The effects of hyperglycaemic hypoxia on rectification in rat dorsal root axons

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1. Electrotonic responses to 150 ms current pulses were recorded from isolated rat dorsal roots incubated for at least 3 h with either normal (5 mM) or high (25 mM) D-glucose solutions, and with either normal (25 mM) or low (5 mM) bicarbonate concentrations.

2. On replacement of O_2 by N_2 for 50 min, all the roots depolarized, but the changes in electrotonus differed systematically. With normal glucose, the depolarization was accompanied by an increase in input conductance. In contrast, for the hyperglycaemic roots the depolarization was slower and accompanied by a fall in input conductance which was exacerbated in low bicarbonate concentrations.

3. The changes induced by hyperglycaemic hypoxia in low bicarbonate could be mimicked by exposure of the roots either to 100% CO_2 or to a combination of 3 mM tetraethylammonium chloride and 3 mM 4-aminopyridine, to block both fast and slow potassium channels.

4. These results indicate that the primary mechanism of hypoxic depolarization of these sensory axons is altered by hyperglycaemia. In normoglycaemia, the changes in electrotonus are consistent with an increase in axonal potassium conductance. The block of potassium channels seen in hyperglycaemic hypoxia is attributed to intra-axonal acidification by anaerobic glycolysis and may contribute to the pathogenesis of diabetic neuropathy.

Neuropathy is a major cause of morbidity in diabetes, and hyperglycaemia is the root cause of diabetic neuropathy (Thomas, 1990). The chain of events by which hyperglycaemia leads to nerve damage, predominantly in sensory fibres, is far from clear but there is much evidence that endoneurial hypoxia due to microangiopathy is involved (for review see Low, 1987; Thomas & Tomlinson, 1993). Using isolated frog nerves, Lorente de Nó (1947) first made the observation that high glucose concentrations cause a lack of functional recovery after hypoxia, and this phenomenon has recently been investigated in rat peripheral nerves and spinal roots *in vitro* (Strupp, Jund, Schneider & Grafe, 1991; Schneider, Jund, Nees & Grafe, 1992; Schneider, Niedermeier & Grafe, 1993*a*; Schneider, Quasthoff, Mitrović & Grafe, 1993*b*). Recovery of membrane and action potentials from hyperglycaemic hypoxia is worse in dorsal than ventral roots (Schneider *et al.* 1992), supporting an association with diabetic neuropathy. The poor recovery of dorsal roots is probably due to intracellular acidification by anaerobic glycolysis, since (a) it is worse when the buffering power and/or bicarbonate-dependent pH-regulating mechanisms of the axons are compromised (Schneider *et al.* 1992), (b) it only occurs with hexoses that can be metabolized rapidly by the glycolytic pathway (Schneider *et al.* 1993*a*), and (c) it is associated with extracellular release of acid (Strupp *et al.* 1991). It is a well-documented observation that hypoxia and/or ischaemia-induced decrease in tissue pH is dependent on the pre-ischaemic blood glucose concentration, being greatest in hyperglycaemic and least in hypoglycaemic animals (e.g. Smith, von Hanwehr & Siesjö, 1986; Siesjö, 1988; Kraig & Chesler, 1990; Tyson, Peeling & Sutherland, 1993).

The present study is concerned with the next link in this chain of pathogenesis: how intracellular acidification causes depolarization and failure to conduct action potentials. Cytoplasmic acidification inactivates fast axonal K^+ channels (Schneider *et al.* 1993*b*), but these channels have not previously been held to be major determinants of the resting potential. To pursue this matter further, we required a technique that would (a) reveal changes in both nodal and internodal channels contributing to the resting potential, (b) provide stable recordings for long periods of hypoxia, and (c) would avoid undefined pathophysiology due to mechanical damage. Neither intracellular microelectrode recording, nor nodal voltage clamp, nor patch clamping meets more than one of these criteria. We,

therefore, turned to recordings of electrotonus, which were previously used to analyse the components of rectification in myelinated axons (Baker, Bostock, Grafe & Martius, 1987), but took advantage of the improved mechanical and DC stability provided by the 'Marsh' Vaseline gap nerve bath (Marsh, Stansfeld, Brown, Davey & McCarthy, 1987). We restricted this study to dorsal roots, since sensory fibres are more readily damaged in diabetes and by hyperglycaemic hypoxia. The results indicate that hyperglycaemic hypoxia depolarizes sensory axons because the acid generated by glycolysis blocks both fast and slow potassium channels that help maintain the resting potential. Preliminary data from this study have been published in an abstract form (Grafe & Schneider, 1993).

METHODS

Animals and preparation

Male Wistar rats, weighing 300–400 g, were obtained from Thomae, Biberach, Germany. The animals were anaesthetized with urethane (1·5 g kg^{-1}, I.P., supplemented as required) for a laminectomy to expose the cauda equina and the spinal ganglia. Spinal roots were removed in their entire length (from the spinal cord to the spinal nerve) for *in vitro* recording. The anatomical relationship of the isolated roots to the spinal ganglia enabled us to differentiate between dorsal and ventral roots. After preparation, the anaesthetized rats were killed by exsanguination and the isolated spinal roots were incubated at room temperature (20–25 °C) from 30 min to about 8 h in solutions with different concentrations of D-glucose or other hexoses. Afterwards these nerves were transferred to the experimental organ bath.

Solutions

The standard solution contained (mM): NaCl, 118·0; KCl, 3·0; $CaCl_2$, 1·5; $MgCl_2$, 1·0. The concentration of D-glucose was either 5 or 25 mM; in some experiments 20 mM D-galactose or 20 mM D-mannose was added to solutions containing 5 mM D-glucose. Normal bicarbonate-buffered solution consisted of the standard solution plus 25 mM $NaHCO_3$ and 1·2 mM NaH_2PO_4, bubbled with 95% O_2–5% CO_2 (normoxia) or 95% N_2–5% CO_2 (hypoxia). In the low bicarbonate-buffered solution, 5 mM HCO_3^- and 20 mM NaCl were added to the standard solution. During normoxia this solution was equilibrated with 20% O_2–79% N_2–1% CO_2 and during hypoxia with 99% N_2–1% CO_2. In some of the experiments, the partial pressure of oxygen (P_{O_2}) in the organ bath was monitored continuously by a Clark-style electrode (Diamond Electro-Tech Inc., Ann Arbor, MI, USA) and found to be less than 2 mmHg within 1 to 2 min after perfusion of the organ bath with the hypoxic solutions. Tetrodotoxin (TTX), 4-aminopyridine (4-AP), and tetraethylammonium (TEA) were purchased from Sigma, Deisenhofen, Germany.

Electrotonus

The organ bath used to record electrotonus and extracellular direct current (DC) potentials has been previously described (Marsh *et al.* 1987; Schneider *et al.* 1992, 1993*b*). It consisted of a three-chambered plexiglass bath compartmentalized by 1 mm partitions (Marsh ganglion bath; Hugo Sachs Elektronik, March-Hugstetten, Germany). Each partition had removable upper and lower sections in which a slot had been cut to allow the spinal roots to pass between chambers without being crushed. Silicone grease was used to seal the root into position and to prevent the free diffusion of solutions between chambers. The central compartment was continuously perfused by positive gas pressure in buffer flasks. The flow rate was 14 ml min^{-1} (volume of the central compartment: 1·5 ml). The nerve end in one of the lateral compartments was drawn into a suction electrode which was used for the application of current pulses (current steps of up to ± 10 μA; duration, 150 ms; stimulation rate, 0·5 Hz). The solution in this lateral compartment was identical to the one in the central compartment. However, it was not made hypoxic. The K^+ concentration in the second lateral compartment was elevated by 30 mM. A pair of DC-stable silver–silver chloride recording electrodes was used to record the potential difference across the resistance between the second lateral and the central compartment of the organ bath. The recordings of electrotonus were made in 0·1 μM TTX, to avoid interference from action potentials.

Conductance

Quantitatively, electrotonus was analysed in terms of slope conductance or input conductance at rest. To find the slope conductance ($\Delta I/\Delta E$) at the nth point (E_n, I_n) on the I–E curve, a quadratic equation was fitted to the three points (E_{n-1}, I_{n-1}), (E_n, I_n), and (E_{n+1}, I_{n+1}). The slope conductance was determined at the end of the current pulses (after 145 ms of constant current injection), which approximated to a steady state. This measurement took account of the contribution of active conductances in the internodal axon to the input conductance of the fibres (Baker *et al.* 1987). Our extracellular recordings only registered an unknown fraction of the intracellular potential changes, so conductances are plotted in arbitrary units and the effects of different treatments were calculated as percentage changes in input conductance.

RESULTS

Effects of hypoxia

The effects of hypoxia were tested on the extracellular direct current (DC) potential (demarcation potential) and on the electrotonic behaviour of isolated rat dorsal spinal roots. Such experiments were performed on spinal roots incubated in either normal (5 mM) or high (25 mM) concentrations of D-glucose before, during and after hypoxia in a solution containing 5 mM HCO_3^-–1% CO_2. The experimental protocol and the effects of these conditions on the DC potential are illustrated in Fig. 1. Initially, the spinal roots were placed into the recording chamber in a solution containing 0·1 μM TTX. After the DC potential had stabilized (30–60 min later), a standardized experimental protocol was started. At the end of a control period of 10 min, the electrotonic behaviour was recorded by means of hyper- and depolarizing current pulses of 150 ms duration. The presence of TTX was necessary to avoid interference from action potentials. The oxygenated solution was then replaced by a N_2-bubbled solution without TTX since we found that TTX reduced the rate of

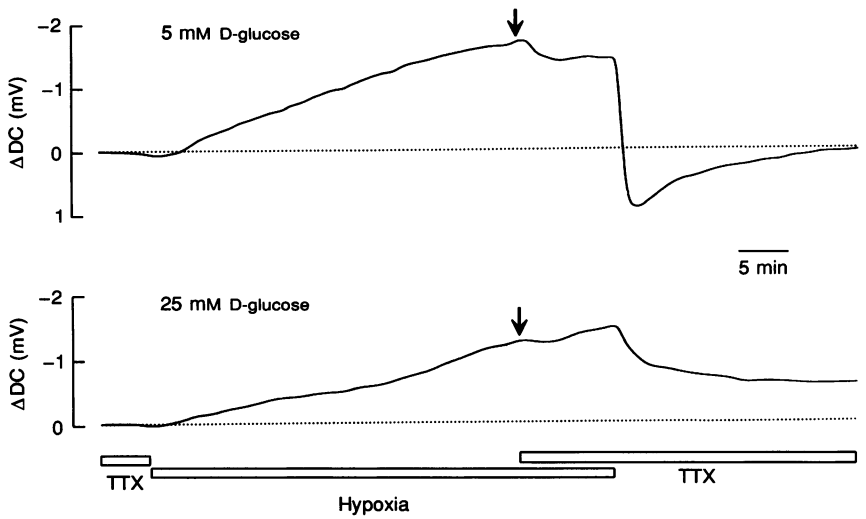

Figure 1. Experimental protocol used for a comparison of electrotonus before and during hypoxia
Illustrated are averages of extracellular direct current (DC) recordings obtained from dorsal spinal roots exposed to hypoxia in 5 or 25 mM D-glucose ($n = 5$ in either condition; pH buffer: 5 mM HCO_3^-–1% CO_2). Recordings of electrotonus were taken 2 min before the start of hypoxia and 1 min before the end of hypoxia (see Fig. 2). Tetrodotoxin (TTX 0·1 μM) was present during both recording periods. In contrast, the first 40 min of hypoxia was performed without TTX in the bathing solution. The arrows indicate the times when TTX was added to the hypoxic bathing solution.

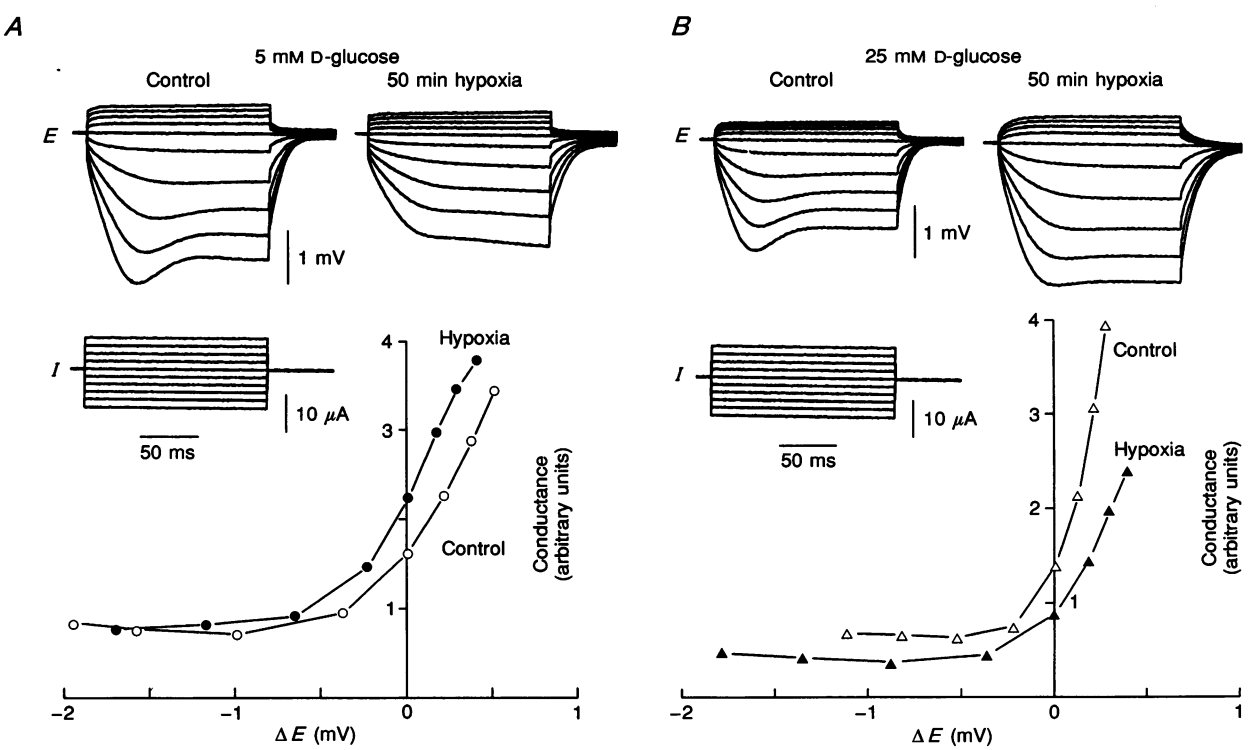

Figure 2. Opposite effects of normoglycaemic and hyperglycaemic hypoxia on the electrotonus of isolated dorsal spinal roots
Recordings of electrotonus were taken 2 min before the start of hypoxia and 1 min before the end of hypoxia in the presence of tetrodotoxin (see Fig. 1 for the experimental protocol). The graphs show conductance–voltage (E) relationships taken from the electrotonus recordings before and during hypoxia. Both experiments were performed in a low bicarbonate (5 mM) concentration. Note the opposite effects of normoglycaemic and hyperglycaemic hypoxia on the slope conductance at the resting potential.

Table 1. Changes in input conductance at resting potential (%) after 50 min hypoxia

	D-Glucose, 5 mM	D-Glucose, 25 mM
	A	B
Bicarbonate, 5 mM	18·5 ± 11·6 (7)	−29·1 ± 3·9 (6)
	C	D
Bicarbonate, 25 mM	43·2 ± 16·8 (5)	−4·2 ± 6·4 (8)

A *vs.* B: $P < 0.005$ B *vs.* C: $P < 0.001$
A *vs.* C: n.s. B *vs.* D: $P = 0.01$
A *vs.* D: n.s. C *vs.* D: $P = 0.01$

Numbers quoted are means ± S.E.M.; number of observations in parentheses. Significance tested using unpaired *t* test; n.s., not significant, i.e. $P > 0.05$.

hypoxic depolarization (observations made on six spinal roots, not illustrated; see also Stys, Waxman & Ransom, 1992). After 40 min of exposure to hypoxia, TTX was added to the hypoxic solution for another 10 min. This resulted in a hyperpolarizing shift of membrane potential similar to the effect of TTX on rat optic nerves recently described by Stys, Sontheimer, Ransom & Waxman (1993). In the presence of TTX, after 49 min of hypoxia, another record of the electrotonic behaviour was recorded. Thereafter, the roots were superfused again with the normal oxygenated, TTX-containing solution and the recovery was observed for a further 30 min.

Normo- and hyperglycaemic hypoxia differed in their effect on the DC potential. The negative-going shifts during hypoxia indicate axonal depolarization during normo- as well as hyperglycaemic hypoxia. However, after hypoxia in 5 mM glucose, a transient membrane hyperpolarization was observed. In contrast, an incomplete recovery from

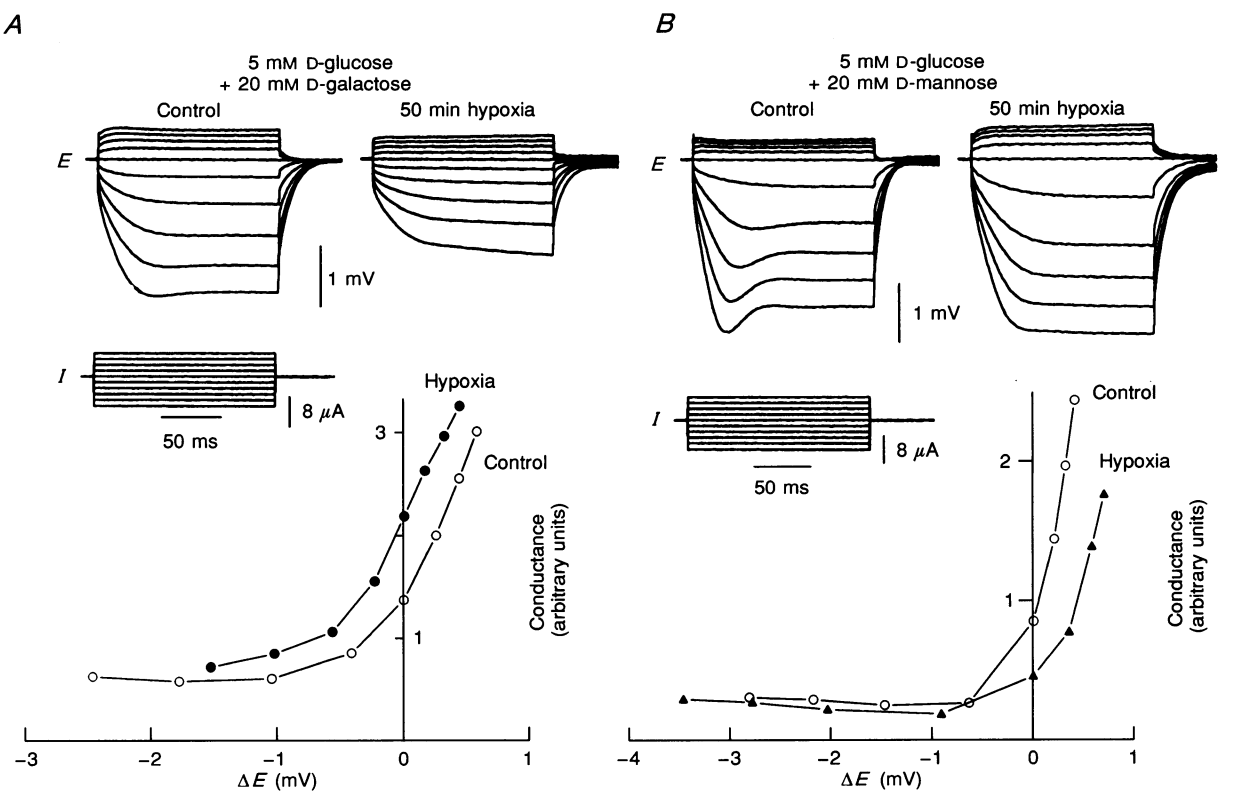

Figure 3. Effects of hyperglycaemic hypoxia are reproduced in high concentrations of D-mannose, not D-galactose

Experimental protocol identical to the one described in Figs 1 and 2. The experiment shown in *A* was performed on a dorsal spinal root, which had been incubated in 5 mM D-glucose plus 20 mM D-galactose 4 h before, during and after hypoxia. The data illustrated in *B* stem from a spinal root incubated in 5 mM D-glucose plus 20 mM D-mannose.

depolarization was noted after hyperglycaemic hypoxia (see also Schneider *et al.* 1992).

Figure 2 illustrates the changes in the electrotonus of dorsal spinal roots during normo- and hyperglycaemic hypoxia in solutions containing 5 mM HCO_3^-–1% CO_2. The electrotonic behaviour induced by hyperpolarizing and depolarizing current pulses of 150 ms duration in normal, oxygenated solutions did not differ systematically with the concentrations of D-glucose used. It revealed the outward rectification and the time-dependent inward rectification previously described for rat spinal roots (Baker *et al.* 1987). However, opposite changes in the electrotonus were observed during normo- and hyperglycaemic hypoxia. This contrast became most clear when electrotonus was transferred in a conductance–voltage relationship (see Methods): hypoxia in 5 mM glucose resulted in an *increase* in input conductance whereas hypoxia in 25 mM glucose produced a *decrease* in input conductance. This difference was most marked at potentials close to, or positive to, the resting potential. The apparent lack of time-dependent inward rectification to hyperpolarizing currents with hypoxia may simply have been due to the depolarization. We have no evidence for a specific effect of anoxia on inward rectification, as has been reported by Duchen (1990) in mouse dorsal root ganglion cells.

Table 1 summarizes the effects of normo- and hyperglycaemic hypoxia on the input conductance of dorsal spinal roots. Such experiments were performed in solutions containing either 5 mM HCO_3^-–1% CO_2 or 25 mM HCO_3^-–5% CO_2. For the statistical analysis, changes in input conductance at the resting potential were compared. The data show that normo- and hyperglycaemic hypoxia produced opposite effects on the resting slope conductance. Furthermore, the decrease in input conductance in high concentrations of D-glucose was much less when the dorsal spinal roots had been incubated in a bathing solution containing 25 mM bicarbonate. This finding supports earlier observations demonstrating that some of the electrophysiological effects seen during and after hypoxia in 25 mM D-glucose depend on the buffering power and/or availability of bicarbonate in the spinal roots (Strupp *et al.* 1991; Schneider *et al.* 1992, 1993*b*).

A comparison with other hexoses

Some spinal roots were incubated in high concentrations of D-mannose or D-galactose before and during hypoxia. These experiments had the aim of separating a metabolic from a possible osmotic effect. According to a previous study, high concentrations of D-mannose, in contrast to D-galactose, imitate the abnormal sensitivity to hypoxia induced by

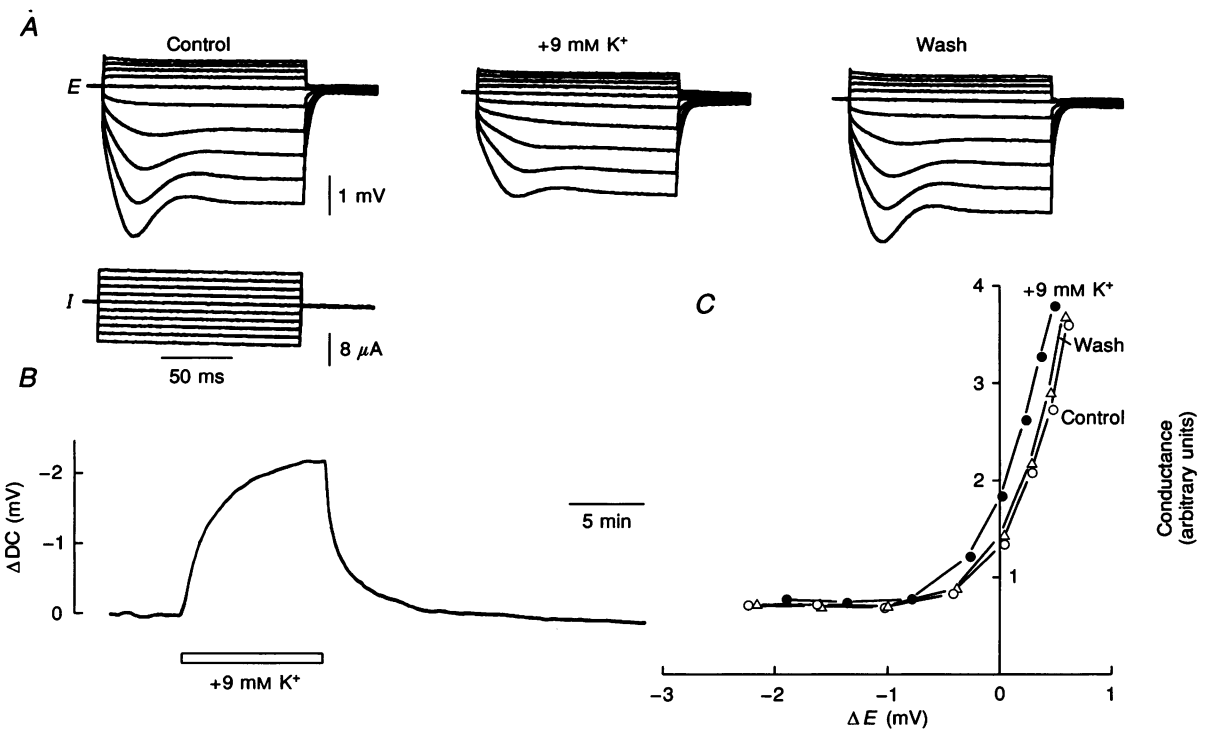

Figure 4. Effects of high concentration of extracellular K^+

In the presence of TTX, the K^+ concentration in the control solution (3 mM) was elevated by 9 mM for 10 min. *A*, the electrotonus was recorded before (control), at the end of exposure to high K^+ and 10 min after the exposure to high K^+ (Wash). *B*, the registration of the extracellular direct current (DC) potential indicates axonal depolarization during exposure to high K^+. *C*, conductance–voltage relationships taken from the recordings of electrotonus illustrated in *A*. Note the K^+-induced increase in slope conductance at the resting potential.

25 mM D-glucose (Schneider *et al.* 1993*a*). In the present study, the effects of these two hexoses were explored on the electrotonus during hypoxia. Figure 3 illustrates the changes in the electrotonus of dorsal spinal roots during hypoxia in solutions containing 5 mM D-glucose plus 20 mM of either D-galactose or D-mannose (pH buffer, 5 mM HCO_3^-–1% CO_2). The electrotonic behaviour induced by hyper- and depolarizing current pulses of 150 ms duration in normal, oxygenated solutions did not differ between roots incubated in D-mannose or D-galactose. However, opposite changes in the electrotonus were observed during hypoxia. Hypoxia in 5 mM D-glucose plus 20 mM D-galactose resulted in an increase in input conductance whereas hypoxia in 5 mM D-glucose plus 20 mM D-mannose produced a decrease in input conductance. As in the case of 25 mM D-glucose, this difference was most marked at potentials close or positive to, the resting potential. On average, input conductance at resting potential was increased by 18·1 ± 6·3% (mean ± S.E.M., $n = 5$) after 50 min of hypoxia in 5 mM D-glucose plus 20 mM D-galactose. On the other hand, input conductance at rest was reduced by 21·0, 45·3 and 51·3% in the three dorsal spinal roots exposed to hypoxia in 5 mM D-glucose plus 20 mM D-mannose.

Effects of depolarization in high K^+

To test the effects of passive depolarization on electrotonus, seven isolated dorsal spinal roots were consecutively exposed to elevations of the extracellular potassium concentration by 3, 6 and 9 mM. The application time of the high-potassium solutions was 10 min in each case. One example of these experiments is illustrated in Fig. 4. High K^+ concentrations depolarized the axons and induced an increase in input conductance. Quantitatively, after 10 min of application, the following depolarizing shifts in the DC potential and increases in the input conductance at the resting potential were observed (means ± S.E.M., $n = 7$): 0·5 ± 0·06 mV, +2·4 ± 0·5% at +3 mM $[K^+]_o$; 1·4 ± 0·17 mV, +11·7 ± 1·3% at +6 mM $[K^+]_o$; 2·1 ± 0·24 mV, 17·2 ± 1·8% at +9 mM $[K^+]_o$.

The effects of high K^+ concentrations on the electrotonus of rat spinal root axons resembled qualitatively the alterations in axonal conductance seen after normoglycaemic hypoxia. However, in order to imitate the effects of hyperglycaemic hypoxia, other types of experiment had to be performed.

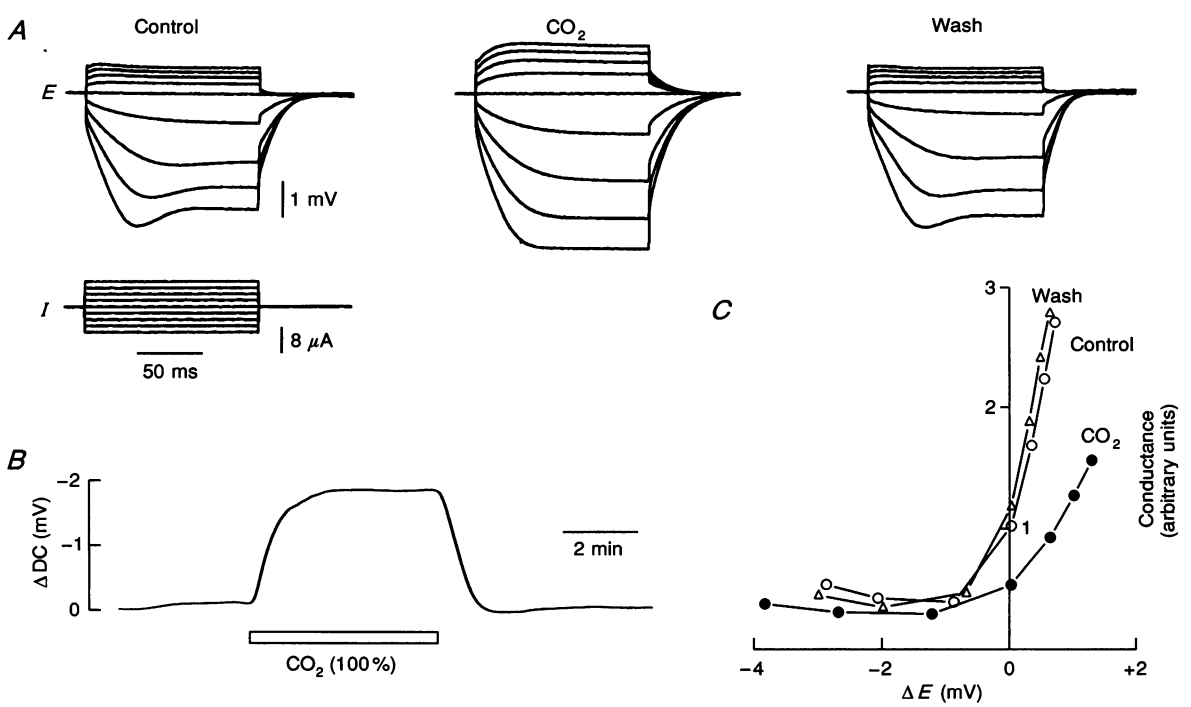

Figure 5. Effects of CO_2

Effect of high concentrations of CO_2 in the presence of TTX on the electrotonus and the extracellular direct current (DC) potential of an isolated dorsal spinal root. The control bathing solution (5 mM HCO_3^-–5% CO_2–95% O_2) was replaced for 5 min by a high CO_2 solution (5 mM HCO_3^-–100% CO_2). *A*, the electrotonus was recorded 1 min before (Control) CO_2, at the end of exposure to high CO_2 and 5 min after exposure to CO_2 (Wash). *B*, the registration of the extracellular direct current (DC) potential indicates axonal depolarization during exposure to 100% CO_2. *C*, conductance–voltage relationships taken from the recordings of electrotonus illustrated in *A*. Note the CO_2-induced decrease in slope conductance at the resting potential.

Effects of axonal acidification

Some of the electrophysiological effects of hyperglycaemic hypoxia on spinal roots have been attributed previously to acidification (Schneider *et al.* 1993*b*). The following experiments were performed to test whether intracellular acidification could also account for the changes in electrotonus during hyperglycaemic hypoxia. As illustrated in Fig. 5, dorsal spinal roots incubated in 5 mM HCO_3^- were exposed to high concentrations of CO_2. CO_2 is highly membrane permeable and known to produce a rapid intracellular, cytoplasmic acidification (Thomas, 1976). In dorsal spinal roots, high concentrations of CO_2 led to a rapid membrane depolarization as indicated by a negative-going shift in the extracellular DC potential (Fig. 5*B*). Furthermore, changes in electrotonus revealed a decrease in membrane conductance similar to the effect of hyperglycaemic hypoxia (Fig. 5*A* and *C*). Quantitatively, the membrane conductance at resting potential decreased by 54·6 ± 6·1% (mean ± S.E.M.; $n = 5$) after 5 min of exposure to 100% CO_2. The effects of CO_2 were quickly reversible.

Another series of experiments was performed with the aim of differentiating between extra- and intracellular acidification as the cause for acidification-related changes in electrotonus. To achieve this, we compared the effects of acidification induced by (a) lowering HCO_3^- at constant CO_2, and (b) raising CO_2 at constant HCO_3^-. The pH in the bathing solution in each case was 6·1; however, only the elevation in CO_2 should induce a strong cytoplasmic acidification (Thomas, 1976; Buckler, Vaughan-Jones, Peers, Lagadic-Gossmann & Nye, 1991)). Figure 6 illustrates one of three such experiments. The electrotonic voltage responses to depolarizing and hyperpolarizing current pulses (± 10 μA) were consecutively recorded from a single dorsal root which was superfused most of the time with a solution containing 25 mM HCO_3^-–5% CO_2 (pH 7·4). Initially, this control solution was exchanged for 10 min with a solution containing 1 mM HCO_3^-–5% CO_2 (pH 6·1). Later, at a constant concentration of 25 mM HCO_3^-, CO_2 was increased to 100% for 5 min. Only in the second case, a clear alteration in the electrotonus was observed. This finding indicates cytoplasmic and not extracellular acidification as the underlying mechanism.

Effects of potassium channel blockers

Finally, the effects of K^+ channel blockers on the electrotonus of dorsal spinal roots were explored. These experiments had the aim of comparing alterations in axonal conductance during hyperglycaemic hypoxia with changes in electrotonus due to a decrease in membrane K^+ conductance. Tetraethylammonium (TEA) and 4-aminopyridine (4-AP) are well-known blockers of axonal K^+ channels; their effects on the electrotonus of rat spinal roots has been described previously (Baker *et al.* 1987). In the present study, we tested the effects of these drugs on the electrotonus, DC potential and input conductance of

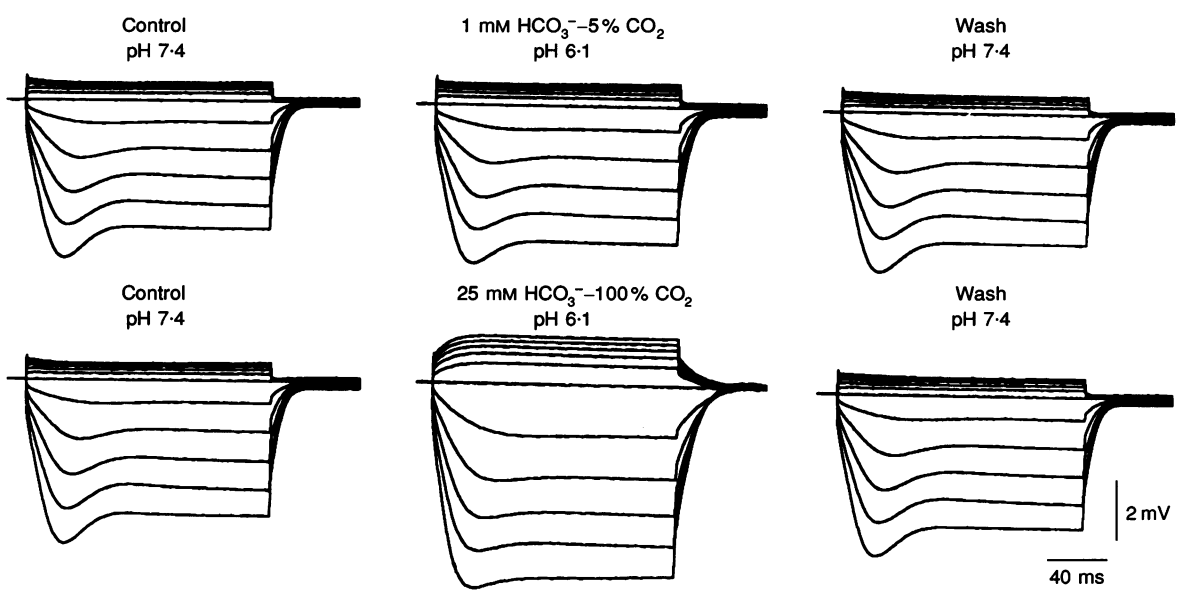

Figure 6. Different effects of extracellular and intracellular acidification on electrotonus
The electrotonic voltage responses to de- and hyperpolarizing current pulses (± 10 μA) were consecutively recorded from a single dorsal root which was superfused most of the time with a solution containing 25 mM HCO_3^-–5% CO_2 (pH 7·4). First, this control solution was exchanged for 10 min with a solution containing 1 mM HCO_3^-–5% CO_2 (pH 6·1). Later on, at a constant concentration of 25 mM HCO_3^-, CO_2 was increased to 100% (pH 6·1) for 5 min. Note the different effects of these two solutions on the electrotonus in spite of an identical bath pH of 6·1. TTX (1 μM) was present in the bathing solution throughout the experiment.

dorsal spinal roots (Figs 7 and 8). Both K^+ channel blockers on their own reduced the input conductance at rest and slightly depolarized the membrane of dorsal spinal roots. The combined effects of TEA and 4-AP were much stronger than the effect of either drug alone. TEA had more effect in the later phase of the electrotonic response, whereas 4-AP reduced the conductance more in the initial part of the electrotonus (compare Figs 7*A* and 8*A*). These observations are consistent with the block of slow and fast K^+ conductances respectively, as previously described for these drugs (Baker *et al.* 1987; Kocsis, Eng, Gordon & Waxman, 1987). The changes in shape of the electrotonus waveforms induced by the combination of TEA and 4-AP resembled those seen during hyperglycaemic hypoxia and high CO_2 much more closely than did those induced by either drug alone.

DISCUSSION

This study has provided new evidence for the mechanism whereby hypoxia depolarizes hyperglycaemic sensory axons, which differs from the mechanism of depolarization in normoglycaemic hypoxia, especially in low bicarbonate. The four conditions tested in Table 1 probably represent four points on a continuum of cytoplasmic acidification, from lowest with 5 mM glucose plus 25 mM bicarbonate to highest with 25 mM glucose plus 5 mM bicarbonate. Irrespective of the bicarbonate concentration, however, high glucose significantly altered the change in input conductance with hypoxia, actually reversing its direction. The probable mechanisms of the conductance changes in normal and high glucose will be considered separately, before discussing the relevance of these findings to diabetic neuropathy.

Normoglycaemic hypoxia

The alterations in the electrotonus during normoglycaemic hypoxia clearly indicate an increase in membrane conductance. At least part of this increase in conductance would be expected simply as a result of the membrane depolarization (due to failure of the electrogenic sodium pump and extracellular potassium accumulation) acting on voltage-dependent K^+ channels (Barrett & Barrett, 1982), and we found that passive depolarization of the axons by elevation of $[K^+]_o$ produced qualitatively similar changes in electrotonus. However, although 12 mM $[K^+]_o$

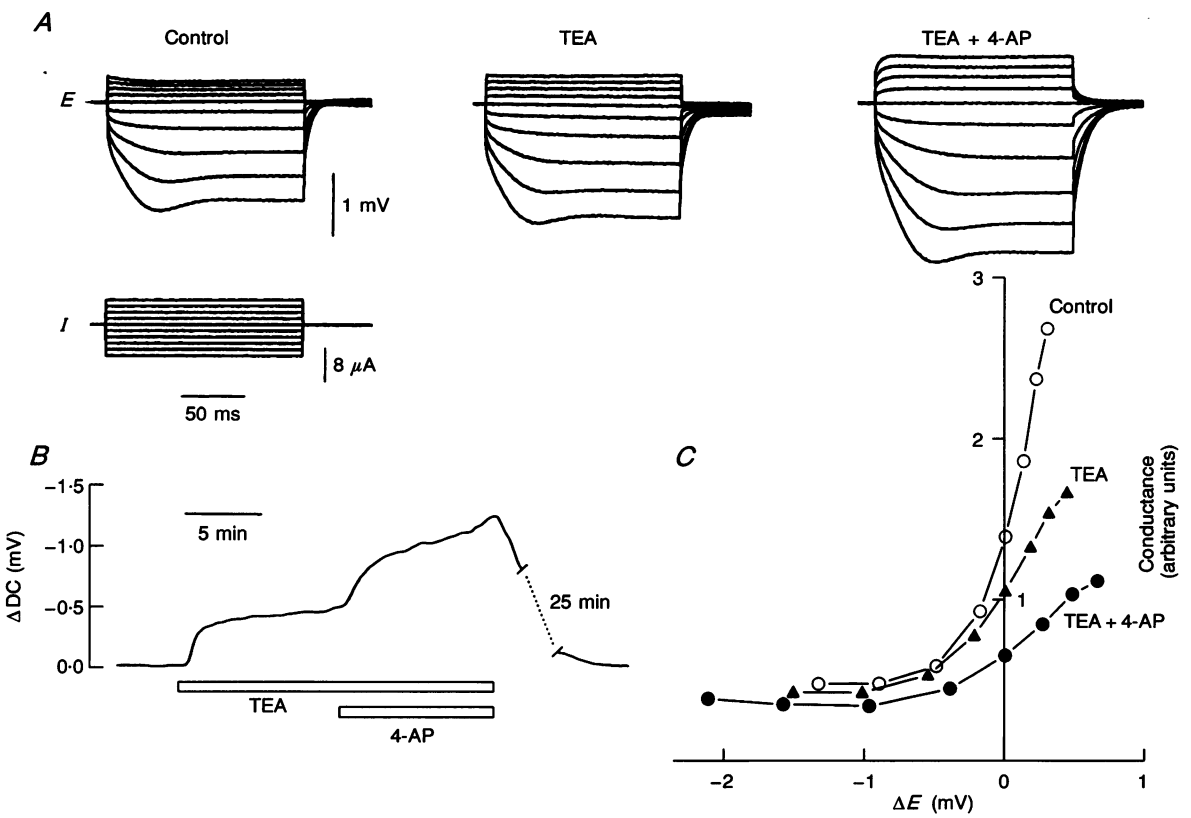

Figure 7. Effects of TEA and 4-AP on electrotonus and membrane potential
A, electrotonic response of a dorsal root to hyper- and depolarizing current pulses of 150 ms duration in the control solution, about 8 min after the addition of TEA (3 mM), and 8 min after 4-AP (3 mM) had been added to the TEA-containing solution. *B*, continuous recording of the extracellullar DC potential before, during and after the application of TEA and 4-AP. *C*, plots of slope conductance *versus* changes in potential (*E*) taken from the electrotonic behaviour shown in *A*.

produced more depolarization than 50 min of normoglycaemic hypoxia, the increase in input conductance was smaller (17%, as against 43% for hypoxia). One possibility for this apparent discrepancy in the extent of the conductance increase is that Ca^{2+}-activated and/or ATP-dependent K^+ channels, which have recently been described in axons (Jonas, Koh, Kampe, Hermsteiner & Vogel, 1991) and dorsal root ganglion cells (Duchen, 1990), are activated during normoglycaemic hypoxia and contribute to the conductance increase.

Hyperglycaemic hypoxia

In contrast to normoglycaemic hypoxia, a decrease in input conductance accompanied depolarization in hyperglycaemic hypoxia. Our data indicate (a) the mechanism underlying this peculiar change in membrane conductance and (b) the type of membrane conductance involved. Clues to the first topic came from the effects of CO_2 on the electrotonus, while blockers of known conductances were used to provide information about the second.

Numerous investigations have been performed concerning the effects of CO_2 on neural structures and functions, which are partly excitatory and partly inhibitory (Caspers & Speckmann, 1990). We know of no descriptions of the actions of high concentrations of CO_2 on axons, but depolarization of rat dorsal root ganglion cells has been described recently (Tegeder & Reeh, 1993). The similarity between the effects of CO_2-induced passive acidification and of hyperglycaemic hypoxia (Figs 2*B* and 5) strongly suggests that acidification is also involved in the latter situation, while the findings in Fig. 6 show that it is the cytoplasmic acidification which is important. However, the observations made by passive axonal acidification alone do not indicate which membrane conductances are involved.

Changes in electrotonus seen in the combined presence of TEA and 4-AP, known blockers of axonal K^+ channels (Waxman & Ritchie, 1993), resemble the effects of hyperglycaemic hypoxia and of passive cytoplasmic acidification. This indicates that an inhibition of pH-dependent K^+ channels is probably involved in both conditions. The existence of K^+ channels blocked by cytoplasmic acidification has been demonstrated in the giant axon of the squid (Wanke, Carbone & Testa, 1979; Clay, 1990) and, recently, in peripheral myelinated rat axons (Schneider *et al.* 1993*b*). The important new finding is that the K^+ channels involved in the generation of the resting potential are also blocked by cytoplasmic acidification.

Differences in osmolarity of solutions containing normal or high concentrations of D-glucose cannot explain our findings. Galactose and mannose are hexoses closely related to glucose. Only D-mannose, however, can enter neuronal

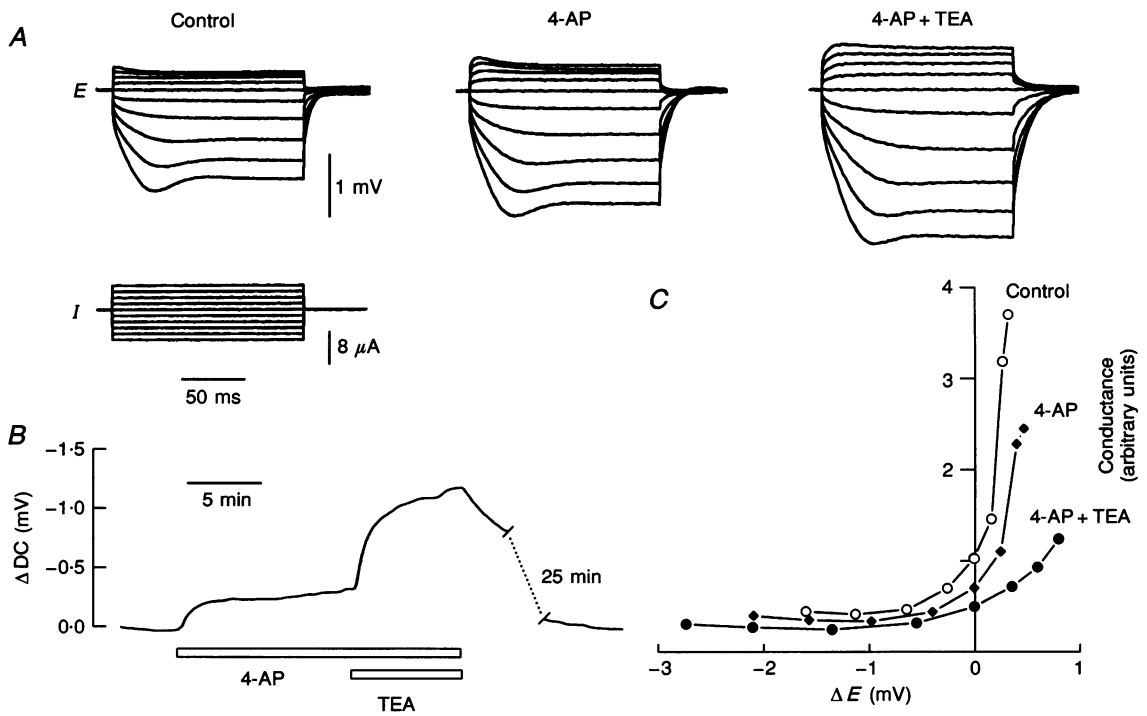

Figure 8. Effects of 4-AP and TEA on electrotonus and membrane potential

A, electrotonic response of a dorsal root to hyper- and depolarizing current pulses of 150 ms duration in the control solution, about 8 min after the addition of 4-AP (3 mM) and 8 min after TEA (3 mM) had been added to the 4-AP-containing solution. *B*, continuous recording of the extracellullar DC before, during and after the application of 4-AP and TEA. *C*, plots of slope conductance *versus* changes in potential (E) taken from the electrotonic behaviour shown in *A*.

glycolysis as quickly as D-glucose (Sokoloff, 1989). The finding that only high concentrations of mannose and not of galactose led to a hypoxia-induced decrease in input conductance is, therefore, a clear indication for the involvement of glycolysis (Fig. 3; see also Schneider *et al.* 1993*a*).

The effects of hyperglycaemic hypoxia differed from those following treatment with CO_2 in one important respect, namely that the effects of hyperglycaemic hypoxia were not readily reversed (Fig. 1). The difference between normoglycaemic and hyperglycaemic axons in the mechanism of depolarization during hypoxia does not therefore account for the differences observed in recovery after re-oxygenation (Fig. 1). A possible explanation for the persistence of the depolarization and conduction block with hyperglycaemia is that the intracellular acidification may continue long after normal oxygen tension is restored. Direct monitoring of intracellular pH will be required to investigate this phenomenon.

Relevance to diabetic neuropathy

Diabetic neuropathy is characterized by 'positive' and 'negative' changes in nerve excitability. Inhibition of fast axonal K^+ channels is a possible mechanism to explain paraesthesia and other positive symptoms (Kocsis, Bowe & Waxman, 1986) and we previously proposed that cytoplasmic acidification due to hyperglycaemic hypoxia might have this effect in diabetic neuropathy (Schneider *et al.* 1993*b*). In the present study, we have shown that (at least in sensory fibres) membrane depolarization is also a consequence of cytoplasmic acidification. The depolarization can directly block impulse conduction and, when maintained, the consequent increase in intracellular calcium (Waxman, Ransom & Stys, 1991) could lead to structural axonal degeneration (Schlaepfer & Bunge, 1973). Membrane depolarization has been proposed before as a step in the pathogenesis of diabetic neuropathy (e.g. Ritchie, 1985; Low, 1987), but this study provides new evidence of how depolarization results from the increased glycolysis in hyperglycaemic hypoxia.

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