for sensory and 12.5 ± 2.33% for motor axons. There was no significant change in supernormality of sensory or motor axons during
Fig. 2 The changes in threshold of sensory and motor axons during ischaemia. The threshold decreased within 30 s and reached its nadir within 2–3 min. Each data point shows the mean ± SD for six subjects normalized to the pre-ischaemic control level. The bottom panel illustrates the reproducibility of the changes in a second series of experiments for the same six subjects.

Supernormality decreased during ischaemia in all subjects, for both sensory ($P = 0.002$) and motor axons ($P = 0.0002$; Figs 3 and 4), such that they became subnormal at the 10-ms conditioning test interval. This contrasts with the lack of a significant change in supernormality during hyperventilation, even though hyperventilation produced slightly greater decreases in threshold. During ischaemia there was also a progressive and significant increase in the latency of the CSAP and CMAP, by $0.2 ± 0.023$ ms ($P = 0.0003$) and $0.17 ± 0.028$ ms ($P = 0.0018$), respectively. These changes could not be attributed to cooling of the limb because skin temperature remained constant.

A further set of experiments was performed to follow changes in the refractory period and early part of the supernormal period. The changes in threshold for a conditioned sensory potential were measured during ischaemia for conditioning–test intervals of 2, 4, 6 and 10 ms. Initially, axons were relatively refractory at the 2-ms interval but supernormal at the other intervals (Fig. 3B). During ischaemia, refractoriness increased greatly at the 2-ms interval by ~100% of the control threshold and extended to the 10-ms interval (Fig. 3B). In three subjects, refractoriness at the 2-ms conditioning–test interval was measured during hyperventilation, and increased by 33%, a smaller increase in all three subjects than occurred during ischaemia (84%).

Rheobase and strength–duration time constant during hyperventilation and ischaemia

Moment-to-moment changes in $I_{th}$ (the threshold current if the stimulus was infinitely long) and $\tau_{SD}$ (chronaxie), were calculated from the unconditioned threshold plots using test stimuli of 0.1 ms and 1.0 ms duration, according to the two equations given in the Methods section. In absolute terms,
Excitability, hyperventilation, ischaemia

Fig. 4 (A) Changes in threshold during hyperventilation and ischaemia for sensory and motor axons in six subjects (mean ± SEM). (B) The changes in supernormality during hyperventilation and ischaemia. Hyperventilation produced no significant change in supernormality, but ischaemia produced significant decreases in supernormality for sensory and motor axons.

$I_h$ was lower for sensory than motor fibres, and decreased significantly during hyperventilation and ischaemia (Fig. 5B). Hyperventilation decreased $I_h$ by 41.4 ± 2.6% for sensory and by 52.3 ± 4.0% for motor axons. During ischaemia $I_h$ decreased by 38.3 ± 3.4% for sensory and 37.5 ± 4.5% for motor fibres.

In relative terms, the changes in $I_h$ produced by the two manoeuvres were proportionally greater than those for threshold measured with brief pulses of 0.1 ms (Fig. 6), indicating that $\tau_{SD}$ was changing. As has been shown previously (Panizza et al., 1994; Mogyoros et al., 1996), $\tau_{SD}$ was longer at rest for sensory axons than for motor axons (Fig. 5A). During both hyperventilation and ischaemia, the time constant for sensory and motor fibres increased significantly (Fig. 5A; $P < 0.05$), by 38.5 ± 3.9% for sensory and 33.5 ± 5.7% for motor axons during hyperventilation, and by 43.5 ± 5.7% for sensory and 25 ± 4.3% for motor axons during ischaemia.

IONIC MECHANISMS CONTRIBUTING TO THE DIFFERENT BEHAVIOUR OF SENSORY AND MOTOR AXONS

To determine whether the ‘threshold channels’ proposed by Bostock and Rothwell (1997) could contribute to the behaviour of sensory and motor axons during hyperventilation and ischaemia, two sets of experiments were performed. In the first, the time course of recovery from 60-µs hyperpolarizing pulses (latent addition), using seven conditioning–test intervals between 0.06 ms and 0.5 ms, was combined with estimates of $\tau_{SD}$. Measurements from sensory axons were made in four subjects at rest, during depolarizing and hyperpolarizing currents pulses and then during hyperventilation. As in the previous study the recovery of threshold was well fitted by the sum of two exponentials (Fig. 7): a fast component with a time constant close to 45 µs, attributed to the passive time constant of the node of Ranvier, and a more variable slow component (time constant ~200 µs), which Bostock and Rothwell (1997) had found to be voltage dependent and interpreted as evidence for threshold channels. Hyperventilation had no appreciable effect on the fast component of recovery, but the increase in $\tau_{SD}$ was accompanied by an increase in the slow component (see curve fitting for one subject in Fig. 7). The findings in the
The changes in different parameters of nerve excitability for sensory axons during hyperventilation and ischaemia. The decrease in $I_{rh}$ was proportionally greater than the decrease in threshold tested using a brief pulse (here, of duration 60 µs). The increase in $\tau_{SD}$ was paralleled by an increase in the relative threshold change produced by a 90% hyperpolarizing conditioning stimulus 0.2 ms before the test stimulus.

In the second protocol (seven experiments on five subjects) the changes in this parameter were compared directly with the changes in $I_{rh}$ and $\tau_{SD}$ for motor and sensory axons during hyperventilation and ischaemia. There was always a close parallel between $\tau_{SD}$ and the threshold increase at 0.2 ms during both manoeuvres (Fig. 6), for both motor and sensory axons. The form of the relationship between $\tau_{SD}$ and threshold increase at 0.2 ms is illustrated in Fig. 8 for the sensory axons in one subject in whom these parameters were varied over a wide range by hyperventilation, ischaemia and polarizing currents. These experiments followed the first protocol, so that the fast and slow components of latent addition could be separated by fitting the recovery curve with two exponentials, as in Fig. 7. In these manoeuvres, the fast component had a time constant of 44.8 ± 1.12 µs (mean ± SEM), while the slow component was more variable (203.6 ± 13.0 µs). The total threshold change (Fig. 8, filled squares) was linearly related to $\tau_{SD}$ throughout, and this relationship was entirely due to the change in slow component (Fig. 8, filled circles); the residual fast component at 0.2 ms (Fig. 8, crosses) remained small and unchanged. This linear relationship was remarkably similar to that reported by Bostock and Rothwell (1997) for their computer model, in which changes in strength–duration behaviour and latent addition were caused by the different degrees of activation of persistent sodium channels.

Fig. 6 The changes in different parameters of nerve excitability for sensory axons during hyperventilation and ischaemia. The decrease in $I_{rh}$ was proportionally greater than the decrease in threshold tested using a brief pulse (here, of duration 60 µs). The increase in $\tau_{SD}$ was paralleled by an increase in the relative threshold change produced by a 90% hyperpolarizing conditioning stimulus 0.2 ms before the test stimulus.

Fig. 7 Changes in threshold produced by a 90% hyperpolarizing pulse at different conditioning–test intervals (latent addition) for sensory axons of a single subject at rest and when hyperventilating to maintain a steady pCO2 level. The data could not be fitted by a single exponential curve. The fit produced using two exponentials (fast and slow) is shown. The fast time constant does not change during hyperventilation. Note that it has decayed almost to zero at the 0.2-ms conditioning–test interval. The hyperventilation-induced change in the data is due to a change in the exponential with slow a time constant.

Other three subjects were similar. These experiments also confirmed the voltage dependence of the slow component.

A convenient measure of the slow component of recovery in the latent addition experiment is provided by the threshold increase 0.2 ms after a hyperpolarizing conditioning pulse, a time when the fast component has decayed almost to zero (Fig. 7; see Bostock and Rothwell, 1997). In the second protocol (seven experiments on five subjects) the changes in this parameter were compared directly with the changes in $I_{rh}$ and $\tau_{SD}$ for motor and sensory axons during hyperventilation and ischaemia. There was always a close parallel between $\tau_{SD}$ and the threshold increase at 0.2 ms during both manoeuvres (Fig. 6), for both motor and sensory axons. The form of the relationship between $\tau_{SD}$ and threshold increase at 0.2 ms is illustrated in Fig. 8 for the sensory axons in one subject in whom these parameters were varied over a wide range by hyperventilation, ischaemia and polarizing currents. These experiments followed the first protocol, so that the fast and slow components of latent addition could be separated by fitting the recovery curve with two exponentials, as in Fig. 7. In these manoeuvres, the fast component had a time constant of 44.8 ± 1.12 µs (mean ± SEM), while the slow component was more variable (203.6 ± 13.0 µs). The total threshold change (Fig. 8, filled squares) was linearly related to $\tau_{SD}$ throughout, and this relationship was entirely due to the change in slow component (Fig. 8, filled circles); the residual fast component at 0.2 ms (Fig. 8, crosses) remained small and unchanged. This linear relationship was remarkably similar to that reported by Bostock and Rothwell (1997) for their computer model, in which changes in strength–duration behaviour and latent addition were caused by the different degrees of activation of persistent sodium channels.
Discussion

In this study we have analysed further the excitability changes in cutaneous afferent and motor axons in peripheral nerve during hyperventilation and ischaemia, extending the previous studies by Macefield and Burke (1991) and Bostock et al. (1994), respectively. The results provide the first direct comparison of the effects of hyperventilation and ischaemia on human peripheral nerve since Kugelberg’s studies in the 1940s (Kugelberg, 1944, 1948a, b), and support a new model of human nerve excitability (Bostock and Rothwell, 1997), according to which some of the different excitability properties of motor and sensory axons are related to differences in the activation at rest of non-inactivating ‘threshold channels’.

Kugelberg (1948a, b) emphasized the similarity in the responses of peripheral nerve to hyperventilation and ischaemia, noting that in each case the fibres to discharge first were those excited first by depolarizing current ramps, and that their firing patterns were similar during both manoeuvres. Most of the tests of excitability used in the present study also showed similar changes with hyperventilation and ischaemia. With the moderate degree of hyperventilation achieved by the subjects (none of whom lost consciousness, as reported by Kugelberg, 1948a, b), not only were the peak threshold changes to 0.1 ms stimuli similar for hyperventilation and ischaemia in both motor and sensory fibres (Fig. 4A), but the changes in the $\tau_{SD}$ and $I_{th}$ were also comparable (Fig. 5A and B). It would appear, therefore, that the Na$^+$ or other channels responsible for determining electrical excitability must be in a similar state in the two conditions. On the other hand, conduction velocity and supernormality were both significantly reduced by ischaemia but not by hyperventilation (Fig. 4B). In addition, refractoriness was increased much more by ischaemia than by hyperventilation. How can these actions of hyperventilation and ischaemia, some indistinguishable and some quite different, be reconciled?

Three types of ion channels are likely candidates to be involved in these excitability changes: Na$^+$ channels, K$^+$ channels and threshold channels. These channels are presumably affected equally by ischaemia, since ischaemia is normally assumed to induce a straightforward membrane depolarization, due to inhibition of the electrogenic Na$^+$ / K$^+$ pump and the consequent increase in extracellular K$^+$ ions, especially in the restricted space under the myelin sheath. This interpretation is supported by the observation that depolarization by applied currents produces similar changes in excitability and accommodation to those occurring in the first 10 min of ischaemia (Baker and Bostock, 1989; Bostock et al., 1991). Hyperventilation, however, does not affect all channels equally. Taking the threshold channels first, according to Bostock and Rothwell’s (1997) model, the $\tau_{SD}$ is closely related to the degree of activation of threshold channels at the resting potential, as indicated by the amplitude of the slow component of latent addition to brief hyperpolarizing currents. There was a similar relationship during both ischaemia and hyperventilation (Figs 6 and 8). The similar increase in $\tau_{SD}$ with the two procedures (Fig. 5) therefore indicates that they produced a similar increase in activation of threshold channels at resting potential. This alone may have been adequate to cause the falls in $I_{th}$, but conventional transient Na$^+$ channels would also have been activated by membrane depolarization. However, the effects of the two procedures on the transient Na$^+$ channels were clearly different: the reduced conduction velocity (despite the increase in excitability) and increased refractoriness during ischaemia can be related to the effects of membrane depolarization on Na$^+$ channel inactivation. This suggests that hyperventilation produced less Na$^+$ channel inactivation than ischaemia. Also, as previously concluded by Macefield and Burke (1991), K$^+$ channels are relatively unaffected by hyperventilation: a reduction in supernormality, seen with ischaemic depolarization and due to the short-circuiting of the depolarizing afterpotential by increased activation of intermodal K$^+$ channels (Barrett and Barrett, 1982; Baker et al., 1987), did not occur during hyperventilation. The present data therefore suggest that only threshold channels were equally affected by the two procedures, and that hyperventilation has a rather selective action on threshold channels.

An alternative explanation would be that hyperventilation increases activation of both threshold and transient Na$^+$ channels, without the effects on inactivation gating of ischaemia, possibly by altering surface charge (see below) closer to the activation gates.

The nature of the environmental change that affects the ion channels in hyperventilation is not fully understood. Because the increase in arterial pH leads (through the
competitive binding of H\(^+\) and Ca\(^{2+}\) ions to plasma proteins) to a fall in the level of free ionized Ca\(^{2+}\) in the plasma (Fanconi and Rose, 1958), and because hypocalcaemia alone can induce paraesthesiae and fasciculation, the effects of hyperventilation on peripheral nerve are usually attributed to hypocalcaemia (e.g. Kugelberg, 1948b; Macfie and Burke, 1991). However, this assumption has been questioned, both on the grounds that when blood pH is altered by ingestion of acid or alkali, hyperventilation tetany depends on pCO\(_2\) rather than blood pH (Shock and Hastings, 1935), and on the grounds that the changes in ionized calcium are too small (Tenny and Lamb, 1965). Certainly, the 5% reduction in plasma ionized calcium estimated by Davis et al. (1970) during hyperventilation which reduced pCO\(_2\) by 51% (comparable to our average of 55%) would not, on its own, be expected to induce ectopic discharges. It is also relevant that while paraesthesiae start, on average, within 1 min of the onset of hyperventilation, they were reported to start ‘almost immediately’ in four out of 15 subjects by Montagna et al. (1995). Axons might be expected to see such rapid changes in pCO\(_2\) and extracellular pH, but not a change in ionized Ca\(^{2+}\) starting on the other side of the blood–nerve barrier. It seems safer to conclude that the excitability changes depend on extracellular H\(^+\) and Ca\(^{2+}\) ions and possibly pCO\(_2\) (Brown, 1953). H\(^+\) and Ca\(^{2+}\) ions can affect the gating of ion channels in two ways, by binding to fixed negative charges on the surface of the membrane and thereby altering the membrane potential gradient acting on the channel, and also by impeding ion fluxes through the channels (Hille, 1991). In the case of frog nodes, the most important action found under physiological conditions was the surface charge effect of Ca\(^{2+}\) ions on Na\(^+\) channel gating (Hille, 1968). However, no studies have yet been made of the effects of H\(^+\) and Ca\(^{2+}\) ions on threshold channels, nor indeed on any other channels in mammalian axons.

Recently, however, a low-threshold, persistent Na\(^+\) current has been found in large rat dorsal root ganglion neurons, and this appears to correspond to the threshold current in human peripheral nerve (Baker and Bostock, 1996a). This current is about an order of magnitude more sensitive to block by H\(^+\) ions than the transient sodium current, with 50% block at pH close to 7.0 (Baker and Bostock, 1996b, and the same authors’ unpublished observations). These findings may explain the selective activation of threshold channels by hyperventilation, but further studies of the effects of H\(^+\) and Ca\(^{2+}\) ions on axonal ion channels will be required to establish which of the possible interactions is primarily responsible for hyperventilation hyperexcitability.

Acknowledgement

This study was supported by the National Health and Medical Research Council of Australia.

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Received August 13, 1996. Accepted October 14, 1996