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A MODEL FOR EXTRACELLULAR SODIUM REGULATION IN THE CENTRAL NERVOUS SYSTEM OF AN INSECT (*PERIPLANETA AMERICANA*)

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The ionic composition of the immediate fluid environment of insect nerve cells is regulated so as to maintain concentrations which may differ considerably from those of the blood. The regulation of the extracellular sodium in the cockroach central nervous system has been suggested to result from a glial-mediated transport of this cation (cf. Treherne, 1974). We here propose a model system for sodium regulation, incorporating such transport, that accounts for the salient experimental observations (Fig. 1).

Intercellular diffusion between the blood and the axonal surfaces is represented as being restricted at the inner ends of the tortuous clefts which traverse the superficial cellular layer, the perineurium. This accounts for the observation that altered external ion concentrations result in extraneuronal potential changes (Treherne *et al.* 1970; Pichon & Treherne, 1970; Pichon, Moreton & Treherne, 1971) – originating at the outer perineurial membranes – before any effects are measured in the axons. The restriction probably results from tight junctions, which limit the penetration of ultrastructural tracers along the clefts (Lane & Treherne, 1972).

Despite the peripheral intercellular diffusion barrier, rapid radiosodium fluxes occur between connectives and the bathing medium (Treherne, 1962; Pichon & Tucker, 1972) indicating that there is a dynamic exchange of sodium between the perineurial cells and the blood. In the model, entry of sodium into the cells occurs by a mechanism that will also accept lithium, a cation which rapidly accumulates in relatively high concentrations in intact connectives (Bennett, Buchan & Treherne, 1975). As proposed for the frog skin, the mechanism could involve carrier-mediated pumping of these two cations (cf. Leblanc, 1972). Alternatively, it could be that the potential gradient across the outer perineurial border is sufficiently large to induce a passive entry of sodium and lithium ions, as is also proposed for the frog skin (Helman & Fisher, 1977; Nagel, 1977*a*, *b*).

The extrusion of sodium ions across the outer perineurial membrane is represented as being largely mediated by conventional pumps which exchange sodium for potassium ions. This explains the effect of externally-applied ethacrynic acid in slowing the eventual decline of the action potentials in connectives exposed to sodium-deficient saline (Schofield & Treherne, 1975). It also accounts for the effect of ouabain or

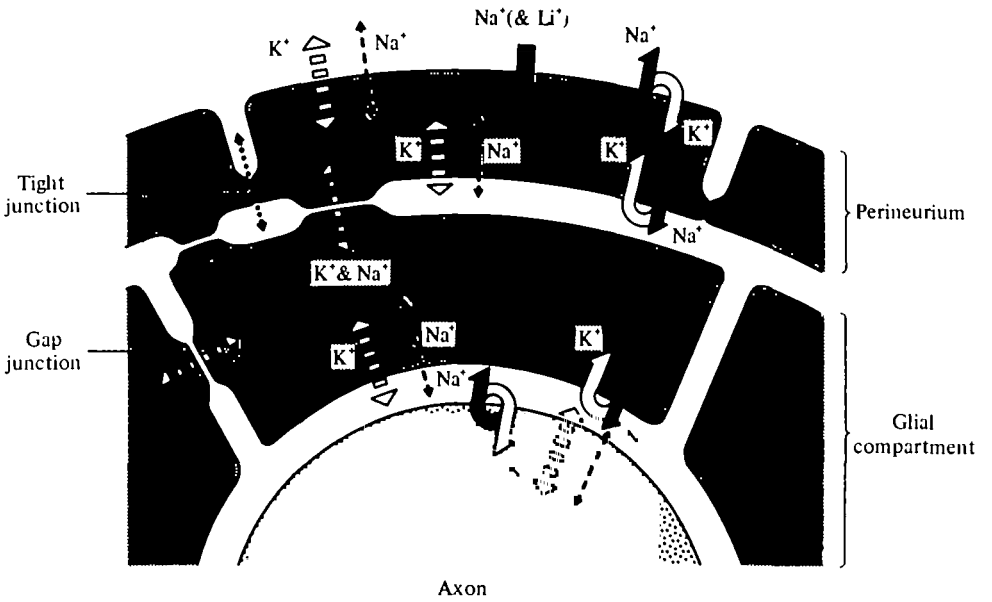

Fig. 1. Model for regulation of extracellular sodium concentration in the cockroach nerve cord. Intercellular passage of sodium between the extracellular fluid and the bathing medium (dotted arrow) is limited by tight junctions at the inner ends of the clefts between the perineurial cells. Passage by a transcellular route, through the perineurial and glial cells, involves diffusion across the cell membranes (broken arrows) and conventional Na/K pumps extruding sodium from the cells (linked arrows). Entry of sodium (and lithium) at the outer perineurial membranes could be carrier-mediated (single solid arrow) or be by diffusion down an electrochemical gradient. The presence of both inward and outward transfer of sodium provides dynamic control of its extracellular concentration. [The neural lamella over the perineurium is not incorporated in the model since it is relatively leaky to small water soluble ions and molecules (cf. Treherne, 1974).]

potassium-deficient saline in reducing radiosodium efflux from the system (Treherne, 1961, 1966).

The perineurial cells and the underlying glia are represented as confluent compartments between which intracellular movements of sodium, and other small water-soluble ions, can occur via gap junctions that link adjacent glial and perineurial membranes (Lane, Skaer & Swales, 1977; Lane & Swales, 1978).

A net transfer of sodium ions across the inner perineurial and glial membranes would be largely mediated by linked Na/K pumps. These pumps would extrude sodium ions into the narrow extracellular clefts and would quickly counteract depletion of extracellular sodium.

According to this model the sodium content of the perineurial cells will be largely determined by the uptake of sodium from the blood and its extrusion into the extracellular clefts and the blood by the Na/K pumps. In the absence of external sodium ions there will be a net outward movement of this cation across the outer perineurial membrane largely mediated by the Na/K pumps. Because of the postulated linkage with the glia the changes in intracellular sodium concentration in the perineurium would be accompanied by equivalent changes within the glia.

The sodium stored in the perineurial and glial elements could account for the substantial losses of sodium ions from connectives in sodium-free saline, in the absence of appreciable changes in the amplitude of the action potentials (Bennett

et al. 1975). Decline in extracellular sodium concentration would be countered by pumping from the cellular reservoir. This would lead to the slow decline in the action potentials seen on initial exposure of connectives to sodium-deficient media (Schofield & Treherne, 1975, 1978).

The rapid recovery of the action potentials on return of sodium-depleted connectives to normal, high-sodium saline, and the rapid loss during subsequent exposure to sodium-deficient and normal salines (Schofield & Treherne, 1975, 1978) can also be explained in terms of an intracellular sodium reservoir. If prolonged exposure to sodium-free media induced an uncoupling of the gap junctions between the perineurial and glial elements, subsequent recovery of extracellular sodium would occur rapidly by pumping of sodium ions through the perineurium alone into the relatively small volume of extracellular fluid. The uncoupled glial compartment would fill only slowly under these circumstances, by passive diffusion through the glial membranes, and would explain why there is only a partial recovery in sodium content of the connectives accompanying full recovery of action potential amplitude (Bennett, Buchan & Treherne, 1975). Uncoupling of the gap-junctions in sodium-free media could result from an increase in intracellular calcium (cf. Baker, Hodgkin & Ridgway, 1971) either by reducing the permeability of the junctional channels directly, as proposed by Rose & Lowenstein, 1975), or by changing intracellular pH (cf. Meech & Thomas, 1977) as proposed by Turin & Warner (1977).

The inability of lithium ions to restore the action potentials in intact, sodium-depleted, connectives (Schofield & Treherne, 1975, 1978) despite substantial accumulation within the connectives (Bennett *et al.* 1975) can be accounted for by the assumption that the perineurial and glial Na/K pumps will not accept lithium ions, as in frog muscle (Keynes & Swan, 1959) and crab neurones (Baker, 1965). This effect would also explain the retention of lithium ions within the connectives during exposure to normal saline (Bennett *et al.* 1975), for this cation would not be pumped out of the cells.

Ethacrynic acid, a supposed sodium-transport inhibitor, is without effect on the ouabain-sensitive axonal sodium pump, but appears to affect extra-axonal (glial and/or perineurial) sodium pumping (Pichon & Treherne, 1974). Ethacrynic acid, or cooling, produces a marked hyperpolarization measured with the tip of the microelectrode located in an extracellular position (Pichon & Treherne, 1974). This effect can be attributed either to the effects on an electrogenic component of inward sodium movement across the outer perineurial membrane or to depolarization of the glial and inner perineurial membranes resulting from extracellular potassium accumulation caused by inhibition of the linked Na/K pumps. In the latter case the glial and/or perineurial depolarization would appear as a hyperpolarizing potential change when measured with an extracellularly-located microelectrode.

The effect of ethacrynic acid and dinitrophenol in slowing the net inward movement of sodium ions to the axonal surfaces, following exposure of sodium-depleted connectives to normal, high sodium, saline (Schofield & Treherne, 1975) could be by action on sodium pumps situated on the inner perineurial or glial membranes. The lack of effect of externally-applied ouabain in reducing the net inward movement of sodium to the axon surfaces (Schofield & Treherne, 1975) can be explained by the inaccessibility of these sodium pumps to this compound.

This provisional model accommodates diverse observations obtained using a variety of experimental approaches. Predictions made on the basis of this model should be susceptible to experimental testing.

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