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POTENTIALS ASSOCIATED WITH THE BLOOD-BRAIN BARRIER OF AN INSECT: RECORDINGS FROM IDENTIFIED NEUROGLIA

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SUMMARY

The blood-brain barrier of the insect nervous system is thought to be formed by the neuroglia. It is associated with a positive interstitial potential relative to the bathing medium ('sheath' potential), and with positive changes in potential that can be induced by raising the potassium level in the bathing medium. In central nervous connectives of the cockroach, *Periplaneta americana*, these potential differences (p.d.s) were measured just below the perineurium at the same magnitude as comparable p.d.s recorded deeper, indicating that they are produced by the perineurium. The interstitial p.d. apparently results from the presence of a paracellular resistance, and from the generation of less electromotive force (e.m.f.) by the basolateral membrane than by the apical (adglial). Recordings from cells identified by injection of peroxidase showed that the initial change in interstitial p.d. induced by high K was accompanied by: a depolarization of greater magnitude across the basolateral membrane, a small depolarization of the apical membrane, and no depolarization of sub-perineurial glial cells or axons. It therefore appears that the depolarization of the basolateral membrane by high K gives rise, by electrical coupling, to the shift in interstitial p.d. and the depolarization of the apical membrane. There was then a gradual depolarization of all membranes, consistent with a leak of K into the sub-perineurial interstitial system, and where the depolarization of the basolateral membrane would be produced by coupling to the apical. If the perineurium contains an intercellular resistance, then it must restrict diffusion of water-soluble substances.

INTRODUCTION

The central nervous system and major nerves of insects contain a blood-brain barrier system that enables the fluid around the neurones to have a different composition from that of the blood plasma which bathes the tissues. The available evidence indicates that this system is formed by the neuroglia (see Treherne & Schofield, 1981). However, no detailed electrophysiological analysis has been made of these cells or of the tissues forming a primary blood-brain interface in any animal species. The present study of the electrophysiological properties of morphologically

Key words: Electrophysiology, glia, blood-brain barrier, insect, *Periplaneta americana*.

identified neuroglia in an insect, and subsequent studies (Schofield & Treherne, 1984 Schofield, Swales & Treherne, 1984), were therefore carried out to provide unique quantitative information about a blood-brain barrier system.

Most of the neuroglia in the insect nervous system envelop the neurones, while the rest form a continuous layer (the perineurium) immediately under the thin sheath of connective tissue which forms the outermost layer of the nervous system (the neural lamella). The blood-brain barrier does not appear to involve the lamella, for this is permeated in seconds by the small ions involved in neuronal conduction and also by macromolecules as large as horseradish peroxidase (see Treherne & Pichon, 1972). Surgical removal of the lamella removes the barrier (see Treherne & Pichon, 1972), but only with accompanying damage to the neuroglia (Lane & Treherne, 1970).

Although net movements of some ions and molecules across the blood-brain barrier system may involve transport through the glial cells, the intercellular diffusion of some water-soluble substances is restricted (see Treherne & Schofield, 1979). Our knowledge of this restriction is limited to that provided by ultrastructural studies on the penetration of externally applied microperoxidase (M_r 1900) and ionic lanthanum (crystal radius, 96 pm; hydrated radius, 2·8 nm). These substances are unable to penetrate the perineurium when applied in normal saline: in the clefts between the lateral membranes of the perineurial cells, septate junctions arrest the passage of microperoxidase (Lane & Treherne, 1972) and, sometimes, lanthanum (L. S. Swales & N. J. Lane, in preparation), while the tight junctions are impermeable to both (Lane & Treherne, 1972; Treherne, Schofield & Lane, 1973). Lanthanum can be induced to pass the perineurial cells by a low external calcium concentration, but does not penetrate far among the sub-perineurial glia (Lane, Leslie & Swales, 1975), which are also joined by tight junctions (see Lane & Skaer, 1980). It also penetrates the perineurium of the insect eye (Shaw, 1983*a*,*b*). When lanthanum and dyes are injected below the perineurium in the insect eye, they do not diffuse extensively in the interstitial system (Shaw, 1983*a*) and are restricted in a sub-perineurial zone (Shaw, 1983*b*). This has been presented as evidence against the tight junction hypothesis for a discrete blood-brain barrier in insects; instead, it has been suggested that the barrier is 'an extensive property of the CNS' (Shaw, 1983*a*), or, more recently, that it lies in some sub-perineurial zone (Shaw, 1983*b*).

Such studies of tracer penetration have severe limitations. Lanthanum, peroxidase and other positively charged tracers will bind to the negative charges which are indicated by cation selectivity to line the path through tight junctions (see Erlij & Martinez-Palomo, 1978) and indicated by flux measurements to be present in the interstitial matrix (Treherne, Schofield & Lane, 1982). Negatively charged compounds would tend to be excluded from an anion matrix by Donnan forces, and reaction with tissue components will occur with dyes, such as Procion (Stead, 1973; Stretton & Kravitz, 1973) and Lucifer Yellow (Stewart, 1978). The tracer studies certainly do not show whether the intercellular junctions limit the free diffusion of the smaller ions, such as sodium or potassium (whose gradients across the barrier might be controlled by active transport). It should be noted that there is no increase in permeation of perineurial intercellular junctions by lanthanum when access of potassium to the axons is increased by stretching nerves (Pichon & Treherne, 1970, 1973) or briefly exposing them to hypertonic urea (Treherne *et al.* 1973). Furthermore, we do no

know whether the junctions in the perineurium are more important than those among the sub-perineurial glia, although this is suggested by their more organized appearance, and also by the observation of axons just below the perineurium which have little or no glial investment (see Lane & Skaer, 1980).

Across the neuroglia, a p.d. can be recorded that indicates the integrity of the barrier that they form. This p.d. can be recorded in the interstitial space outside the neurones in intact preparations, with a positive value relative to the bathing medium, and has been termed the sheath potential (Pichon & Boistel, 1967) or extraneuronal potential (Pichon & Treherne, 1970). Changes in the p.d. are produced by alterations in ionic composition of the bathing medium, in particular an elevation of the potassium level (Treherne, Lane, Moreton & Pichon, 1970), and may also be recorded with the sucrose-gap technique (Pichon & Treherne, 1970). When the barrier is abolished by surgical removal of the lamella, there is no interstitial p.d. (Pichon & Boistel, 1967) and no p.d. can be induced by high K (Treherne *et al.* 1970). The K-induced changes are also much reduced when the barrier is damaged by drying, stretching (Pichon & Treherne, 1970) or brief exposure to hypertonic urea (Treherne *et al.* 1973). Changes in p.d. are also produced by ethacrynic acid (Pichon & Treherne, 1974), which is known to reduce the rate of sodium movement across the barrier (Schofield & Treherne, 1975).

The rapidity of the changes induced by high K indicates that they arise from a diffusion potential (Pichon, Moreton & Treherne, 1971). This, together with the sensitivity to ethacrynic acid, suggests that the interstitial p.d. arises, at least in part, from a difference in membrane potential upon opposite sides of some layer of neuroglia (Pichon & Treherne, 1974). In this investigation, the origin of the interstitial p.d. in the central nervous system of the cockroach is sought by recording at different depths in the interstitial system, and by recording from glial cells identified by injection of peroxidase.

METHODS

Microelectrode recordings were made from one of the penultimate pair of connectives of the abdominal nerve cord of adult male cockroaches, *Periplaneta americana* L., reared in laboratory culture. The nerve cord was mounted in a Perspex chamber, essentially as used in earlier investigations (e.g. Treherne *et al.* 1973). Saline flowed continuously over the penultimate connectives, and could be rapidly changed by operating a multi-way non-return valve (Holder & Sattelle, 1972). This saline was earthed, downstream from the connectives, by a bridge of agar in 3 M-KCl, and was separated by seals of petroleum grease from saline bathing the rest of the preparation. Action potentials were evoked by electrical stimulation near the terminal ganglion. Experiments were carried out at room temperature (18–24 °C).

The saline was that of Treherne *et al.* (1982), except for making 0 mM-Na or 0 mM-Cl salines, and was chosen for ease of comparison of some results in this and subsequent papers (Schofield & Treherne, 1984; Schofield *et al.* 1984) with those of Treherne *et al.* (1982). It has the following composition: 120 mM-Na, 10 mM-K, 2 mM-Ca, 2 mM-Mg, 55 mM-mannitol, 5 mM-trehalose, 131·7 mM-Cl, 2·5 mM-HCO_3, 1·8 mM-HPO_4, 0·2 mM-H_2PO_4 (pH 7·8). High K saline (130 mM-K) was made by substitution of K for Na.

Saline for experiments to test the effect of removing external Na or Cl had 8·6 mM-HEPES, 3 mM-OH (pH 7·2), and 3·3 mM-Cl in place of the bicarbonate and phosphates, and mannitol concentration was 50 mM. To make the 0 mM-Na saline, the 120 mM-Na was replaced with 120 mM-tris(hydroxymethyl)methylamine and 107·3 mM-Cl. The 0 mM-Cl saline was made by substituting 127 mM-methyl sulphate for 127 mM-Cl, and 4 mM sulphate for the remaining 8 mM-Cl.

Microelectrodes were glass micropipettes. For experiments in which no peroxidase injection was made, electrodes were pulled from thin-walled glass (Clark Electromedical) to obtain fine-tipped electrodes which had a low resistance when filled with 3 M-KCl (5–10 MΩ). Tip potentials of such electrodes were less than 2 mV. Where peroxidase injection was attempted, electrodes for intracellular recording and marking were filled with a filtered (0·2 μm Millipore) solution of 2% horseradish peroxidase (HRP; Sigma type VI) in 0·5 M-KCl and 0·2 M-Tris buffer (pH 7·2) (15–30 MΩ), while electrodes for recording from the interstitial system were made from thick-walled glass (15–30 MΩ).

Leitz micromanipulators were used to lower electrodes into a connective. The depth of the electrode tip below the surface of the connective was measured from the calibrated scale of the manipulator as the electrode was withdrawn. Recordings made at depths greater than 20 μm were always made near the mid-line of the connective. To assist penetration, and hence keep dimpling of the connective to a minimum, the penultimate connectives were pre-treated for 10 min with 1% collagenase (Sigma type I), and initial penetration was made by advancing the electrode in steps of a few microns, followed by oscillation of the capacity compensation control in the recording amplifier (WPI M701). To help maintain steady recordings, the deepest electrodes were inserted first.

Cells were injected with HRP by passing positive current pulses (10 nA, 500 ms at 1 s intervals) into the HRP electrode over a period of 5–20 min. The electrode was then removed. After allowing 30 min for diffusion of peroxidase, the penultimate connectives were cut from the preparation and fixed for 30 min using 2·5% glutaraldehyde in 0·1 M-phosphate buffer (pH 7·2).

HRP was visualized for the electron-microscope by the polymerization of 3,3′-diaminobenzidine (DAB) using a procedure essentially as suggested by Itoh *et al.* (1979). After fixation, the connectives were placed for 10 min in each of the following: phosphate buffer, 0·5% $CoCl_2$ (Adams, 1977), and 0·2 M-Tris (pH 7·2). The material was then incubated at 37 °C in a medium consisting of 25 mg DAB tetrahydrochloride (Sigma), 7 units glucose oxidase (Sigma V), 100 mg β-D-glucose, 20 mg NH_4Cl, and 50 ml of 0·1 M-phosphate buffer (Lundquist & Josefsson, 1979). The reaction product was usually sufficiently dense after 1 h incubation, as judged under the binocular microscope. Connectives were then placed in phosphate buffer, where they could be kept for several days, before staining in 2% uranyl acetate, dehydration, and embedding in Araldite. Thin sections were cut on an LKB Ultratome III, stained with uranyl acetate and lead citrate, and examined in a Phillips EM300.

Probabilities of differences between values recorded simultaneously (denoted as 'pair *P*') were calculated by Wilcoxon matched-pairs signed-rank test. Other probabilities ('*P*') were calculated by Mann-Whitney U-test. All tests were two-tailed

RESULTS

Magnitude of p.d.s in the peripheral and deep interstitial system

Recordings made outside an axon, deep in the connective, were compared with recordings made simultaneously at the most peripheral interstitial position encountered (Fig. 1A). The size of the peripheral interstitial channels appears to be adequate for such recordings since they can be 100 nm in width (Fig. 3C), at least as large as channels outside axons where recordings may be made without attenuation (Treherne *et al.* 1970). Nine such paired recordings were made in nine preparations. An electrode was inserted into the axon, usually a giant, and then slowly retracted until a steady positive p.d., the sheath potential (Pichon & Boistel, 1967), was recorded (△ in Fig. 1B), with a mean value of 17 mV (S.E. 1·9) at a depth of 52 μm (S.E. 10·6). This position was about half-way to the central longitudinal axis of the connective (radius

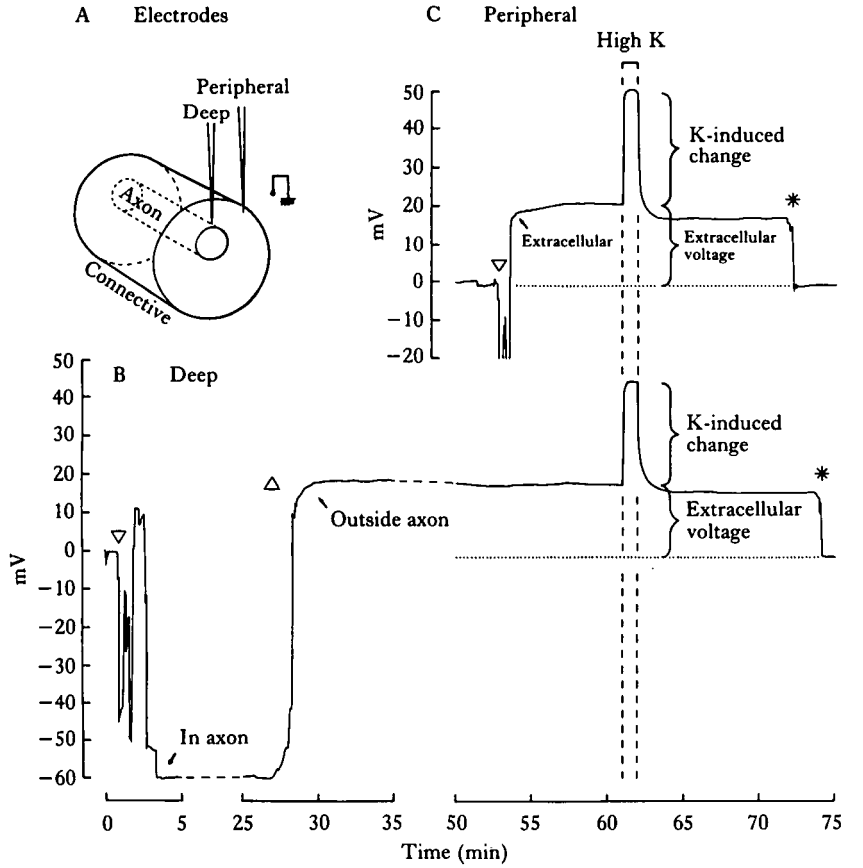


Fig. 1. Simultaneous recording at two depths in the interstitial system. (A) Connective in schematic cut-away view to show the deep electrode tip outside an axon, and the peripheral one just inside the connective. (B) Recording from the deep electrode shows insertion (▽), resting potential in an axon, and partial withdrawal to obtain the interstitial p.d. (△). High K then induces a positive, reversible change in p.d. (C) Recording from the peripheral electrode shows insertion (▽), with some transient negative p.d. before the appearance of the interstitial p.d., followed by the effects of high K. At the end of recording, both electrodes were withdrawn into the bath (*).

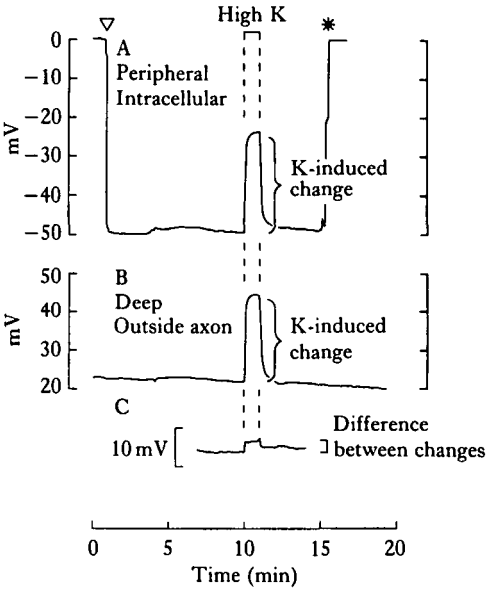


Fig. 2. Simultaneous recording of the K-induced change in p.d. (A) in an apparent perineurial cell, and (B) in the interstitial system outside an axon. (C) The difference between the magnitudes of the changes was significant (see text).

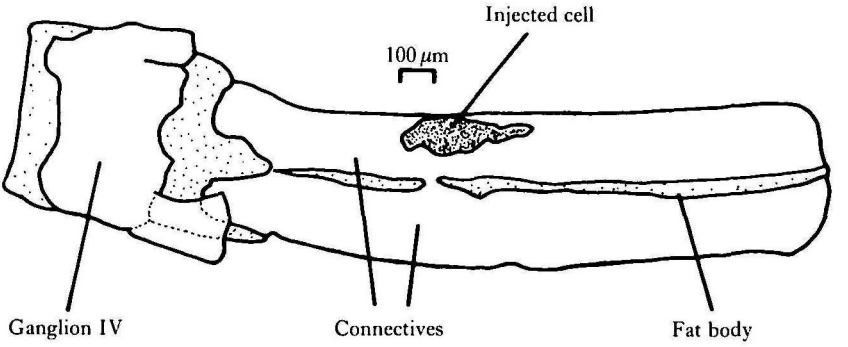
about 100 μm). A second electrode was then gradually advanced into the connective until the tip was indicated to be in an interstitial position by the recording of a positive p.d. (Fig. 1C). Although the recording was in a more peripheral position (pair $P = 0{\cdot}0039$), at a mean depth of 12 μm (S.E. 1·3), the p.d. was no different to that at the deeper position (pair $P = 0{\cdot}46$), averaging 18 mV (S.E. 2·4). The K level in the saline was then elevated to induce a positive change in p.d. (Fig. 1B, C), an extracellular potential change (Pichon & Treherne, 1970). The initial step in p.d. recorded at the periphery (18 mV, S.E. 3·3) was no different (pair $P = 0{\cdot}84$) to that recorded deeper (18 mV; S.E. 3·0).

The interstitial p.d. and the K-induced change were thus recorded at full magnitude just below the perineurium.

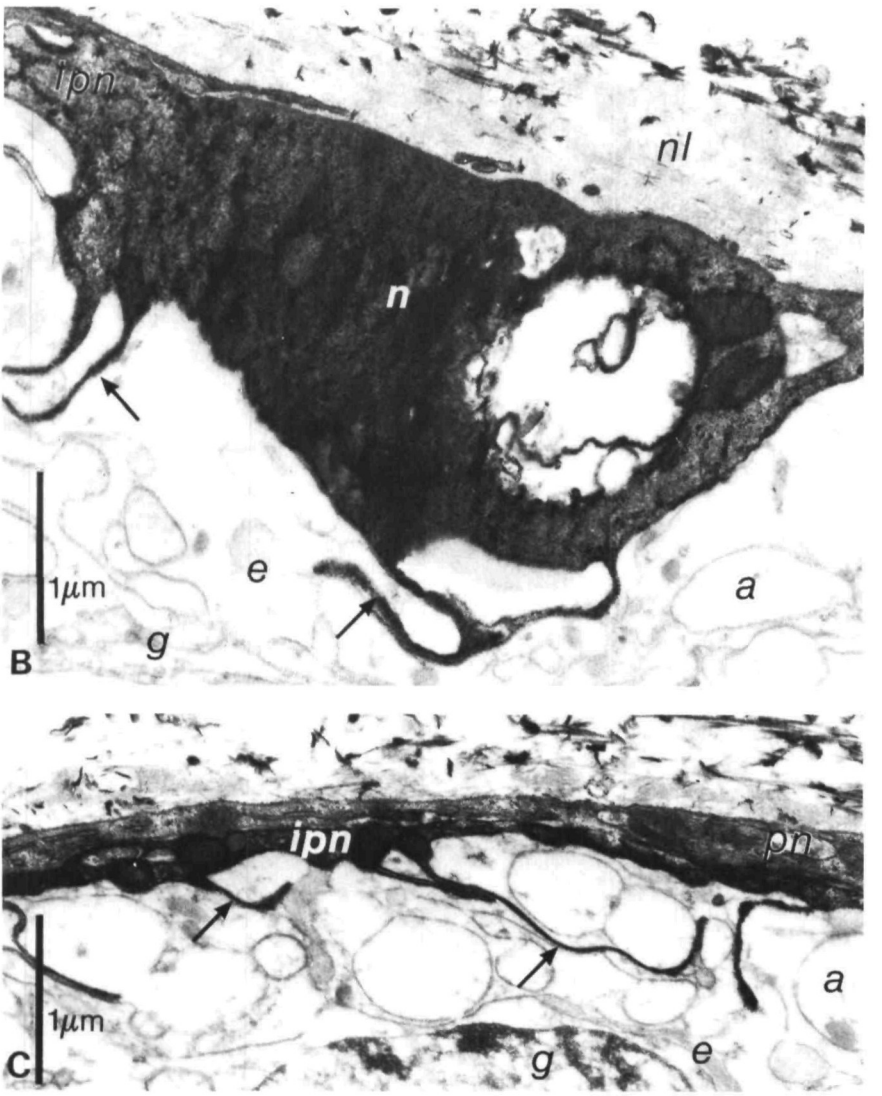
Recordings from perineurial cells

While making the above observations, a negative p.d. was often recorded as the electrode entered the preparation (▽ in Fig. 1B, C). The electrode tip could be held in this peripheral, apparently intracellular position (Fig. 2A). As the electrode was

Fig. 3. (A) Portion of nerve cord to show perineurial cell injected with HRP, in one of the connectives between ganglia IV and V. Drawn after DAB development and fixation (×40). The fat body stained during DAB development. (B) Transverse thin section showing the above injected perineurial cell (*ipn*) lying below the neural lamella (*nl*). In the region of the nucleus (*n*), there is a hole apparently made by the electrode. Processes from the cell (arrows) descend among axons (*a*) and glia (*g*). Interstitial system (*e*). ×22 400. (C) Serial section of the same cell (*ipn*) showing attenuation compared to the nuclear region above. Interstitial channels have widths of up to about 100 nm (*e*). Other symbols as above. ×22 400. Scale bars, 1 μm.


Injected cell
100 μm
Ganglion IV
Connectives
Fat body

3A


ipn
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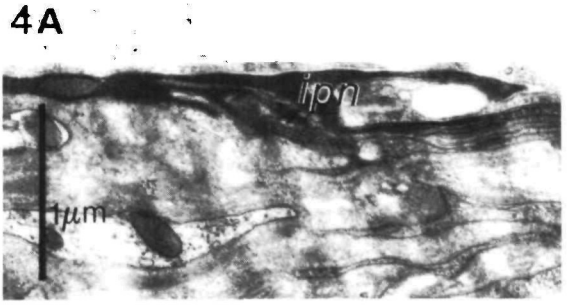
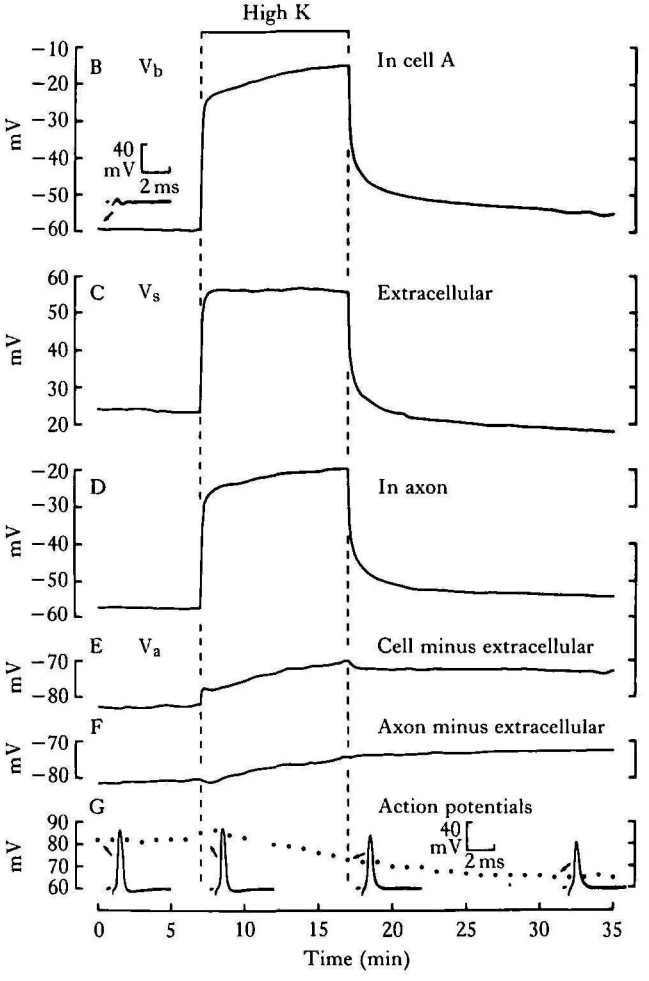

4A
1μm


High K
B Vb
In cell A
40 mV
2 ms
C Vs
Extracellular
D
In axon
E Va
Cell minus extracellular
F
Axon minus extracellular
G
Action potentials
mV
Time (min)

withdrawn, there was no appearance of a positive p.d. during the return to the bath (▽ in Fig. 2A), unlike when an electrode was withdrawn from an axon (△ in Fig. 1B). In later experiments, such recordings of apparent resting potential were identified as recordings from perineurial cells by injection of peroxidase just before withdrawing the electrode.

For each injection, only one cell was found to be marked. Under the light microscope, the cell could be seen to be superficial, extending up to 400 μm along the longitudinal axis (Fig. 3A). Using the electron microscope, the cell was found to be up to 2 μm in thickness near the cell nucleus, and it is likely that most recordings were made in this region (Fig. 3B), for there would be little room for the electrode tip in the rest of the cell, which was generally less than 0·2 μm thick (Figs 3C, 4A). The basolateral membrane, facing toward the saline, extended smoothly under the neural lamella (Figs 3B, 4A) and against the adjacent perineurial cells (Fig. 3C). The apical membrane, facing the interstitial system below the perineurium, appeared more extensive, being folded into many pleats parallel to the axons, as previously described in uninjected cells (Maddrell & Treherne, 1967). These folds projected among both sub-perineurial glia and axons, to a depth of up to 2 μm (Figs 3C, 4A).

Magnitude of p.d.s in perineurial cells and interstitial system

Recordings from seven apparent perineurial cells (Fig. 2A) were compared with recordings made simultaneously from outside axons (Fig. 2B) in six preparations. Recorded depth of the cells was 3 μm (S.E. 3·8), not significantly different from the depth of the peripheral interstitial recordings of the preceding section ($P => 0{\cdot}05$). Recordings at the deeper level (pair $P = 0{\cdot}016$), outside the axons, were made at a depth of 56 μm (S.E. 10·0), very similar to that for the preceding section ($P > 0{\cdot}10$). Since the p.d. recorded from the cell was made relative to the bath, it represents the p.d. across the basolateral membrane, and had a resting level of −50 mV (S.E. 2·0). This value could be in error due to damage of the cell by the electrode, but such damage is not indicated by the abrupt achievement of stable resting potential (e.g. Fig. 2A) and by the retention of peroxidase within injected cells, even near the apparent injection site (e.g. Fig. 3B). The interstitial p.d. had a value of 19 mV (S.E. 3·0), similar to that obtained above in the deep position ($P > 0{\cdot}10$), and peripherally ($P > 0{\cdot}10$). The perineurium is formed by the folds of only one layer of cells. Since the opposite faces of these cells are often less than 0·2 μm apart (Figs 3C, 4A), the resistance between these faces would not be high enough to support gradients of potential as large as the interstitial p.d. (Gradients of less than 1 mV μm^{-1} have been reported in other cells: Zeuthen, 1976.) It follows that the potential of the cell relative to the interstitial system, i.e. the p.d. across the apical membrane, is found to rest at −69 mV (S.E. 3·5). (Any error due to junction potential will be different for

Fig. 4. (A) Transverse thin section of connective (×22 400) to show perineurial cell identified by peroxidase injection (*ipn*) after simultaneous recordings of effects of high K upon p.d.s recorded: (B) across the basolateral membrane (V_b), (C) in the interstitial system (V_s), (D) in an axon. (E) Voltage across the apical membrane (V_a) was obtained from V_s and V_b. (F) Axon resting potential was obtained from V_s and the p.d. in the axon. (G) Action potentials were recorded in the axon following extracellular stimulation of the nerve cord, whereas there was no obvious response in the perineurial cell (inset of B).

this p.d. than for the recording of basolateral p.d. because the reference electrode is in a different medium. Note that tip potentials were low: see Methods.)

When the connective was exposed to high K, the initial depolarization recorded from the cell averaged 24 mV (S.E. 3·6), significantly different to the step recorded outside the axon (pair $P = 0{\cdot}016$), which averaged 20 mV (S.E. 3·4). This difference (e.g. Fig. 2C) means that there was a depolarization of the apical membrane by an average of 4 mV (S.E. 0·6). A hyperpolarization was never recorded, unlike for the smaller, background fluctuations in p.d.

Effects of 0 mM-Na and 0 mM-Cl

The effect of removal of Na or Cl from the saline upon the p.d. recorded from three apparent perineurial cells, in three preparations, was compared with the effects upon simultaneous recording of interstitial p.d. When Na was removed, the basolateral membrane was depolarized by 1·6 mV (S.E. 0·44), not significantly different from the change of 1·4 mV (S.E. 0·23) in interstitial p.d. (pair $P = 0{\cdot}75$). There was also no difference between the hyperpolarization of the membrane by −1·7 mV (S.E. 0·78) induced by 0 mM-Cl and the simultaneous reduction of −1·2 mV (S.E. 0·13) in interstitial p.d. (pair $P = 0{\cdot}75$).

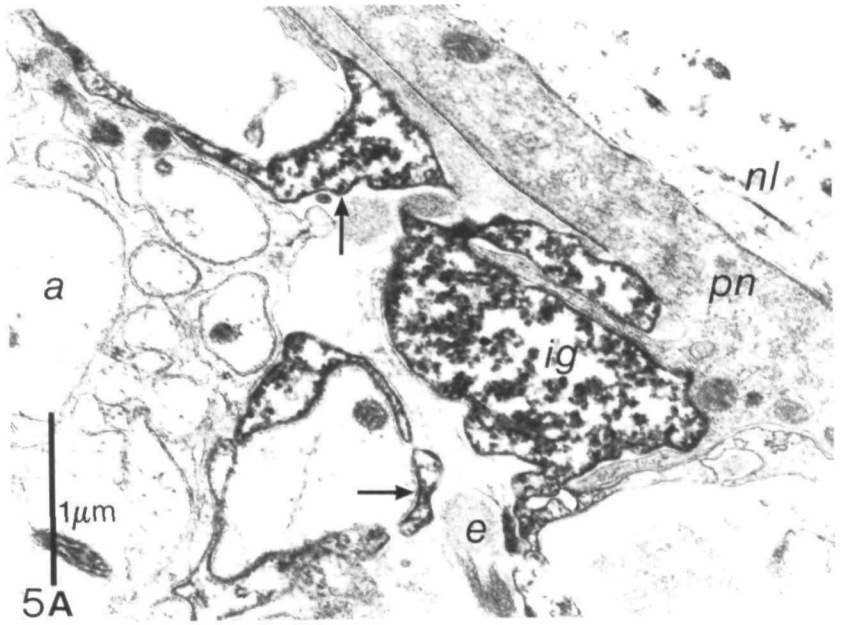
Since these substitutions produced relatively little change in p.d., no control was made upon any secondary effects they may have had, for example of Tris upon metabolism, or of methyl sulphate upon free calcium concentration.

Time course of p.d. changes induced by high K

The initial changes in p.d. induced by high K were completed in under 15 s. They were followed by a slower, progressive increase in the difference between the p.d. across the basolateral membrane (V_b) and the interstitial p.d. (V_s), which meant a slow positive shift in the p.d. across the apical membrane (V_a). As in the case of the identified cell shown in Fig. 4A, this slow change (Fig. 4E) usually involved a positive shift in V_b (Fig. 4B). V_s either remained steady (Fig. 4C) or fell (Fig. 1A of Schofield *et al.* 1984). In this section of the Results, and the next, the electrode used to impale the glial cell contained an HRP solution (see Methods). For all impalements which lasted long enough to inject the cell, the marked cell had the appearance under the light microscope of a perineurial cell, and some cells were later identified as such under the electron microscope.

The time course of the recordings was compared with events monitored in an axon, in four preparations. The axon membrane potential was deduced from the difference between the interstitial p.d. (Fig. 4C) and the p.d. recorded in the axon (Fig. 4D). The initial depolarization of the apical membrane was accompanied by an apparent hyperpolarization of the axon (Fig. 4F) or by no change. Axon hyperpolarization was associated with an increase in amplitude of the evoked action potentials (Fig. 4G). In contrast, the slow depolarization of the apical membrane was closely followed by a depolarization of the axon (Fig. 4F) and the concomitant decline in action potential amplitude (Fig. 4G).

The initial abrupt depolarization in V_a is thus not accompanied by a similar change in the axon, whereas the slow depolarization of V_a is associated with an axon



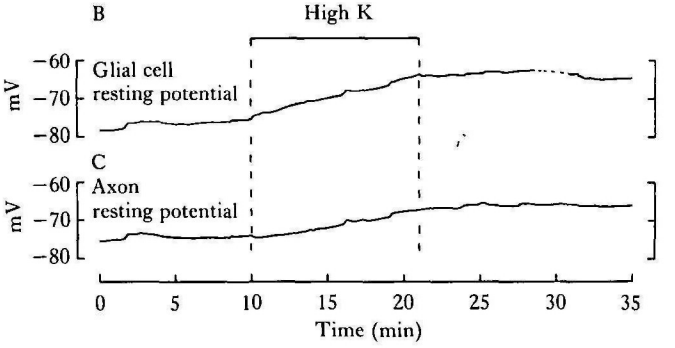


Fig. 5. (A) Transverse thin section of connective (×22 400) to show glial cell injected with peroxidase (*ig*), below the perineurium (*pn*). Processes of the cell are arrowed. Other symbols as Fig. 4. (B) Effect of high K upon resting potentials in the above glial cell and an axon, derived from p.d.s recorded simultaneously in the interstitial system, glial cells and axon.

depolarization, indicating an accumulation of potassium, leaking in from the high concentration outside the connective.

Recordings from glial cells below the perineurium

Recordings were made below the level of the perineurium from some cells that did not show spikes at times when spikes were recorded from an axon. Two of these cells were identified as sub-perineurial glial cells (Fig. 5A). Upon raising the K level in the saline, the glia showed a depolarization (Fig. 5B) that had a time course similar to that recorded in the axon (Fig. 5C); the initial depolarization was not as rapid as for the apical perineurial membrane.

DISCUSSION

The sheath potential in the nervous system of the cockroach, and the changes induced by high K, can now be seen to be generated across the perineurium. Since the perineurium consists of a single layer of cells, these p.d.s must be determined by the resistances and e.m.f. sources within the basolateral membrane (facing the medium), the apical membrane (adjacent to the glia below the perineurium), and the intercellular channels across the perineurium. The neural lamella can be expected to generate no e.m.f., for example, none is indicated for the stroma of the rabbit corneal endothelium (Lim & Fischbarg, 1981). Characteristics of the apical membranes could also reflect those of some sub-perineurial glia, by coupling through the gap junctions (see Bennett, 1978) that are present (see Lane & Skaer, 1980), but the lack of a rapid shift in membrane potential in these glia at a time when a depolarization of the apical membrane can be recorded (Fig. 5B) suggests that such coupling has a long time constant or may be absent. (Further investigation is needed to establish whether these cells are coupled.) The membranes can be expected to generate resting potentials that are chiefly sensitive to potassium gradient, in common with other glial cells (see Somjen, 1975), including apparent glia of another insect (Coles & Tsacopoulos, 1979). Any e.m.f. generated along the intercellular channel, on the other hand, is likely to be small, since selectivity is probably low (see Erlij & Martinez-Palomo, 1978) and ionic composition of the saline is unlikely to be greatly different from that of the interstitial fluid (Thomas & Treherne, 1975).

A difference between the e.m.f.s generated by the basolateral and apical membranes, together with some small e.m.f. generated within the intercellular channels, could thus give rise to the interstitial p.d. A contribution of membrane potential is indicated by the effects of high K (Treherne *et al.* 1970; Fig. 1) and ethacrynic acid (Pichon & Treherne, 1974). For the p.d. across one membrane to be different from that across the other, there would have to be a resistance in the intercellular channels. Tight junctions are present at this point (see Lane & Skaer, 1980) and are known to provide intercellular resistance in other tissues (see Erlij & Martinez-Palomo, 1978). Different e.m.f.s could be generated if the two membranes had similar ionic sensitivities, for the K concentration in the sub-perineurial interstitial system can be expected to be less than the 10 mM in the saline (Thomas & Treherne, 1975). Alternatively, a difference in e.m.f. might arise from different ionic sensitivities, or differences in the distribution of electrogenic pumps (Pichon & Treherne, 1974). In

preparations maintained in saline of lower K concentration, there could be less difference in generated e.m.f. than in the present study, which would explain why the interstitial p.d. observed previously in 3 mM-K saline (7 mV; Pichon & Boistel, 1967) is less than that observed here (18 mV). It is important to realize that the presence of the interstitial p.d. means that there has to be a resistance or e.m.f. source in the channels, and that this paracellular shunt and the membranes would be electrically coupled; in such a system, the resistance and e.m.f. of any component can influence the p.d.s recorded across the other components (see Boulpaep, 1971). A quantitative analysis of these effects is made in the subsequent paper (Schofield & Treherne, 1984).

The initial changes in interstitial p.d. that are induced by high K can be predicted to result largely from the effect of K upon the basolateral membrane, and to appear across the perineurium by electrical coupling. The shunt in many epithelia is not much more sensitive to potassium than to sodium (see Erlij & Martinez-Palomo, 1978), although a relatively high selectivity has been reported for one K-selective tight epithelium (Frizzell, Koch & Schultz, 1976). An origin in the membrane rather than the shunt is supported by the recordings from identified perineurial cells, made simultaneously with recordings of interstitial p.d., which showed that high K induced a larger shift in p.d. across the membrane (24 mV) than across the perineurium. Changes in concentration of Na or Cl produced small changes in p.d. that were similar at the two sites, and it is therefore not clear whether they originate primarily from the membrane or the intercellular channel.

Accumulation of potassium in the sub-perineurial interstitial system, following elevation of the level in the saline, was indicated by depolarization of axons (Fig. 4F, G), as in a previous study (Treherne *et al.* 1970). The observed two-stage depolarization of the apical membranes might, therefore, indicate an initial rapid accumulation of K at the apical surface, followed by a slower one. An initial rapid rise in potassium level has been suggested to account for the relationship between the apparent change in interstitial p.d. and the external K level (Pichon *et al.* 1971), but this proposal was based on the assumption that the p.d. change would be directly proportional to a diffusion potential generated in the blood-brain barrier, whereas the electrical coupling would not yield such a relationship (Schofield & Treherne, 1984). There is no known mechanism to account for a sudden fall in the rate of K accumulation, and no initial rapid depolarization was observed in axons or sub-perineurial glia. (Instead, the recordings from axons indicated an initial hyperpolarization, accompanied by an increase in amplitude of action potentials, which may be an artefact, but could be taken to indicate a decrease in interstitial K level.) An alternative explanation for the initial rapid depolarization of the apical membrane is that it results from electrical coupling to the basolateral membrane.

A similar argument applies to the slow depolarization of the basolateral membrane which accompanies the slow depolarization of the apical membrane (Fig. 4E), axons (Figs 4F, 5C) and sub-perineurial glia (Fig. 5B). This depolarization might result from a slow accumulation of potassium outside the basolateral membrane, but it is difficult to see how such could still be occurring some minutes after K starts accumulating at the axon surface. The depolarization might also occur by loss of potassium from the cell, but this is unlikely with increased external K concentration

Again, the depolarization may be explained by electrical coupling, but this time it is the depolarization of the apical membrane which has an effect upon the basolateral membrane. (It is also possible that there is some depolarization due to swelling, as discussed in a later paper: Schofield *et al.* 1984.)

The electrical resistance in the intercellular channels of the perineurium, indicated by these observations, must represent a restriction to diffusion. Among the glia below the perineurium, there could be further resistance, and hence more restriction. Recording of interstitial p.d.s with the same magnitudes at different depths among the sub-perineurial glia does not preclude the presence of such resistance, given that the p.d.s are generated across the perineurium. To establish whether the perineurium forms the principal resistance across the neuroglia, and hence the principal barrier to diffusion, it is therefore necessary to make direct measurement of resistance, as in the subsequent paper (Schofield & Treherne, 1984).

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