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OSCILLATIONS OF GLIAL MEMBRANE POTENTIAL IN A LOCALIZED REGION OF THE BLOOD–BRAIN BARRIER OF AN INSECT

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Summary

In an abdominal ganglion of the American cockroach, *Periplaneta americana*, electrophysiological characteristics of the perineurium, the glial layer that forms the insect blood–brain barrier, were investigated by microelectrode recording. The potential across the barrier was positive relative to the bathing medium. Its value increased when the external K concentration was raised, as to be expected from a depolarization of the perineurial cell membrane facing the saline, the basolateral membrane. A negative 'all-or-nothing' transient, having an amplitude of some 30 mV and a half-amplitude duration of about 1.5 min, was also induced, either during the K elevation or after. Given sufficient K exposure, a series of these transients occurred, with a periodicity of 3–15 min, indicating that a cellular oscillator had been activated. The delay between application of high K and the appearance of the first transient was variable, and could be as long as 60 min for a threefold increase in K level. The transients could persist upon return of normal saline, for at least 85 min, with little change in amplitude. By recording transperineurial potential simultaneously with recording from superficial cells, one of which was identified as a perineurial cell by peroxidase injection, the transients were found to be generated by a depolarization of the membrane adjacent to the underlying nerve cells, the adglial membrane. Analysis using a simple electrical model yielded values for the resting electromotive force (e.m.f.) generated by the membranes, −50 mV for the basolateral, −71 mV for the adglial, and indicated that the paracellular pathway had a resistance 4.3 times larger than the transcellular resistance. These results reveal a cellular oscillator, apparently of cytosolic type, in the perineurial glia of an insect ganglion, and demonstrate physiological differences between the perineurium of the ganglion and that of other regions of the insect nervous system.

Introduction

The ganglia of the insect nervous system are the sites of much synaptic and metabolic activity. This activity is supported by the superficial layer of neuroglia,

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the perineurium, that forms the principal component of the blood–brain barrier (Schofield and Treherne, 1984; see Treherne, 1985). The barrier is important in conservation of the nerve cell microenvironment and is involved in the transfer of various metabolites (see Wigglesworth, 1972; Treherne, 1985). The organization of the perineurium in the ganglion is unlike that in the central nervous system and major peripheral nerves: studies made chiefly upon cockroach show that the layer is thicker over many areas of the ganglion, often being formed by the interleaving of several cell processes; and the cells show signs of more intense metabolic activity, having a higher concentration of glycogen granules and mitochondria (Wigglesworth, 1960; Smith and Treherne, 1963; Maddrell and Treherne, 1967). It is therefore of interest to compare the physiology of the perineurium in the ganglion with that in other regions.

In this paper, electrophysiological characteristics of the perineurium are investigated in an abdominal ganglion of a cockroach, *Periplaneta americana*. Recordings of the potential across the perineurium are made, and the potassium sensitivity is determined, as in previous investigations of permeability of the insect blood–brain barrier (e.g. Schofield and Treherne, 1986). A potassium-induced oscillatory response is described, and its origin is determined by recording from identified glia.

Materials and methods

Adult male American cockroaches, *Periplaneta americana*, were obtained from laboratory culture. A length of abdominal nerve cord, comprising the third unfused ganglion to the terminal ganglion, was dissected out under saline. A polyamide ligature (Ethilon 11/0: Ethicon Ltd) was tied around each large nerve root of the fourth ganglion (ganglion V of Shankland, 1965), except where noted in the second section of the Results. Experiments were carried out at room temperature.

To make recordings from ganglion V, the preparation was mounted, ventral surface uppermost, in an acrylic chamber, similar to that used in previous investigations (e.g. Treherne *et al.* 1973). The chamber had three compartments, joined by a transecting channel lined with grease (high vacuum Glisseal: Borer A.G.). The preparation was laid in the channel so that ganglion V lay in the central recording compartment, with the two posterior ganglia in one side compartment, and the anterior ganglion in the other. Ganglion V was positioned above a supporting arch formed from acetate plastic sheet (Fig. 1). There was a hole on top of the arch, about 0.6 mm in diameter, to allow access of saline to the under face of the ganglion. Grease covered the rest of the arch and was applied around the peripheral nerves, and the connectives leading from the ganglion, so that the surface of the ganglion would be electrically isolated from the rest of the tissue. The recording compartment and adjacent compartments were filled with normal saline (the time from setting up the preparation noted for some experiments was measured from this point). For the next 10 min, collagenase

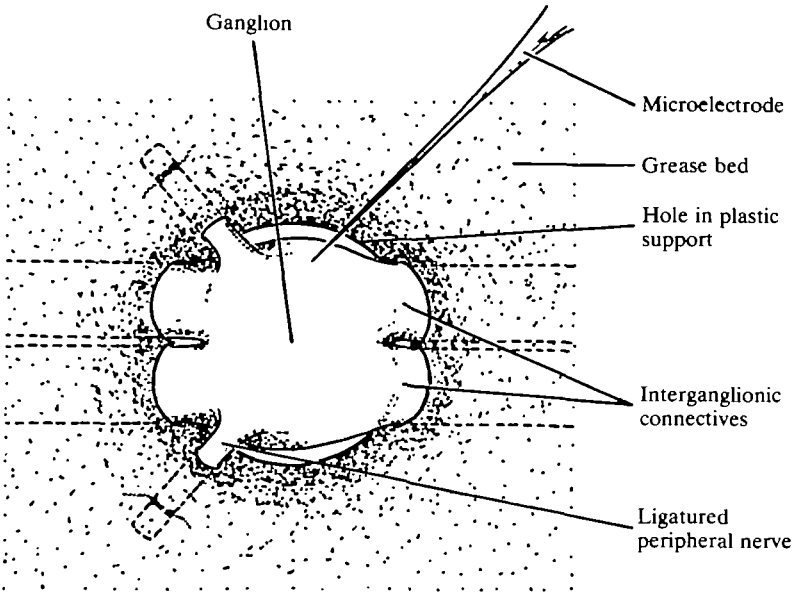

Fig. 1. Plan view of the abdominal ganglion positioned over a hole in a plastic support, for microelectrode recording. The interganglionic connectives and the peripheral nerves were embedded in grease on the support.

(type 1-S: Sigma) was applied to the ganglion only, as a 1 % solution in normal saline, to render the neural lamella, the fibrous outer sheath, more easily penetrable by microelectrodes. During the rest of the experiment, saline flowed continuously through the recording compartment, coming from a non-return manifold (Holder and Sattelle, 1972) which was gravity-fed from reservoirs by use of solenoid-operated valves.

Normal saline, modelled on a previous version (Schofield *et al.* 1984), was composed as follows (mmol l^{-1}): Na, 120; K, 10; methylglucamine, 22; Ca, 2; Mg, 2; glycerol, 10; trehalose, 5; Cl, 130; benzenesulphonate, 25; OH, 5; Hepes, 14.3 (pH 7). (Choline was used instead of methylglucamine where noted in the second section of the Results). Phenol Red was usually added as monosodium salt at a final concentration of 0.0001 % (2.7 μmol l^{-1}). A 100 mmol l^{-1} K saline was made by replacement of 90 mmol l^{-1} Na by the equivalent amount of K. To make 30 mmol l^{-1} K saline, keeping Na concentration at 120 mmol l^{-1}, K was substituted for 20 mmol l^{-1} methylglucamine. All salines were filtered (0.22 μm: Millipore).

Microelectrodes were drawn from thin-walled aluminosilicate glass tubing, 1.0 mm o.d. This type of glass was used, rather than borosilicate, to reduce breakage of the tip when penetrating the nerve sheath. Except for peroxidase-marking, the electrodes contained filtered (0.1 μm: Millipore) 3 mol l^{-1} KCl at pH 7.2 (5 mmol l^{-1} Hepes), and had a resistance of 3–6 MΩ. For injection of peroxidase a recording electrode was filled with a filtered (0.1 μm: Millipore) 2 % solution of horseradish peroxidase (HRP) (Type VI: Sigma) in 0.2 mol l^{-1} Tris

buffer (pH 7.2) containing 0.5 mol l^{-1} KCl: resistance was about 40 MΩ. Leitz micromanipulators were used to insert electrodes into the preparation, essentially as in an earlier investigation (Schofield *et al.* 1984), with the apparatus supported on an air-table (AVT-701: Wentworth Laboratories Ltd).

Electrophysiological recordings were made conventionally, using amplifiers of high input impedance connected to an oscilloscope and chart recorder. The saline in the recording compartment was held at earth potential using a voltage-clamp circuit. For most experiments, a microcomputer (PC/ATe: IBM) fitted with an A/D convertor (DT2821-F: Data Translation) was used to digitize the signals and to operate the solenoid valves controlling saline flow. Programs were written in Asyst (v2.1).

For injection of HRP, positive current pulses (10 nA, 500 ms, 1 s intervals) were passed for about 5 min. Subsequent fixation and polymerization of 3,3'-diaminobenzidine were carried out as previously described (Schofield *et al.* 1984). Dr P. J. S. Smith of this laboratory kindly completed the procedure, as follows. The preparations were dehydrated and embedded in Araldite (CY212: TAAB), following a standard procedure (Smith *et al.* 1984), and transverse sections were cut (Ultracut: Reichert-Jung). After localization of the injection site in 2 μm sections examined with Nomarski optics (Labovert: Leitz), thin sections were cut serial to the thick sections. These were then stained, using uranyl acetate and lead citrate, for examination in the electron microscope (STEM 420: Phillips).

Data values are given as mean and standard error. For statistical tests, a significance level of 5 % was adopted.

Results

Electrical model

Potentials recorded from the perineurium (Fig. 2) were interpreted in terms of an electrical model (Schofield and Treherne, 1984). In this model, the paracellular pathway across the perineurium contains intercellular occlusions (principally tight junctions) which are represented by a shunt; the cellular component consists of the basolateral membrane, bathed by the saline, and the adglial membrane, next to the underlying nerve cells. It must be noted that the model represents the perineurium of the ganglion in a much simplified fashion: all intercellular occlusions are lumped as a single shunt; the interdigitating membranes above the level of the paracellular shunt are considered as one basolateral membrane; the membranes below the level of the shunt are termed the adglial membrane; and the cytosol is assumed to be isopotential by virtue of low-resistance gap junctions linking the cells.

In electrical terms, the circuit consists of a loop composed of the shunt and the two membranes. The shunt has a resistance R_s and electromotive force (e.m.f.) E_s; the basolateral membrane has a resistance R_b and e.m.f. E_b; and the adglial membrane has a resistance R_a and e.m.f. E_a. Since E_s should be small, it can be ignored for many purposes. In the absence of E_s, any difference between e.m.f.s

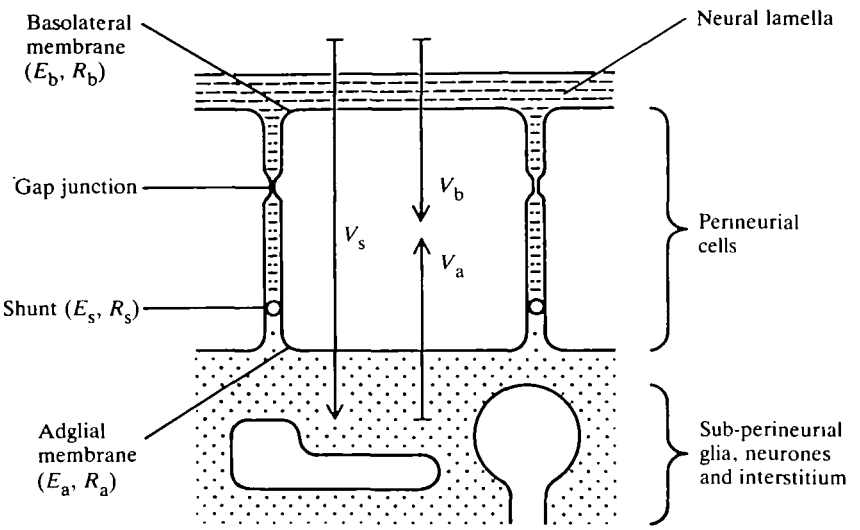

Fig. 2. Schematic section through the perineurium in the insect ganglion (not to scale), showing where the potentials were recorded. The perineurium is represented by the principal components of an electrical model (Schofield and Treherne, 1984), as described in the text. The perineurium lies under the neural lamella (the outer connective tissue layer of the nervous system), and lies over glial cells and neurone cell bodies. The transperineurial potential V_s was recorded with an electrode inserted in the sub-perineurial interstitium; the basolateral membrane potential V_b was recorded with an electrode in a perineurial cell; and the adglial membrane potential V_a was obtained as the difference between V_s and V_b.

generated by the basolateral and adglial membranes will produce current flow across the cell, with a return path through the shunt. The current will induce a potential across the shunt, i.e. the potential V_s across the barrier, and will also contribute to basolateral membrane potential V_b and adglial membrane potential V_a. By virtue of this current, a change in value of a parameter will change the potential recorded across any component.

The transperineurial potential (V_s)

To record the potential across the blood–brain barrier in the ganglion, a microelectrode was inserted carefully so that the tip lay just below the perineurium, and was then repositioned to avoid cell resting potentials, to place the tip in the interstitial system, among the nerve cells (Fig. 2). The transperineurial interstitial potential (V_s) was then recorded relative to the bathing medium. It had a peak value of 15–25 mV, and showed a decay of about 10 mV in the first hour, then a more gradual decline.

K-induced oscillations in V_s

When the external concentration of potassium was raised, there was a positive shift in V_s (Fig. 3), as to be expected from a depolarization of the basolateral

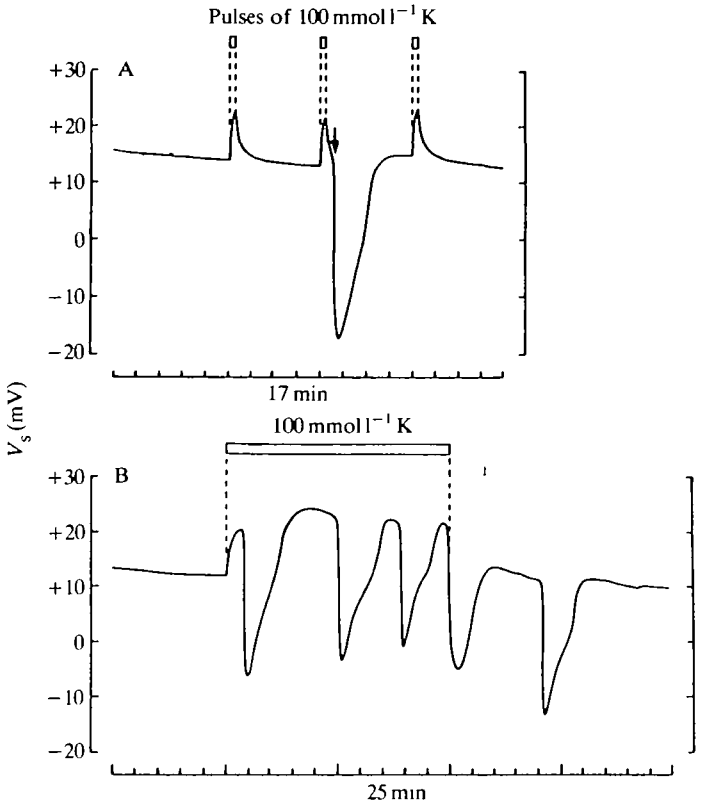

Fig. 3. Recordings of transperineurial interstitial potential (V_s) in the fifth abdominal ganglion to show the effects of elevating K concentration from 10 to 100 mmol l^{-1}. (A) Effect of three 15 s pulses of high K. Each application induced a positive shift in potential. The positive shifts induced by the first and third applications to the preparation were each followed by a smooth restoration of the potential to the resting value; the second was followed by a transient negative shift (arrow) before recovery. (B) Effect of 10 min exposure to high K. During the high-K exposure, there was a positive shift in potential on which three negative transients of decreasing amplitude were superimposed. A further transient commenced as the K concentration of 10 mmol l^{-1} was restored, followed by a fifth.

membrane (Schofield and Treherne, 1984). For a series of K pulses, elevating the K concentration 10-fold for 15 s, the positive shift produced by each pulse was sometimes followed by a negative transient (Fig. 3A, arrow), rather than a smooth recovery of potential (Fig. 3A, first and third pulses). The transients showed an initial negative shift that developed rapidly, followed by a slower multiphasic recovery that sometimes involved an overshoot above the baseline. They had an amplitude of some −30 mV and a duration at half-amplitude of about 1.5 min.

For K elevations of several minutes in duration, a complete transient could occur during the exposure, and a series of transients could be initiated (Fig. 3B) with a period of 3–15 min between each transient. The amplitude of the transients

declined in high K, and recovered when normal saline was restored (Fig. 3B). The induction of a series of regenerating transients indicates that high K activates a cellular oscillator.

(The data shown in Fig. 3 were obtained from ganglia without ligatures on the peripheral nerves, using choline instead of methylglucamine in the saline: see Materials and methods. This had no obvious effect on the results, but in all other experiments, ligatures were applied to try and reduce any leakage at the cut nerve ends, and choline was replaced, to avoid possible stimulation of cholinergic receptors.)

Effects of 30 mmol l^{-1} K

Elevation of the K concentration in the preceding experiments involved a reduction in Na level by two-thirds. To see whether transients could be induced by a smaller K elevation, with no alteration of Na level, the K concentration was raised three-fold by substitution of K for methylglucamine.

Six ganglia were exposed to 30 mmol l^{-1} K for 60 min, starting 30 min after setting up the preparation. Transients were induced (Fig. 4) in all six. In significant contrast (Fisher exact probability test), six ganglia exposed to normal saline for 120 min showed no tendency to oscillate. V_s at 30 min in the ganglia exposed to high K had a value of 18.0±1.0 mV (S.E.), no different (*t*-test) from that in the controls, 18.3±0.88 mV.

For five of the K-exposed ganglia (the sixth was excluded because of an abnormally small transient), the first transient had an amplitude of −30.3±0.86 mV, and a duration at half-amplitude of 112±14.6 s.

The delay between application of high K and the onset of the first transient varied widely among the preparations, ranging between 16.5 and 60 min in duration. The positive shift reached a peak much earlier, with a latency of 7.8±2.3 min. There was a general indication that initiation of the transient was related to the leakiness of the barrier, based on kinetics of V_s during the K exposure. Detailed analysis was impossible because of drift, but when the K-induced positive shift decayed rapidly, indicating some leakage of K into the sub-perineurial interstitium (Schofield and Treherne, 1984), the transient usually occurred early and the periodicity was short (Fig. 4A); when the decay was slow, the transient was late and the periodicity was long (Fig. 4B).

These observations show that elevation of external K concentration from 10 to 30 mmol l^{-1} is sufficient to induce transients in the abdominal ganglion. Initiation does not occur immediately following depolarization of the basolateral membrane, but appears to require a 'priming' period that may involve leakage of K across the blood–brain barrier.

The nature of a series of transients during high K contrasted with that in normal saline. When there were several transients during the K exposure, the periodicity and the amplitude decreased, followed by recovery when the normal K level was restored (Fig. 4A), similar to the observations in the 100 mmol l^{-1} K saline (Fig. 3B). If a series started near the end of the high-K exposure, the transients in

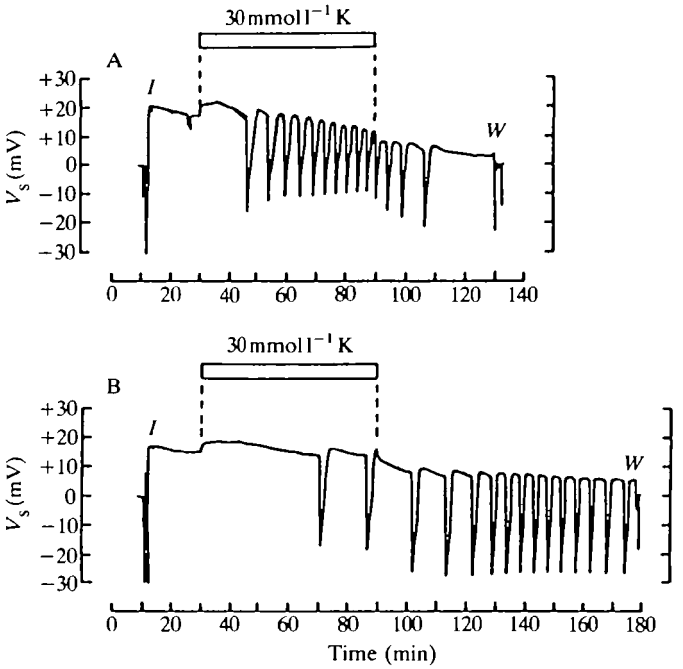

Fig. 4. Two examples of the effect upon V_s in a ganglion of 60 min exposure to 30 mmol l^{-1} K. (A) When the high K was applied, there was an initial positive shift which started to decay after less than 10 min and was followed after about 20 min by a series of transients of decreasing amplitude. (B) In this ganglion, the K-induced shift in V_s decayed after about 20 min, and two transients occurred after about 40 min of high K. In the normal saline, there was a series of transients showing a transitory decrease in period. *I* marks insertion of the electrode, *W* indicates withdrawal. Time scale indicates time from setting up the preparation.

normal saline showed a transitory decrease in periodicity with little loss of amplitude, and were free-running long after the K exposure, for as much as 85 min (Fig. 4B).

The amplitude of an initial transient in this high-K, normal-Na saline was similar to that obtained after recovery in normal saline (Fig. 4), as observed for the high-K, low-Na exposure in the previous section (Fig. 3B). This shows that, whereas K is clearly important in inducing a series of transients, the mechanism involved in the generation of each transient is not very sensitive to the level of K or Na outside the basolateral membrane.

Effect of low Cl on transients

If the negative transient were to be produced by the basolateral membrane, this would require the membrane to hyperpolarize (Fig. 2) (Schofield and Treherne, 1984). Since chloride is an obvious candidate to mediate such a shift, and since the transient did not appear to be affected by the levels of K or Na in the saline, a te

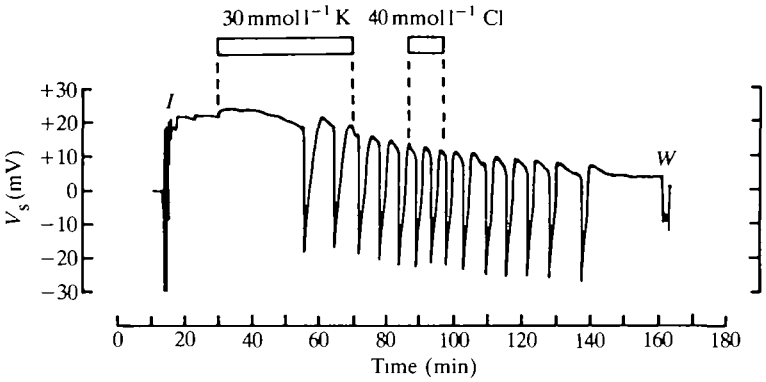

Fig. 5. Recording of V_s in a ganglion, with transients induced by exposure to 30 mmol l^{-1} K, showing that reduction of Cl level to 40 mmol l^{-1} had no obvious effect on the transient pattern. Display as in Fig. 4.

was made of the effect of lowering Cl concentration during a series of transients that had been induced by prior exposure to an elevated K level. When the Cl concentration was lowered from 130 mmol l^{-1} to 40 mmol l^{-1} during the series, for as long as 10 min, there was little change in the transient pattern (Fig. 5: compare with Fig. 4B).

Localization of the origin of the transient

Generation of the transients was investigated by recording from the perineurial glia, while recording simultaneously from the interstitial system, as in previous studies upon interganglionic connectives (Schofield *et al.* 1984; Schofield and Treherne, 1984). The recording from the cell, relative to the bathing medium, gave the potential V_b across the basolateral membrane. The potential V_a across the adglial membrane was obtained by subtraction of V_s from V_b (Fig. 2).

In six preparations, recordings were made from seven cells at a recorded depth of 14±4.7 μm, and peroxidase was injected into three of these. The appearance of injected cells in whole-mount was similar to that observed for perineurial cells in the connective (Schofield *et al.* 1984). By sectioning one of the injected ganglia, the marker was found in a superficial location using the light microscope (Fig. 6A), and observation under the electron microscope confirmed that a perineurial cell had been injected (Fig. 6B). The recordings from the injected cell (Fig. 7) showed effects that were essentially as found in the recordings from the other cells.

Upon elevation of K concentration from 10 to 100 mmol l^{-1}, the positive shift in transperineurial potential was accompanied by a depolarization of the basolateral membrane of slightly larger magnitude and a slow depolarization of the adglial membrane (Fig. 7, left-hand side). (A transient could occur during the K exposure as shown in Fig. 7, left arrow, and discussed in the next paragraph.) Observations after 15 s of high K, from four cells in four preparations (Table 1), showed

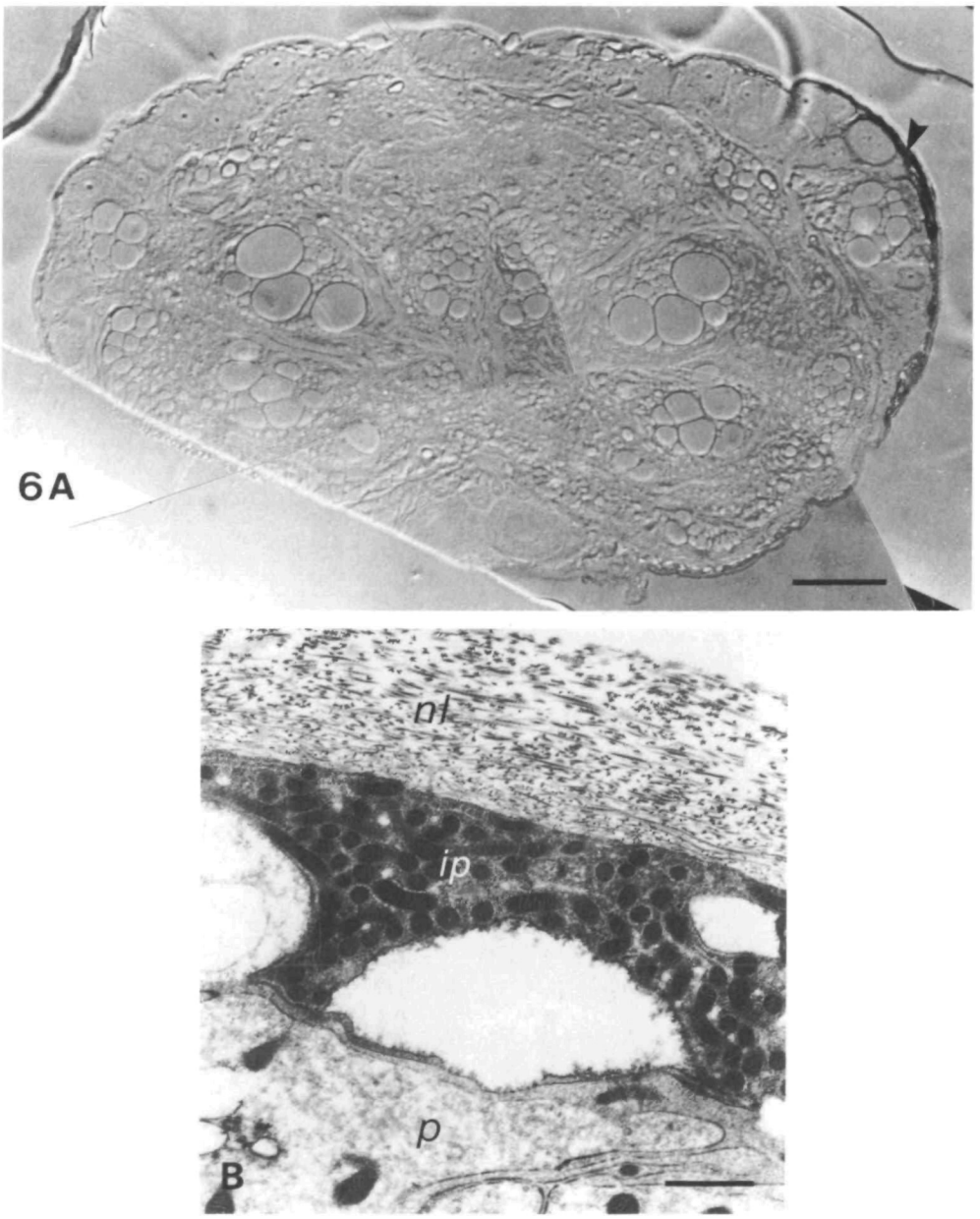

Fig. 6. (A) Transverse thick section (2 μm) of the abdominal ganglion (ventral surface at top) from which the recordings shown in Fig. 7 were made, locating the peroxidase marker (arrowhead). (B) Thin section through the edge of the injection site located in A, showing a process of a perineurial cell injected with peroxidase (*ip*) lying just below the neural lamella (*nl*) and above an unfilled perineurial cell (*p*). Scale bars, A 50 μm; B 1 μm.

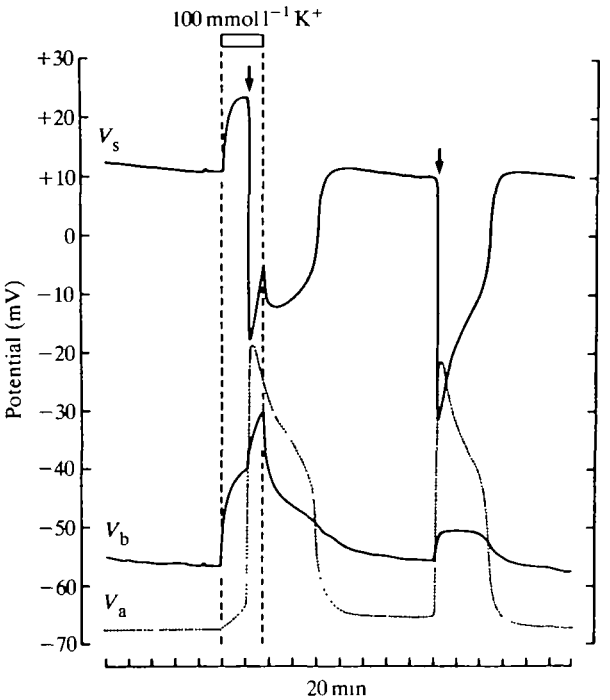

Fig. 7. Simultaneous recording, in a ganglion, of interstitial potential (V_s) and of basolateral membrane potential (V_b) a perineurial cell (identified in Fig. 6). Subtraction of these potentials yields the adglial membrane potential (V_a). During application of 100 mmol l^{-1} K, there was a positive shift in V_s, V_b and V_a. One negative transient in V_s started during the K exposure, and one came after (arrows). Both these transients were accompanied by a positive swing in V_a and a much smaller positive shift of V_b.

significant differences between the changes in V_s (dV_s), in V_b (dV_b), and in V_a (dV_a) (paired *t*-test). Because of the large size of the shift in V_b compared with the other changes, and the polarity of these alterations, the electrical model of the perineurium (Fig. 2) (Schofield and Treherne, 1984) indicates that the effects result from a depolarization of the basolateral membrane, with the changes across the other components being produced by electrical coupling.

During the initial phase of a K-induced transient in normal saline (Fig. 7, right-hand arrow), the negative change in V_s was found to occur at the same time as a larger positive shift in V_a and a much smaller positive shift in V_b. Such effects were observed for six cells in six preparations (Table 1) and the differences were significant (paired *t*-test). Essentially similar results were observed for a transient during the K exposure (Fig. 7, left-hand arrow). Since the change in V_a was of greater amplitude than the other shifts, and was of the same polarity as the change in V_b, these results are indicated by the model (Schofield and Treherne, 1984) to be produced by a depolarization of the adglial membrane.

The recovery phase of the transient in V_s and V_a was not immediately

Table 1. *Values recorded simultaneously in an abdominal ganglion during an elevation of K concentration from 10 to 100 mmol l^{-1}, and during a K-induced transient in normal saline*

	K exposure (N=4)	Transient (N=6)
V_s (start)	16.5±2.40	13.5±1.84
V_s (end)	23.4±1.52	−20.3±4.12
dV_s	6.9±1.46	−33.9±2.35
V_b (start)	−51.8±4.01	−51.6±3.18
V_b (end)	−43.9±4.96	−48.0±3.43
dV_b	7.9±1.65	3.6±0.41
V_a (start)	−68.3±5.11	−65.1±2.84
V_a (end)	−67.3±5.34	−27.7±3.63
dV_a	1.0±0.24	37.5±1.95
dV_s:dV_a	7.3±1.41	—
dV_s:dV_b	—	10.5±1.98

Potentials are given in mV and values are mean±S.E.
'Start' values represent the level as the K exposure or transient commenced.
For the K exposure, 'end' values were obtained after 15 s of high K; for the transient they were obtained at the point of maximum dV_s.

accompanied by a recovery in V_b, which showed a plateau (Fig. 7, right-hand transient), and hence a longer duration at half-amplitude. For the transients used for Table 1, the duration at half-amplitude was 107±8.6 s for V_s; 147±9.7 s for V_b; and 111±7.3 s for V_a. The value for V_b was significantly different from the others (paired t-test). This delayed reversal of the change in V_b indicates that recovery of the transient involves more than a simple repolarization of the adglial membrane, and the multiphasic nature of the return in V_a (Fig. 7, right-hand side) indicates that recovery involves more than one process. Overshoot of V_s above the baseline at the end of recovery (as in Fig. 2A) was observed during recordings from two cells and appeared to result from an undershoot of V_a (data not shown).

Quantitative analysis

From the values recorded simultaneously (Table 1), a quantitative assessment of some electrical parameters was made, by use of the electrical model (Schofield and Treherne, 1984).

Considering the changes obtained after 15 s of high K to result from a depolarization of the basolateral membrane, and hence to involve no change in resistance or e.m.f. of shunt or adglial membrane, the ratio dV_s:dV_a for the K exposure (Table 1) gives a value of 7.3 for the ratio R_s:R_a (equation 7, Schofield and Treherne, 1984).

Similarly, since the initial changes observed during a K-induced transient are indicated to result from a depolarization of the adglial membrane, with no concomitant changes in e.m.f. or resistance of the shunt or basolateral membrane, $dV_s:dV_b$ for the transient (Table 1) yields a value of 4.3 for $R_s:R_b$ (equation 9, Schofield and Treherne, 1984).

From $R_s:R_a$ and $R_s:R_b$, we can obtain $R_b:R_a$, 0.7, and $R_s:(R_b+R_a)$, 4.3.

Also, with the additional assumption of a zero value for E_s, the same equations (equations 7 and 9, Schofield and Treherne, 1984) yield values for E_a and E_b. From the mean values of potential observed during high-K exposure (Table 1), we obtain a value for E_a of -70.6 mV before the pulse and -70.5 mV after 15 s, i.e. no obvious change; and corresponding values for E_b of -50.2 mV rising to -41.7 mV. From the data for the initial phase of a K-induced transient, in normal saline, we obtain a value for E_a of -66.9 mV, rising to -24.9 mV; and a value for E_b of -50.3 mV scarcely changing to -49.9 mV. Thus the adglial membrane may have been slightly more depolarized during the K-induced transient than during the prior K exposure.

Discussion

The blood–brain barrier of the abdominal ganglion in the cockroach was found to have a unique oscillatory property. There were also some striking contrasts in further electrophysiological characteristics compared with the barrier in other regions of the nervous system.

The presence of an oscillator in the ganglion was revealed by transient shifts in transperineurial potential of about -30 mV, with a duration at half-amplitude of about 1.5 min, occurring in series, with a periodicity of 3–15 min. A threefold elevation of the external potassium level was sufficient to induce these oscillations (Figs 3–5). By recording from perineurial cells (Fig. 7, right-hand side; Table 1), the transients were shown to result from a depolarization of the adglial membrane of the perineurium (Fig. 2). This was deduced from comparison of the simultaneous recordings: the positive shift in adglial membrane potential (V_a) was slightly larger than the accompanying negative shift in transperineurial potential (V_s) and much larger than the positive shift in basolateral membrane potential (V_b). The opposite phase of the change in V_b compared with the change in V_s showed that these changes could not result from current flow from an external source, such as the underlying nerve cells (Fig. 2). These characteristics, and the persistence of the oscillations in normal saline, also argue against any artefactual basis, such as a change in conformation of the ganglion due to osmotic pressure. (There are no contractile elements in the ganglion.) Generation of the transients by the adglial membrane can explain why they were unaffected by changes in concentration of Cl outside the preparation (Fig. 5), and why the initial amplitude in either of the high-K salines was similar to that in normal saline (Figs 3B–5). It might also explain why the size declined during the K exposure, then returned afterwards (Figs 3B, 4A), because K can be expected to leak across the barrier

during the exposure, gradually depolarizing the adglial membrane and thus altering its electrical characteristics.

Each transient involved an abrupt initial depolarization of the adglial membrane, followed by a slower multiphasic recovery (Fig. 7, right-hand side). The rapidity of the depolarization suggests that it was produced by a change in conductance rather than a change in ion pumping. The multiphasic repolarization of V_a suggests that recovery involved more than just a simple reversal of such a conductance change. Recovery of V_a was not fully tracked by the recovery in V_b, which showed a plateau (Fig. 7, right-hand side), perhaps indicating some alteration in parameters of the basolateral membrane. Further study could be made of the mechanism generating the transient, but identification of the ionic species involved, and the nature of any ion pumps, will be hampered by the difficulty of access to the adglial membrane.

The perineurial oscillator could have been activated in two main ways: either potassium had a direct effect on the cells, or it acted by stimulating the release of an agonist within the ganglion. A direct action seems most likely, for the oscillator could be activated by brief exposure to high K (Fig. 3), with little apparent access of potassium to the sub-perineurial interstitium, as indicated by the small size of the depolarization of the adglial membrane (Fig. 7, left-hand side), which was in any case presumed to be due largely to electrical coupling.

Cellular oscillators can be categorized into those where the primary location of the oscillator is in the membrane and those where it is in the cytosol (see Berridge and Rapp, 1979). The long periodicity of the perineurial oscillator suggests that it is of the cytosolic type (see Berridge *et al.* 1988; Berridge and Galione, 1988). Although potassium could act rapidly at high concentrations, it was observed with threefold elevation of potassium concentration (Fig. 4) that induction could require a potassium exposure of up to 60 min. During this priming period, potassium may have stimulated the accumulation of some messenger in the perineurial cells. This might explain why periodicity decreased when K exposure was continued after the transients began (Figs 3B, 4A). It may be that potassium induces calcium influx to drive the oscillations, as in frog sympathetic neurones (Lipscombe *et al.* 1988), in which case the oscillations should be sensitive to the electrochemical gradient for calcium across the cell membranes.

Such oscillatory properties are not known for other blood–brain barrier systems, or for other insect glia. In particular, oscillations are not found in the blood–brain barrier of cockroach connective and peripheral nerve (see Treherne, 1985), and this has been confirmed under identical conditions to those employed here (P.K. Schofield, unpublished observations). Negative shifts of extraneuronal potential in connectives can be induced by cooling or exposure to ethacrynic acid, for cockroach (Pichon and Treherne, 1974), and by exposure to nitrate, for stick insect (Schofield, 1979), but these shifts are not of the same transient nature, and do not regenerate. The negative phase of the electroretinogram, recorded in the interstitium of the insect retina, across the blood–eye barrier, can be explained in terms of current flow induced by photoreceptor activity (see Laughlin, 1981).

Because the oscillations involved a depolarization of the adglial membrane potential, they provided a tool for electrical analysis of the perineurium using the electrical model of Schofield and Treherne (1984).

The opposite faces of the perineurium were each shown to generate a different resting e.m.f. by the positive value of the transperineurial potential. From the electrical analysis, the calculated values were about −50 mV for the basolateral membrane e.m.f. (E_b) and −71 mV for the adglial (E_a), i.e. a difference of 21 mV. Connectives show a greater disparity, of 47 mV, but they have a similar transperineurial potential, presumably because the resistance ratios (see below) are different (Schofield and Treherne, 1984).

A potassium sensitivity of the basolateral membrane of the perineurial cells in the ganglion is sufficient to explain the positive shifts in transperineurial potential that were induced by elevation of the external K concentration (e.g. Fig. 3A, first pulse), as in connective nerve (Schofield and Treherne, 1984). There are at present no appropriate data to compare sensitivities of the basolateral membranes in these two regions. The resting e.m.f. of the basolateral membrane in the ganglion was more negative than that observed in the connective (at a lower external K level), which could mean that the potassium sensitivity is higher, but the changes in transperineurial potential, 6.9 mV after an increase of 90 mmol l^{-1} for 15 s (Table 1), were much smaller than the increases seen in connectives (e.g. more than 30 mV after 64 mmol l^{-1} increase for the same time: Schofield and Treherne, 1986). This dissimilarity between these two regions may not apply in some other insects, for the K-induced positive shift is small in connectives of the stick insect *Carausius morosus* (Schofield, 1979).

The resistance of the basolateral membrane was calculated to be slightly less than that of the adglial, by a factor of about 0.7. Assuming a similarity in resistivity of these two membranes, the area of the basolateral membrane is then indicated to be 143 % that of the adglial. This is quite unlike the value of 9 % obtained for connective (Schofield and Treherne, 1984), suggesting a higher degree of basolateral interleaving and less extensive adglial projections (Maddrell and Treherne, 1967).

The paracellular pathway was calculated to have 4.3 times the resistance of the cell, indicating that the barrier is relatively 'tight' to intercellular diffusion (see Erlij and Martinez-Palomo, 1978). This ratio is about five times higher than that measured for interganglionic connectives in this insect (Schofield and Treherne, 1984). A restriction to the diffusion of potassium down the basolateral clefts is also suggested by the time for a K-induced positive shift to reach a peak, about 8 min, which is much slower than the period of about 1 min for connective (e.g. Schofield *et al.* 1984) or for peripheral nerve (P. K. Schofield, in preparation).

These properties of the blood–brain barrier of the ganglion should have distinct physiological effects. The high resistance of the paracellular pathway across the perineurium relative to the transcellular resistance shows the importance of the perineurium as a diffusion barrier in the ganglion. This characteristic must be important in maintaining transport through the cells (see Erlij and Martinez-

Palomo, 1978), which is involved in ionic homeostasis of the nerve cell environment (see Treherne, 1985) and in the nutrition and protection of the ganglion (see Wigglesworth, 1972; Treherne, 1985). Large fluctuations in the concentration of potassium in the blood, such as those which occur daily in cockroaches (Lettau *et al.* 1977), should cause little positive shift in transperineurial potential in the ganglion, but should induce oscillations. Quite different effects can be expected in the blood–brain barrier in other regions. This would set up current flow between the ganglion and these other areas, passing over the surface of the nervous system, and through the sub-perineurial interstitium, with important consequences for both metabolism and homeostasis (see Nicholson, 1980). If these oscillations are induced *via* the action of K upon the adglial membrane, they might also be induced by such an action resulting from activity of the underlying nerve cells. (It should then be borne in mind that d.c. recordings from these cells made relative to the external medium, as is common practice when recording from non-spiking neurones, will contain a contribution from any potential across the barrier.) Oscillations in membrane potential, which can be induced by phorbol ester, have been observed in vertebrate astrocytes (MacVicar *et al.* 1987; Walz and MacVicar, 1988), and it has been suggested that they could influence neuronal excitability (MacVicar *et al.* 1987). The oscillations in the glia of the insect ganglion might have such a role: the barrier lies just above the neuronal somata; and each transient involves a dramatic alteration of the electrical gradient across the adglial membrane, with reversal of the direction of current flow through the paracellular pathway. It is possible that the perineurial oscillator is involved in the control of the respiratory rhythm, for the oscillator has the same order of periodicity as the intermittent breathing pattern of the cockroach and several other insects (see Mill, 1985). The function of the oscillations could be investigated by making simultaneous recordings from neurones, glia and interstitium.

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