J. exp. Biol. (1981), **95**, 61–73
With 6 figures
Printed in Great Britain

MECHANISMS OF IONIC HOMEOSTASIS IN THE CENTRAL NERVOUS SYSTEM OF AN INSECT

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SUMMARY

Extracellular ionic homeostasis in an insect central nervous system involves a peripheral intercellular diffusion barrier, an extracellular matrix and neuroglial cation transport.

The peripheral location of the barrier in the superficial neuroglia is confirmed by intracellular recording from glial cells identified by peroxidase injection. This barrier protects the underlying neurones from large changes in ionic composition of the blood-plasma, but renders them more susceptible to fluctuations in ion composition resulting from neuronal signalling within the very restricted extracellular system. Because of the peripheral intercellular barrier, sodium movements between the blood and the extracellular fluid are largely transcellular and are mediated by ion pumps on the perineurial and underlying glial membranes. It is suggested that the homeostatic role of the neuroglial ion pumps is augmented by an anion matrix which functions as an extracellular sodium reservoir. It is proposed that during depletion of extracellular sodium, this cation is released by the matrix to maintain the sodium activity in the fluid at the axon surfaces.

INTRODUCTION

We have used insect nervous systems to study three related aspects of glial–neurone interactions: the contribution of neuroglia to blood–brain barrier systems, the nature and functional significance of the neuronal microenvironment delimited by the neuroglia, and the homeostatic role of glial cells in regulating the ionic composition of this environment.

Insects provide convenient preparations for these investigations, for (unlike most invertebrates) they possess well-developed blood–brain barriers that differ from those of mammals in being associated with glial rather than vascular elements (Treherne & Pichon, 1972; Abbott & Treherne, 1977; Lane & Treherne, 1980). Furthermore, insects exhibit an extreme form of ionic homeostasis by maintaining ionic concentrations at the neuronal surfaces that can be very different from those of the blood plasma (see Treherne, 1974).

Our research has concentrated on the central nervous system of the cockroach, *Periplaneta americana*. This avascular organ is delimited by a connective tissue sheath, the neural lamella (Fig. 1). The neural lamella contains collagen embedded in a matrix of glycosaminoglycans which, in histochemical tests, are identified as a mixture of chondroitin, dermatan and keratan sulphates (Ashhurst & Costin, 1971*a*).

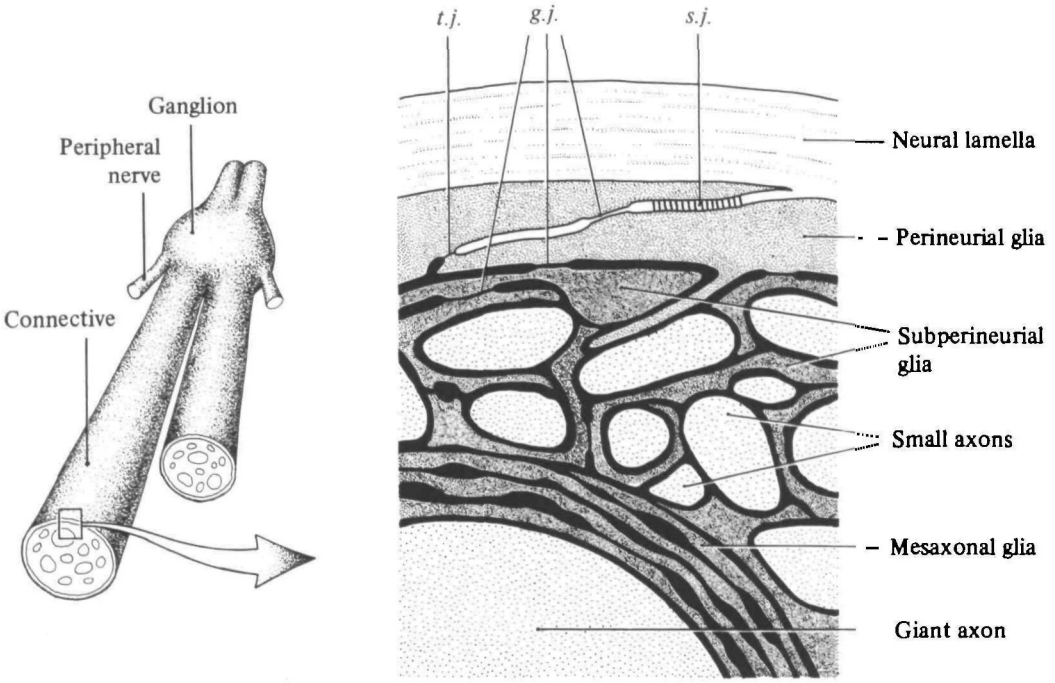

Fig. 1. Connectives of the cockroach central nervous system (left) contain an association of axons and neuroglia which is ensheathed by a layer of glial cells termed the perineurium, overlaid by the neural lamella (right). Tight junctions (*t.j.*) and septate junctions (*s.j.*) are found between perineurial cells. Gap junctions (*g.j.*) connect perineurial and glial cells. Giant axons are surrounded by many glial folds, the mesaxon; smaller axons have less glial envestment. The subperineurial extracellular system, around axons and glia, is indicated in black. Schematic drawing, not to scale.

Beneath the ensheathing neural lamella is the perineurium, a layer of overlapping neuroglia attached to one another by septate and tight junctions. The tight junctions are found predominantly at the inner ends of the clefts between the cells. The perineurial cells are also linked to each other and to the underlying neuroglia by gap junctions (Lane & Treherne, 1972; Skaer & Lane, 1974; Lane, Skaer & Swales, 1977; Lane, 1981). The neuroglia are packed around the neurones with a separation of 10–20 nm, so that these cells lie in a complex of narrow interconnecting channels. As originally described by Smith & Treherne (1963) these extracellular channels contain electron-dense material (see Lane, 1981) which has been histochemically identified as hyaluronate in the larger glial lacunar spaces of the ganglia (Ashhurst & Costin, 1971*a*).

In this review we examine research upon the cockroach central nervous system that indicates both passive and active roles for neuroglia in maintenance of the ionic composition of the neuronal environment. We also present recent electrophysiological data that demonstrate the importance of the perineurium as a diffusion barrier, and radioisotopic evidence of an anion matrix which acts as an extracellular cation reservoir.

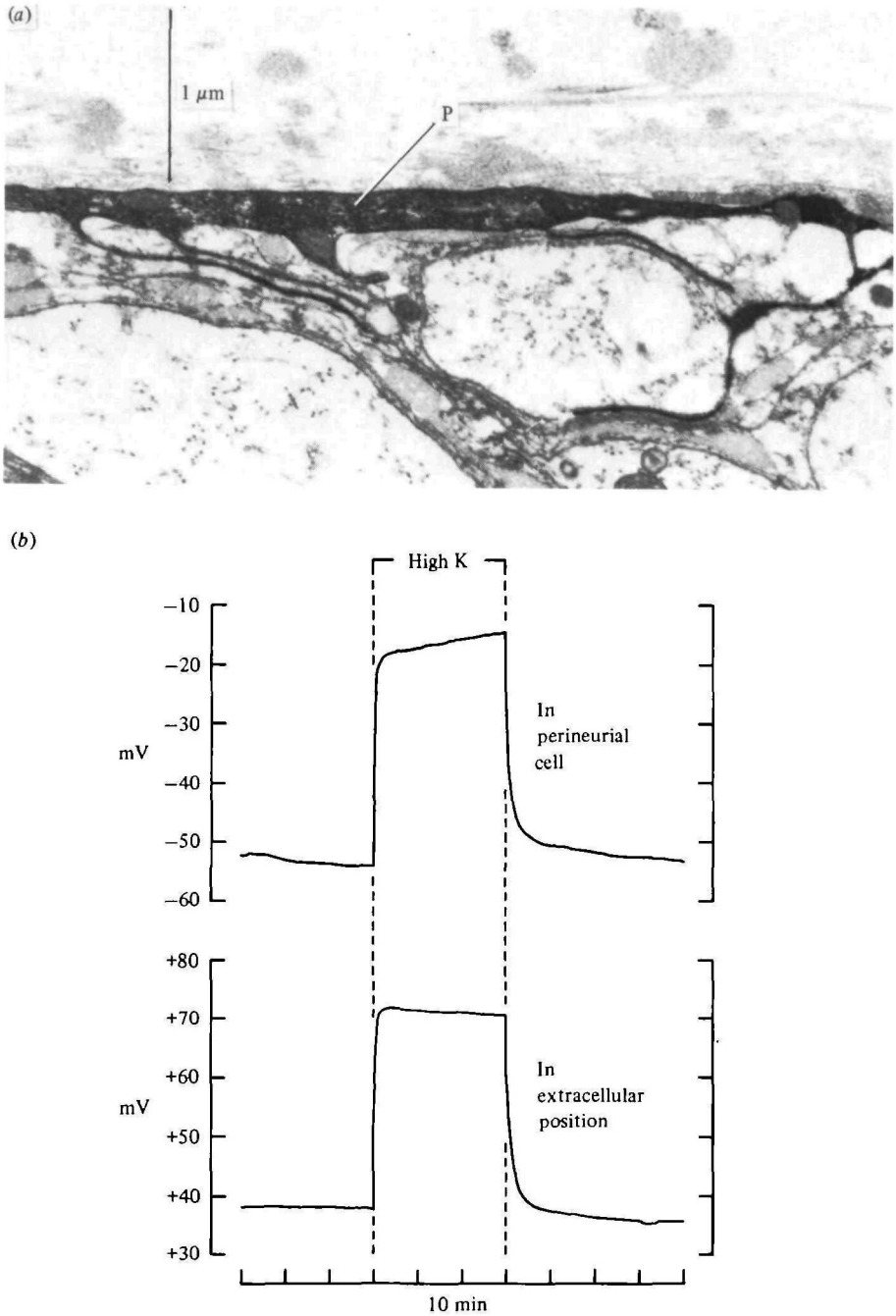

Fig. 2. (*a*) A perineurial cell (P), injected with peroxidase from a microelectrode, shown in an electron micrograph of a transverse section of a connective in the cockroach C.N.S. Injection was made after obtaining the recording shown below (*b*). Peroxidase was visualized using 3,3′-diaminobenzidine. ×22400. (*b*) Potentials recorded simultaneously from the perineurial cell shown above (*a*), and from an extracellular position immediately below the perineurium. Depolarization was produced by substitution of potassium for sodium (120 mM) in the saline bathing the nerve cord. Recordings were made conventionally, using microelectrodes, with reference to the bath at ground potential. (From P. K. Schofield, L. S. Swales & J. E. Treherne, in preparation.)

THE PERIPHERAL DIFFUSION BARRIER

Localization of the barrier

A critical factor in our analysis is the localization of the blood–brain barrier system. This system limits the movements of water-soluble ions and small molecules between the blood and the neuronal surfaces, as has been demonstrated in electrophysiological experiments (e.g. Hoyle, 1953; Twarog & Roeder, 1956; Treherne *et al.* 1970). It has been supposed to be located in the perineurium since extracellular tracers (peroxidase, microperoxidase and ionic lanthanum) permeate the neural lamella, but are unable to penetrate beneath the superficial layer of neuroglia. The tracers are capable of only limited entry into the clefts between the perineurial cells, which is suggested to be due to the intercellular junctional complexes, notably tight junctions (Lane & Treherne, 1972).

The ultrastructural studies do not necessarily indicate an impermeability to smaller substances. It is known, for example, that brief exposure of cockroach connectives to hypertonic urea increases access of sodium, potassium and lithium ions to the axon surfaces (Treherne, Schofield & Lane, 1973; Schofield & Treherne, 1978) but does not facilitate penetration of ionic lanthanum (Treherne *et al.* 1973) or microperoxidase (J. E. Treherne, P. K. Schofield & N. J. Lane, in preparation).

A superficial diffusion barrier, at the perineurial level, is also difficult to reconcile with the rapid fluxes of radiocations which have been observed between the plasma and the central nervous tissues (Treherne, 1961; Tucker & Pichon, 1972).

We have, however, recently provided further evidence that the blood–brain barrier is located in the perineurium, by recording with microelectrodes from perineurial cells, identified by injection of peroxidase (Fig. 2*a*).

The cockroach blood–brain barrier may be characterized electrophysiologically, by recording the diffusion potentials that are generated across the barrier when the ionic composition of the external saline is altered. These potentials have been measured within giant axons, in the extracellular spaces immediately outside them, and with sucrose-gap recordings (Treherne *et al.* 1970; Pichon & Treherne, 1970; Pichon, Moreton & Treherne, 1971). We have now shown that these potential changes are at their largest amplitude within the perineurial cells, and only slightly attenuated immediately below this neuroglial layer (Fig. 2*b*). These observations show that the potentials are generated across the outermost membranes of the perineurial cells, and the junctions joining the cells, and that this interface may therefore be identified as the blood–brain barrier for monovalent cations.

Experimental disruption of the barrier

Brief exposure of cockroach connectives to hypertonic urea, in the manner of Ussing (1968), greatly increases the access of water-soluble cations to the axon surfaces. This is deduced from the accelerated electrical responses of the axons to changes in external sodium, potassium and lithium concentrations (Treherne *et al.* 1973; Schofield & Treherne, 1978), by the reduction in the amplitude of extraneuronal potential changes (Treherne *et al.* 1973), and by the substantial increase in the fast component of ^{22}Na efflux (see Fig. 6).

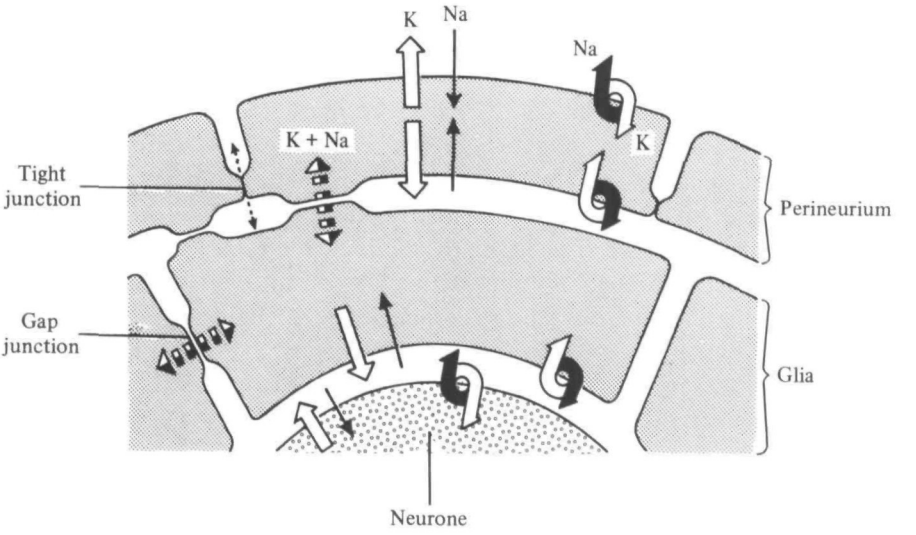

Fig. 3. Model for cation regulation in the cockroach central nervous system (C.N.S.). Transfer of sodium and potassium between blood and the C.N.S. will occur through the perineurium. Intercellular diffusion through the perineurium (dotted arrow) is restricted by tight junctions, so transfer is largely transcellular, by means of Na/K pumps (linked arrows), and by diffusion down electrochemical gradients (single arrows). Diffusion will also occur through gap junctions (broken arrows) between perineurial and glial cells. The sodium concentration of the extracellular compartment is maintained high, and the potassium low, by the pumps on the inwardly facing perineurial membranes and the membranes of underlying glia and neurones.

The increased access induced by urea treatment does not appear to result from appreciable cellular damage, for extracellular tracers are excluded from the perineurial cytoplasm of urea-treated preparations (Treherne *et al.* 1973) and the uptake and efflux of ^{14}C-xylose and inulin are unaffected by urea treatment (J. E. Treherne, unpublished observations). Furthermore, brief exposure to hypertonic urea does not reduce the membrane potentials of perineurial cells, or the resting and action potentials of surgically exposed giant axons (Treherne *et al.* 1973).

Despite the increased intercellular access of monovalent cations induced by urea treatment, the conventional extracellular tracers, ionic lanthanum (Treherne *et al.* 1973) and microperoxidase (J. E. Treherne, P. K. Schofield & N. J. Lane, in preparation) still fail to penetrate into the subperineurial extracellular system. This suggests that the effect of urea treatment on intercellular cation movements may be to alter the charge characteristics of the perineurial junctional complexes in the absence of appreciable structural changes that may be required to affect the intercellular diffusion of larger charged particles.

CATION TRANSPORT BY THE NEUROGLIA

The presence of the peripheral, neuroglial, blood–brain barrier ensures that the movements of sodium ions between the external medium and the extracellular fluid are largely transcellular. As suggested in the model illustrated in Fig. 3, the escape of sodium across the blood–brain interface is mediated by sodium pumps on the

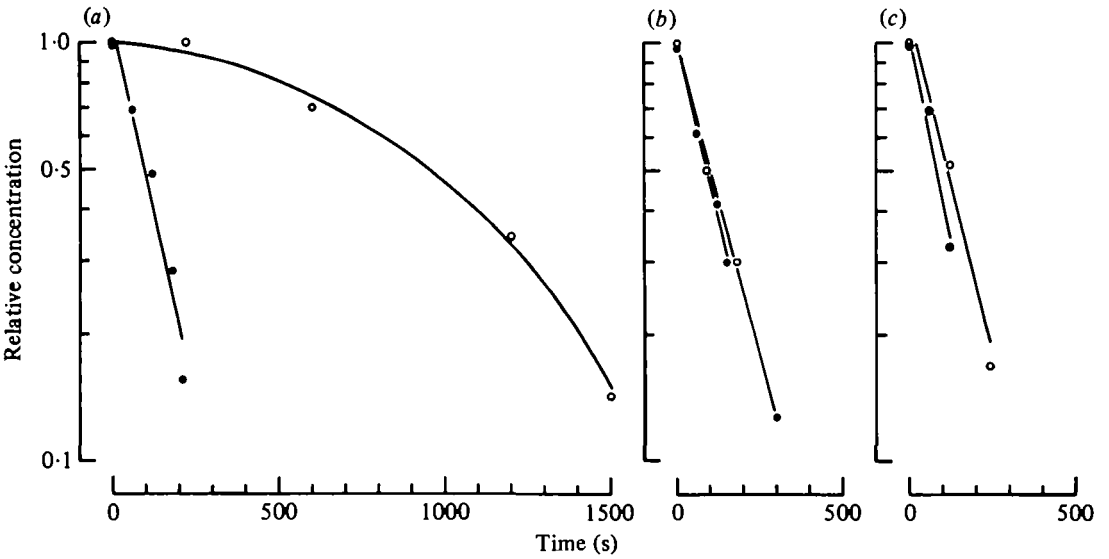

Fig. 4. Changes in extracellular sodium concentration in relatively 'leaky' cockroach connectives, resulting from successive exposures to sodium-free (sucrose-substituted) saline. (*a*) Initial exposure to sodium-free results in a decline in extracellular concentration (○) that is slower than the recovery observed upon return of normal saline (●). (*b*) After 5 min in normal saline, exposure to sodium-free produced a decline that was as rapid as the recovery. (*c*) After a further 40 min in normal saline, another exposure to sodium-free saline still produced rapid and symmetrical movements. Concentrations were estimated from changes in action potential amplitude (recorded with sucrose-gap) and are expressed relative to the concentration before each sodium-free exposure. (From Schofield & Treherne, 1978.)

outer perineurial membranes. This accounts for the observed effect of ethacrynic acid in slowing the rapid, terminal, decline in amplitude of the action potentials in preparations exposed to sodium-free saline (Schofield & Treherne, 1975). Similarly, the net uptake of sodium appears to be mediated by pumping at the inwardly facing perineurial and glial membranes. This can be concluded from the effect of ethacrynic acid and dinitrophenol in slowing the net inward movement of sodium ions to the axon surfaces, following exposure of sodium-depleted connectives to normal, high-sodium saline (Schofield & Treherne, 1975). It is also indicated by the inability of lithium ions to gain access to the extracellular fluid in sodium-depleted connectives despite substantial accumulation of this cation within the nervous tissues (Bennett, Buchan & Treherne, 1975), for Na/K pumps appear not to accept lithium ions (Keynes & Swan, 1959; Baker, 1965).

According to the model illustrated in Fig. 3 the sodium content of the perineurial cytoplasm and that of the underlying glia (to which it is supposed to be linked by gap junctions) will be determined by a balance between the passive sodium permeabilities and sodium pumping. The pump on the inwardly facing perineurial and glial membranes will tend to re-cycle sodium ions in the vicinity of the axon surfaces. The decline in extracellular sodium concentrations (in sodium-free saline) will therefore primarily result from reduced sodium pumping as the intracellular concentration in the glial compartment falls.

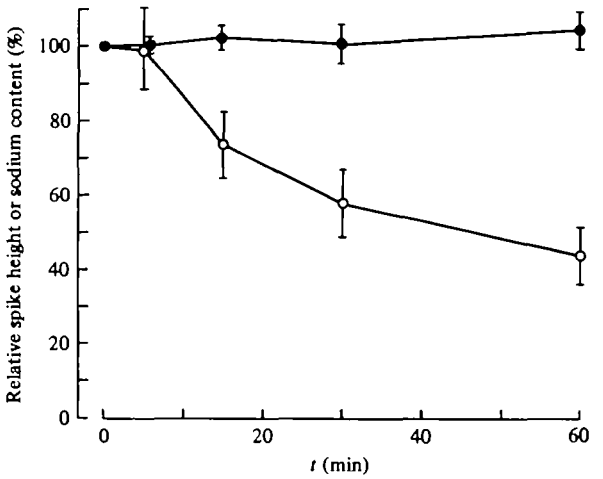

Fig. 5. The effects of exposure to sodium-free (tris-substituted) saline on the sodium content (○) of cockroach connectives which showed no significant change in extracellular sodium concentration, as indicated by the amplitude of compound action potentials (●). Sodium content was measured by flame photometry and action potentials were recorded by sucrose-gap. (From Bennett, Buchan & Treherne, 1975.)

If the sodium pumps on the outer perineurial membranes have a higher sodium affinity than those on the inwardly directed perineurial and glial membranes then at low intracellular sodium levels net pumping would tend to be largely outwardly directed. Such a system could give rise to the accelerated terminal decline in extracellular sodium concentration, on initial exposure to sodium-free saline (Fig. 4), which can be slowed by sodium-transport inhibitors (Schofield & Treherne, 1975).

The slow decline in extracellular sodium which occurs on initial exposure of cockroach connectives to sodium-free saline (Fig. 4) can be accounted for by the provision of sodium from a reservoir (Treherne & Schofield, 1978). This is indicated by the observation that connectives can lose around 50% of their total sodium content with no appreciable decline in sodium concentration at the axon surfaces (Fig. 5). The reservoir would not appear to be easily refilled since the return of sodium to the bathing medium results in a rapid recovery of extracellular sodium (Fig. 4) that is accompanied by only partial recovery of the sodium content of the connectives (Bennett *et al.* 1975). Furthermore, subsequent outward and inward movements induced between the external medium and the extracellular fluid occur at similar, rapid, rates (Fig. 4). The existence of a sodium reservoir (either extracellular or intraglial) has also been proposed by Fulpius & Baumann (1969) to account for the persistence of receptor potentials in drone photoreceptors during prolonged exposure to sodium-deficient saline.

The inability to recharge the postulated reservoir in the cockroach central nervous system following exposure to sodium-free saline could result from uncoupling of the glial compartment from the perineurial cytoplasm. Under these circumstances the glial compartment would fill only slowly by passive diffusion through the glial membranes. Such uncoupling in sodium-free media could result from an increase in intracellular calcium (cf. Baker, Hodgkin & Ridgeway, 1971) either by reducing

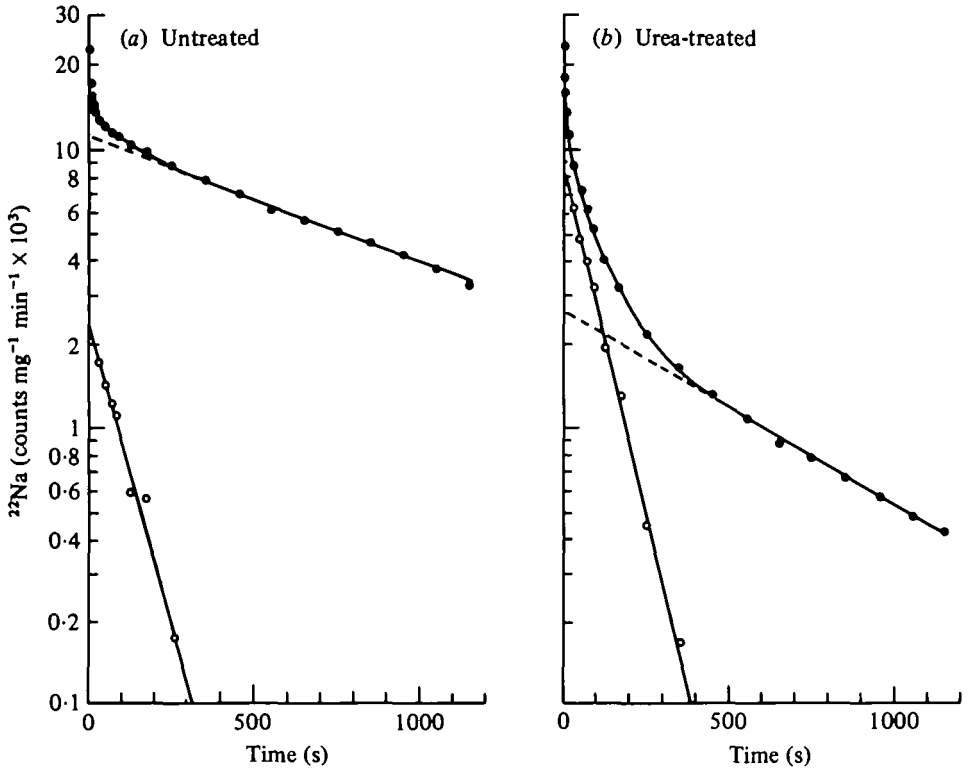

Fig. 6. (*a*) Efflux of ^{22}Na from the cockroach nerve cord into normal saline (●) can be separated into a slow component (extrapolated by dotted line) and a fast component (○). (*b*) Efflux from cords loaded after exposure to 3 M urea for 30 s (see text) shows a smaller slow component and larger fast component than in the untreated cord (*a*). Cords were loaded with ^{22}Na *in normal saline for* 1½ *h before efflux.* (From J. E. Treherne, P. K. Schofield & N. J. Lane, in preparation.)

the permeability of the junctional channels directly, as proposed by Rose & Rick (1978), or indirectly by changing intracellular pH (cf. Meech & Thomas, 1977), as proposed by Turin & Warner (1977) and, in the case of neuroglia, by Orkand, Orkand & Tang (1981).

IONIC COMPOSITION OF THE EXTRACELLULAR ENVIRONMENT

The perineurial junctional complexes divide the extracellular system into two fractions: a superficial one, in the neural lamella and underlying perineurial clefts, and a subperineurial one, between the closely applied glial and neuronal membranes, which is effectively isolated from the blood plasma.

We have recently studied the ionic composition of these extracellular environments using radioisotopes. As previously shown (e.g. Treherne, 1962), the efflux of radiocations and ^{36}Cl can be represented as a two-stage process (Fig. 6*a*). In the case of sodium ions the fast component comprises 21·6% (6·3 ± 0·4 mmol/kg tissue) of the total exchangeable sodium. This component does not exhibit the characteristics of

an intracellular cation fraction. For example, both the rate and the size of the fast component of sodium efflux are unaffected by dinitrophenol and sodium transport inhibitors (ouabain, ethacrynic acid). Furthermore, cation/chloride ratios of the fast components are not as would be predicted for intracellular ion fractions. For example, the calcium/chloride ratio of the fast components is exceptionally high for an intracellular fraction (0·20/6·0 mmol kg^{-1} = 0·033) and exceeds that for the external medium (2·0/131·7 mmol kg^{-1} = 0·015). The ratio between the fast components of sodium and chloride efflux (5·7/6·7 mmol kg^{-1} tissue = 0·85) is close to the sodium/chloride ratio for the bathing medium (120·0/131·7 mmol l^{-1} = 0·91) J. E. Treherne, P. K. Schofield & N. J. Lane, in preparation).

The above observations imply that the fast component of sodium efflux is largely extracellular as was originally supposed (Treherne, 1962). This fraction must apparently be located above the level of the perineurial tight junctions (i.e. largely in the neural lamella and superficial perineurial clefts) because there is an effective intercellular diffusion barrier in the peripheral neuroglia (see above). There could, however, be a small intracellular contribution from the sodium contained within the relatively thin perineurial glia. It should be noted that the half-time for radiosodium efflux ($t_{0.5}$ = 63·5 s) into sodium-free saline (i.e. net efflux) approximates to that for the net sodium movements between the external medium and the axon surfaces calculated from electrophysiological observations (Fig. 4*b*, *c*). Disruption of the blood–brain barrier, by brief exposure to hypertonic urea, results in a substantial increase in the fast component of sodium efflux (Fig. 6). Chloride movements are less affected, so that the additional ion fraction released by urea treatment contains considerably less chloride than sodium ions: the Na/Cl ratio of this fraction is 9·6/3·6 mmol kg^{-1} tissue = 2·7 as compared with a ratio of 120·0/131·7 mmol l^{-1} = 0·91 for the bathing medium and 5·7/6·7 mmol kg^{-1} tissue = 0·85 for the fast fraction in untreated cords. Such an excess of sodium over chloride would be expected if the fast fraction revealed by urea treatment consisted of ions which had been in Donnan equilibrium with fixed anionic sites within the extracellular system. The existence of such sites can be correlated with the nature of the sub-perineurial extracellular material. This material has been found to bind lanthanum ions (J. E. Treherne, P. K. Schofield & N. J. Lane, in preparation) and contains hyaluronate (Ashhurst & Costin, 1971*a*) which could thus constitute, or contribute to, an extracellular anion matrix. This system bears some similarities to the proposed Ca-binding matrix which has been postulated to overlie the plasma membrane of the squid axon and to influence Ca efflux into the bathing medium (Baker & McNaughton, 1978).

CONCLUSIONS AND SPECULATIONS

Regulation of the ionic composition of the immediate fluid environment of insect nerve cells appears to be achieved by a combination of passive and active processes involving the neuroglia and an extracellular anion matrix.

The intercellular diffusion barrier in the superficial layer of neuroglia, the perineurium, protects the underlying neurones from the immediate effects of the large fluctuations in ionic composition which occur in the blood plasma (Pichon & Boistel

1963; Pichon, 1970; Treherne, Buchan & Bennett, 1975; Lettau *et al.* 1977) and, also, from the adverse effects of extraneous pharmacologically active and toxic substances. However, it also renders them more susceptible to fluctuations in chemical composition of their immediate environment, which might result from electrical and metabolic activities. In insects, there are no large extracellular fluid compartments equivalent to the vertebrate cerebrospinal fluid. The neurones are thus confined in a very restricted microenvironment, frequently consisting of narrow clefts of between 10 and 20 nm in width. They will, therefore, be potentially vulnerable to rapid alterations in extracellular ion composition (resulting from neuronal activity), as well as from slower changes (resulting from variations in the blood).

The available evidence indicates that the neuroglia play an important role in regulating the sodium content of the brain microenvironment in the cockroach. We also have circumstantial evidence for the involvement of an anion matrix which could act as an extracellular cation reservoir.

The possible involvement of anion matrices in extracellular ionic homeostasis has been previously suggested on the basis of limited physiological evidence in some invertebrate nerves (e.g. Chamberlain & Kerkut, 1967; Treherne, Carlson & Gupta, 1969; Treherne & Moreton, 1970; Sattelle, 1973; Abbott, Pichon & Lane, 1977) and, as a consequence of histochemical or biochemical demonstrations of extracellular glycomolecules, in arthropod nerves (Lemire & Deloince, 1970; Ashhurst & Costin, 1970*a*, *b*), at the node of Ranvier (Langley & Landon, 1969; Langley, 1970; Landon & Langley, 1971) and in vertebrate brain (e.g. Szabo & Roboz-Einstein, 1962; Bondareff, 1967; Nicholson, 1980). There is, however, very little information to enable quantitative assessments to be made of the physiological roles of extracellular anion matrices in nervous tissues. An obvious possibility, in the cockroach nervous system, is that extracellular ions associated with the anion matrix contributes to the postulated sodium reservoir (see p. 66). Now the Na/Cl ratio from the subperineurial extracellular fraction is 2·7, as compared with 0·91 in the external medium. This suggests that for every free sodium ion there are two associated with the matrix which could be released as the concentration of free sodium ions decreases during initial exposure to sodium-free saline. Such a release would result in a threefold increase in the quantity of extracellular sodium available for re-cycling by glial sodium pumping.

The possibility also exists that the matrix could be involved in short-term homeostasis necessitated by the restricted extracellular environment. The effects of the tiny microenvironment can be illustrated in the case of the cockroach giant axons. In these the sodium entry which mediates the inward current of the action potential has been estimated to be 6·3 pmol cm^{-2} $impulse^{-1}$ (Narahashi, 1963). Now with extra-axonal clefts of 15 nm in width containing 120 mmol Na^+ (plasma concentration) the sodium immediately available to carry the increased current would be 180 pmol cm^{-2} (i.e. enough to support only about 30 action potentials). The sodium within the extra-axonal space is unlikely to be rapidly augmented by that contained within the long, single, mesaxon cleft. Thus even if this cleft acted as an infinite reservoir it can be calculated, using the approach of Treherne *et al.* (1970), that with an axon of 50 μm diameter, it would take about 1·2 s for half equilibration with the ions

contained in the extra-axonal space, which is long when considered in relation to stimulation regimes with spike intervals of only a few milliseconds (Parnas *et al.* 1969).

The above considerations indicate that the free sodium ions in the fluid immediately bathing the axon surfaces is likely to support only a limited number of action potentials. This situation is essentially similar to that calculated for the photoreceptor cells of the locust eye, in which a minimal estimate indicates that there is only about 20 times more sodium immediately available than is required for one full-sized response (Shaw, 1977). However, unlike the cockroach giant axon system, the fluid bathing the photoreceptor membranes communicate with additional extracellular spaces via channels of short path length (12 μm). This, it is postulated enables a rapid redistribution of sodium so as to recharge the fluid layer at the receptor surfaces (Shaw, 1977). In the cockroach giant axon system, on the other hand, there is only a single, long mesaxon cleft which could only slowly refill the extra-axonal clefts by diffusion. The abdominal giant axons can nevertheless conduct at 200 impulses s^{-1} (Narahashi & Yamasaki, 1960), and can fire for some minutes at 50 s^{-1} (Parnas *et al.* 1969).

In the cockroach central nervous system additional sodium (twice as much as in free solution) would be provided by that contained in the extracellular anion matrix. This sodium will increase the time (by up to three times) during which full-sized action potentials are maintained during rapid axonal firing. The presence of the matrix would also ensure that potassium ions released during neuronal activity would undergo a reduction in activity coefficient in the vicinity of the axon surfaces. Such an effect could, for example, explain why the decline in potassium activity (deduced from the decay of the negative after-potential) is more rapid at the surface of the cockroach giant axon ($t_{0.5}$ = 9·2 ms; Narahashi & Yamasaki, 1960) than that of the squid ($t_{0.5}$ up to 100 ms; Frankenhaeuser & Hodgkin, 1959) despite the relatively leaky glial covering of the latter (Villegas & Villegas, 1964, 1968).

Extracellular polyanions could thus function as a short-term homeostatic device to maintain the overshoot of the action potential: first, by releasing sodium ions in the vicinity of the axon surfaces and, secondly, by reducing axonal depolarization to reduce the degree of inactivation of the sodium channels.

An important feature of this mechanism is that the release of sodium from the matrix would tend to *maintain* the concentration of this cation at the axon surfaces whilst enabling an *increase* in sodium concentration to occur beneath the axonal membrane during excitation. This increase could stimulate linked Na^+/K pumping (cf. Baker *et al.* 1969) which, consequently, would remove excess extracellular potassium and maintain the sodium concentration at the axon surfaces (see Varon & Somjen, 1979). Such axonal, rather than glial, cation pumping appears to play a significant role in ionic homeostasis in *Necturus* optic nerve (Tang, Cohen & Orkand, 1980).

Now in the case of a cockroach giant axon a firing frequency of 50 s^{-1} would require the relatively large increase in sodium extrusion of $50 \times 6{\cdot}3 = 315$ pmol cm^{-2} s^{-1} to maintain the sodium gradient across the axon membrane: a value which by itself exceeds the maximal rate of sodium pumping of around 150 pmol cm^{-2} s^{-1} measured in squid axons with raised intracelllar sodium concentrations (Brinley &

Mullins, 1968). In the insect central nervous system, therefore, it seems likely that the effects of axonal ion pumping must be augmented by movements of sodium ions induced by extracellular current flow during spatial buffering of released potassium (see Nicholson, 1980; Gardner-Medwin, 1981) as well as by sodium extrusion by linked Na/K pumps in the neuroglia, as suggested by the results of Coles & Tsacopoulos (1981).

The available evidence thus suggests that ionic homeostasis of the brain microenvironment in this insect is achieved by a combination of passive and active processes involving the neuroglia and an extracellular anion matrix. The intercellular diffusion barrier in the superficial neuroglia protects the neurones from the large changes which occur in the ionic composition of the blood plasma. This passive role of the superficial neuroglia is reinforced by glial and axonal cation pumping which, it is postulated, tend to recycle sodium ions so as to maintain the extracellular concentration during prolonged exposure to sodium-deficient saline. This effect could be augmented by a sodium reservoir associated with an extracellular anion matrix. This matrix could also serve an important role in short-term ionic homeostasis by buffering extracellular sodium ions, to enable increases in intra-axonal sodium ions to occur and thus to stimulate the axonal Na/K pump, without depleting the extracellular concentration of free sodium ions.

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