

CATION ACCESSIBILITY OF THE PERIPHERAL NERVOUS SYSTEM IN *LIMULUS POLYPHEMUS* – AN ELECTROPHYSIOLOGICAL STUDY

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The nervous systems of many vertebrates, and of some invertebrate phyla, are known to possess a 'blood-brain barrier' conferring a selective impermeability to certain ionic and molecular constituents of the body fluids and thus protecting the excitable membranes within a relatively constant micro-environment; this subject has been recently reviewed by Abbott & Treherne (1977). In vertebrates the barrier may be a property of the tight junctions between capillary endothelial cells (Brightman & Reese, 1969). Similarly in insects, at least to the level of the large peripheral nerves, electrophysiological evidence (Treherne *et al.* 1970; Treherne, 1974) and fine-structural studies (Lane & Treherne, 1972; Lane, 1972) indicate the existence of a barrier preventing free diffusion, residing in the tight junction complexes of the perineurium. However, investigations of crustacean nervous systems (Abbott, 1970; Abbott, Moreton & Pichon, 1975) show rapid access of ions to the peripheral nerves, but a delayed entry to the central nervous tissue that may be related to gap junctions and/or to *maculae occludentes* between the perineurial sheath cells (Lane & Abbott, 1975). An analogous situation may occur in some gastropod molluscs, where a partial 'neurone-haemolymph barrier' has been described (Reinecke, 1976), though in many members of this phylum the nervous tissue is freely accessible to ions (Sattelle & Lane, 1972; Willmer, 1978).

This communication considers the peripheral nerves of a marine Xiphosuran arthropod, applying similar techniques to those used in other invertebrate systems to assess the accessibility of the tissue to physiologically important cations. A parallel study will consider the structural correlates of accessibility in both central and peripheral nerves of *Limulus* (J. B. Harrison & N. J. Lane, in preparation).

Electrophysiological records were obtained from isolated ligatured leg nerves, by a modified sucrose-gap technique; results are therefore expressed throughout using relative amplitude scales.

The effects of reduced sodium concentrations are summarized in Fig. 1*a*. The action potential was progressively reduced as $[Na]_o$ declined, and the effects are consistent with a predominantly sodium-dependent signal; the simple logarithmic relation of spike amplitude to $[Na]_o$ indicates a conventional Nernst relationship, although the calculation of a Nernst gradient is not appropriate with extracellular

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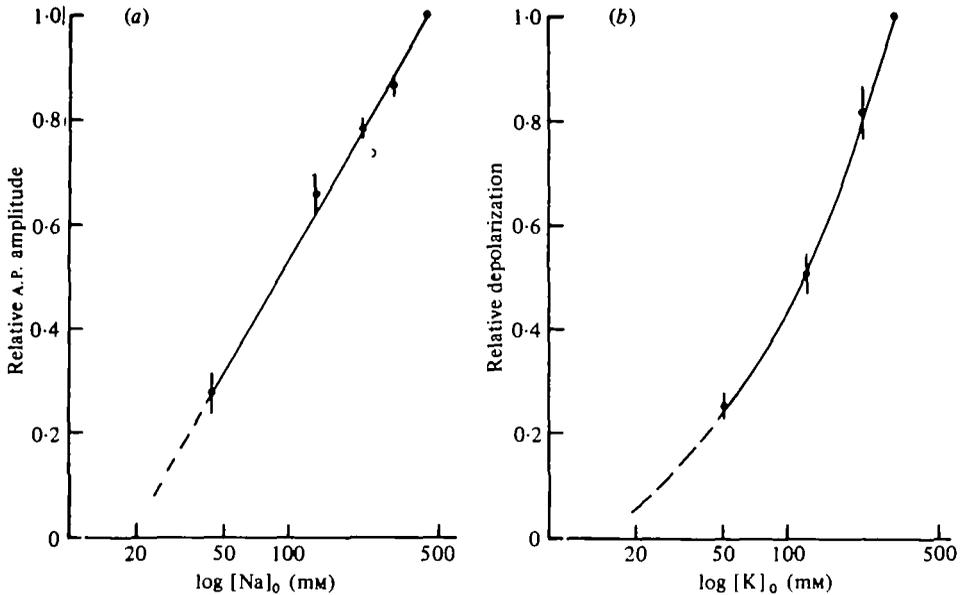


Fig. 1. (a) The effects of decreased levels of $[Na]_0$ on the action potential and (b) of potassium concentration on the membrane polarization, in *Limulus polyphemus* leg nerve. Vertical bars represent $2 \times S.E.M.$

recording techniques where the signal is variably attenuated (Schofield & Treherne, 1978). Responses to sodium were rapid and reversible, suggesting free entry and exit for this cation. Furthermore the responses were unaffected both quantitatively and qualitatively by 'desheathing' the nerve, confirming the lack of a peripheral barrier effect in association with the neural lamella.

The apparently complete sodium-dependence of the spike in *Limulus* was confirmed using tetrodotoxin (Sigma). A dose-response curve for this drug is shown in Fig. 2; the concentration required to completely block the nerve is of a similar order to that found in vertebrates (Hille, 1966) and in marine invertebrates (Narahashi, Moore & Scott, 1964; Nakamura, Nakajima & Grundfest, 1965), suggesting that a conventional sodium channel mediates cation entry during spike initiation.

As with other invertebrate nerve preparations, raising the levels of external potassium induced a depolarization in *Limulus*. The relationship of the relative polarization incurred to $[K]_0$ was again logarithmic (Fig. 1b) and potassium is thus likely to be the main determinant of the resting potential. Once again responses were similar whether recorded from intact or from desheathed nerves; the neural lamella presented no real barrier to potassium movements.

A fuller analysis of the rates of potassium movement within the tissue is given in Table 1. For all the responses tested, half-times for entry and exit of this cation were fairly short; though at all but the highest concentrations used exit of potassium was significantly slower than entry. This could indicate some binding of introduced cations to the mucopolysaccharide thought to constitute the extracellular matrix, as has been suggested for crustacean nervous systems (Abbott, Pichon & Lane, 1977), rather than a real 'restriction' of cation movement by a specific structural phenomenon.

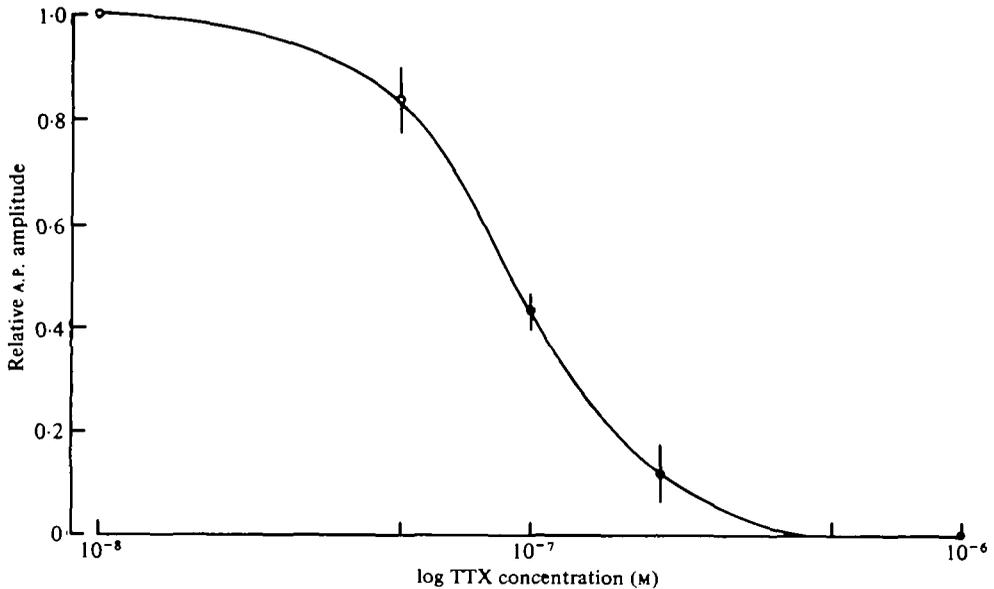


Fig. 2. The response of *Limulus* nerves to varying concentrations of tetrodotoxin.

Table 1. Values of $T_{0.5}$ for movements of potassium ions within the leg nerve of *Limulus* during exposure to varied external K^+ concentrations

$T_{0.5}$ (s)	K_o (mM)			
	50	124	233	321
Entry	35.6 ± 4.4	41.0 ± 1.2	46.3 ± 10.5	57.3 ± 11.2
Exit	64.3 ± 2.4	68.7 ± 7.2	69.0 ± 7.0	58.7 ± 13.5

The results clearly indicate the lack of any fundamental limitation of cation movements in *Limulus* peripheral nerve. This concurs with the finding that lanthanum readily penetrates throughout the extracellular spaces of the nerve (J. B. Harrison & N. J. Lane, in preparation), and correlates with the lack of junctional complexes revealed by electron microscopy (Dumont, Anderson & Chomyn, 1965; J. B. Harrison & N. J. Lane, in preparation). *Limulus* nerve is also shown to be conventional in its cation requirements, so that it will only function normally when presented with media whose cationic constituents are comparable to the seawater which the animal habitually encounters and to which its blood approximately corresponds (Cole, 1940; Robertson, 1970). Thus it resembles the majority of marine and littoral invertebrates, with which it shares common environmental problems, in having a nervous system freely accessible to the circulating body fluids; the insects, faced with the greater hazards of terrestrial existence and more variable haemolymph composition, remain the only invertebrate group known to possess a true blood-brain barrier.

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