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AN ELECTROPHYSIOLOGICAL STUDY OF THE SODIUM AND POTASSIUM PERMEABILITIES OF INSECT PERIPHERAL NERVES

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INTRODUCTION

The central nervous systems of the insect species which have been investigated have been shown to possess a well-developed blood–brain barrier system which severely restricts the movements of water-soluble ions and molecules between the neuronal surfaces and the blood or bathing medium (see Treherne & Pichon, 1972, for references). Recent investigations suggest that the restricted access to the extra-axonal fluid in cockroach central nervous connectives results from the presence of perineurial tight junctions which occlude the intercellular clefts that traverse the perineurium (Maddrell & Treherne, 1967; Lane & Treherne, 1970; 1971, 1972). It has also been shown that alterations in the concentrations of inorganic cations in the bathing medium is accompanied by appreciable extraneuronal potential changes obtained using intracellular or extracellularly located microelectrodes (Treherne *et al.* 1970; Pichon & Treherne, 1970; Pichon, Moreton & Treherne, 1971). In the case of elevated external potassium concentrations, for example, it has been postulated that the extraneuronal potential changes result from the effect of this cation in depolarizing the outwardly facing perineurial membrane, access to the inwardly directed one being restricted by the perineurial tight junctions (Lane & Treherne, 1972). Essentially similar explanations have been subsequently applied to the equivalent phenomena observed in the central nervous connectives of a lepidopteran (Pichon, Sattelle & Lane, 1972) and a crustacean species (Abbot & Pichon, 1973; Pichon & Abbot, 1973).

The situation in insect peripheral nerves has not been elucidated, and at the present time it is not clear to what extent the hypotheses erected for the central nervous system can be applied to them. The classical experiments of Hoyle (1952, 1953) showed a delayed effect of elevated potassium concentrations in reducing the amplitude of extracellularly recorded action potentials in the crural nerve of *Locusta migratoria*. These observations were interpreted in terms of a peripheral diffusion barrier associated with the nerve sheath and the overlying 'tracheolated membrane'. These conclusions contrast with those obtained for crustacean species in which peripheral nerves have been shown to be easily accessible to sodium and potassium ions, whereas an apparent restriction to intercellular diffusion was associated with the perineurium in

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central nervous connectives (Abbott & Pichon, 1973). It has also been observed that in small peripheral nerves of the cockroach there may not be any discrete peripheral cellular layer equivalent to the perineurium (Lane & Treherne, 1973). In these peripheral nerves it appears that the extracellular fluid bathing the axonal surfaces is accessible to externally applied lanthanum (Lane & Treherne, 1972).

The present electrophysiological investigation was undertaken to assess the degree of accessibility of axons of large peripheral nerves to water-soluble cations in two insect species.

MATERIALS AND METHODS

Adult specimens of the American cockroach *Periplaneta americana* and the desert locust *Schistocerca gregaria* Forsk. have been used for these experiments. The cockroaches were reared in the laboratory at a temperature of approximately 30 °C on a standard diet of dog biscuits, linseed cake and rolled oats; the locusts were kept at a daily cycle of 12 h at 35 °C (in daylight) and 25 °C (in darkness) and were reared on potted wheat and bran.

The crural leg nerve (5 B 1) of the locust and nerve 5 of the third thoracic appendage of the cockroach were used in this investigation.

The normal physiological solution used for the cockroach was that devised by Yamasaki & Narahashi (1959) and had the following composition: 214·0 mM/l Na^+, 3·1 mM/l K^+, 1·8 mM/l Ca^{2+}, 216·9 mM/l Cl^-, 0·2 mM/l $H_2PO_4^-$ and 1·8 mM/l HPO_4^{2-}. Sodium-deficient saline was produced by substitute of $tris^+$ for Na^+. A similar normal solution was used for the locust except that the Na^+ was reduced to 155 mM/l and K^+ elevated to 10 mM/l, with equivalent alteration in concentration of Cl^-.

The first series of electrophysiological experiments were performed on isolated metathoracic legs of both the cockroach and the locust. Two windows were opened in the cuticle in the coxa and the femur. A pair of hooked chlorided silver electrodes, placed under the nerve in the coxa, were used to stimulate the preparation. Short-duration rectangular current pulses were delivered to these electrodes by a Farnell stimulator through an RF isolating unit. The electrical activity of the nerve was recorded in the femur between an indifferent electrode connected to the ground and a suction electrode as shown in Fig. 1. This portion of the leg was continuously perfused by the test solution. Very rapid changes of solutions could be obtained using a multiway non-return valve (Holder & Sattelle, 1972) placed close to the preparation to reduce the dead space. This was connected to the micropipette through which the perfusion was made by means of a short length of flexible nylon tubing.

In the second series of experiments one branch of the crural nerve was dissected from the femur of the jumping-legs of the locust and mounted in a sucrose-gap chamber analogous to that devised by Treherne *et al.* (1970) and Pichon & Treherne (1970) for the isolated nerve cord of the cockroach. The preparation was stimulated at one end, between two pools of saline. The compound action potential was recorded across a sucrose-gap between the middle (test) compartment, which contained the experimental solution flowing at a regulated rate, and the left-hand compartment which was filled with isotonic KCl. Changes of the solution in the test compartment were achieved using a multiway non-return valve arranged close to the nerve chamber.

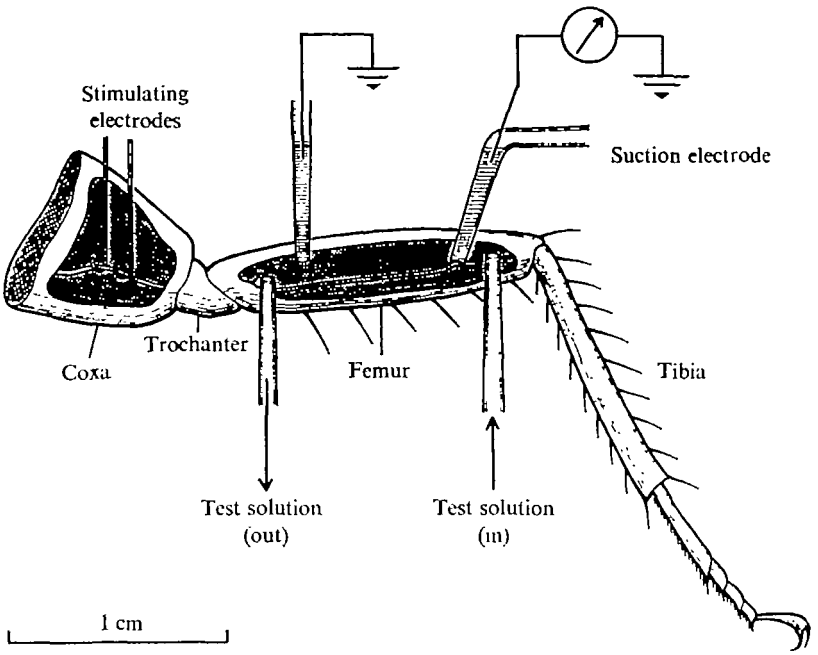

Fig. 1. Schematical representation of the metathoracic leg of the cockroach as used in the *in situ* experiments on the effects of alterations of the external concentrations of Na^+ and K^+ on conduction of compound action potentials.

For both kinds of experiments perfusion was achieved using a gravity-feed system from a series of elevated reservoirs.

All recordings were made via a unity-gain high-impedance FET amplifier connected to a Tektronix 561 oscilloscope and to a Smith Servoscribe potentiometric recorder. As in previous experiments on the central nerve cord, variable degrees of tension were applied to the nerves. In some experiments the fat body sheath was removed using small scissors and watchmaker forceps. This dissection was in some cases followed by a resection of the nerve sheath using carefully sharpened steel needles. Care was taken to avoid any drying of the preparation.

Experiments were carried out at room temperature ranging from 22 to 24 °C.

RESULTS

Effects of sodium-free, tris, saline on cockroach nerve

The response of the metathoracic nerve 5 to sodium-deficient conditions in the bathing medium depended upon the state of the preparation. With preparations which had been stretched (to approximately $1\frac{1}{8}$ times normal length) exposure to sodium-free solution resulted in a rapid decline in the amplitude of extracellularly recorded action potentials (Fig. 2). A rapid recovery was also obtained on restoration of normal sodium concentrations in the bathing medium. These results contrast with those obtained with intact, unstretched, preparations in which action potentials could be elicited for extended periods in sodium-free saline (Fig. 3).

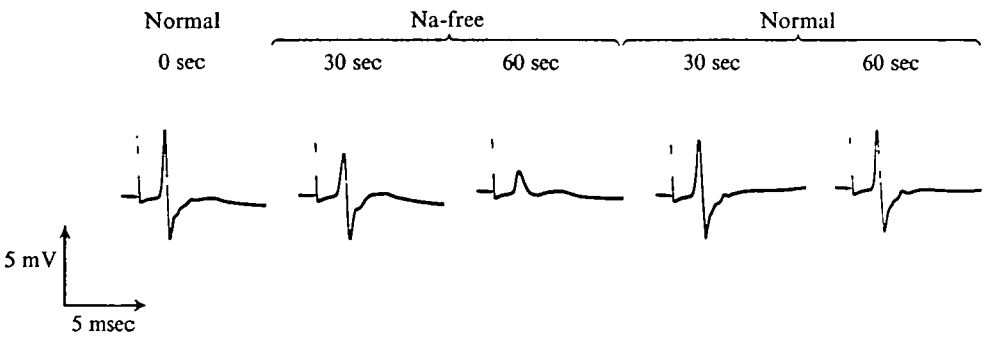

Fig. 2. Effect of sodium-free, tris, saline on conducted action potentials in stretched cockroach crural nerves recorded using a suction electrode.

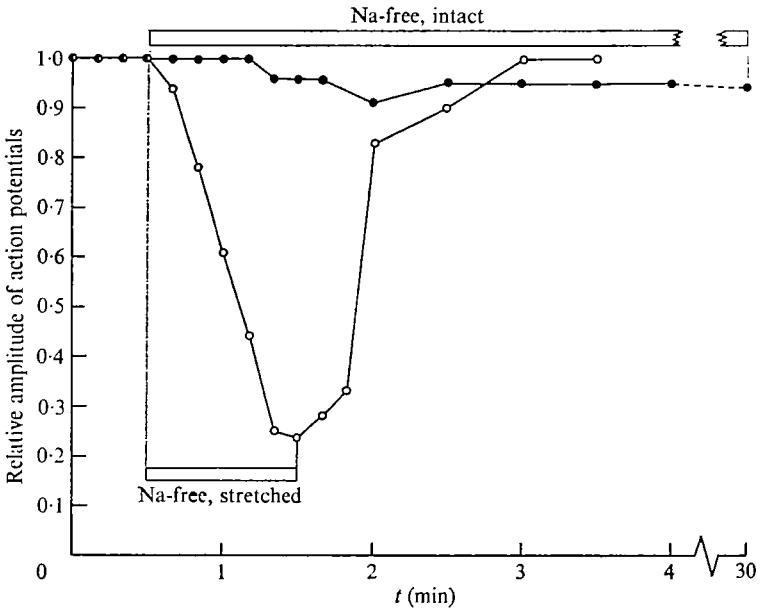

Fig. 3. Effect of sodium-free, tris, saline on the amplitude of compound action potentials recorded with suction electrodes in stretched (open circles) and intact crural nerves (closed circles) of the cockroach.

Effects of sodium-free, tris, saline on locust crural nerve

Fig. 4 illustrates the electrical response of locust crural nerve, which was de-sheathed following removal of the fat body sheath (see Fig. 5), during exposure to sodium-free saline. In this preparation substitution of tris for sodium ions in the bathing medium resulted in a rapid decline in amplitude of the action potentials, followed by an equivalent restoration of the action potentials on return to normal sodium concentration in the bathing medium.

Intact nerves, contained in the neural fat body sheath, showed no appreciable decline in amplitude of the action potentials during periods of exposure to sodium-free saline (Fig. 6a). This is apparently not related to the fat body sheath for essentially

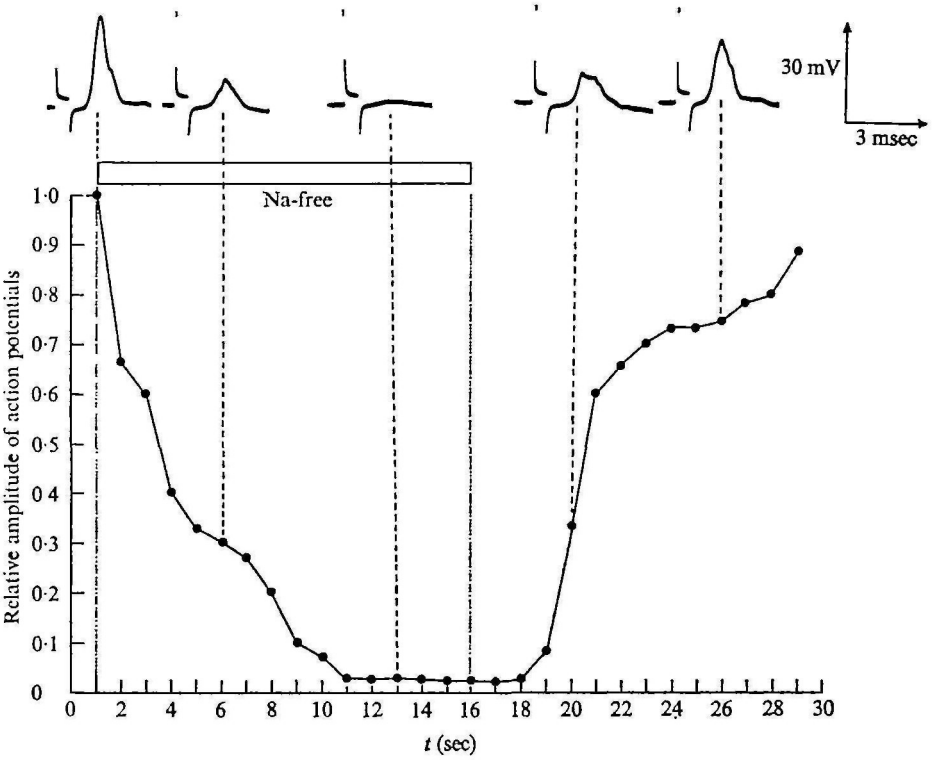

Fig. 4. Effect of substitution of sodium ions for those of tris on the compound action potentials recorded across a 'sucrose-gap' in the desheathed crural nerve of the locust.

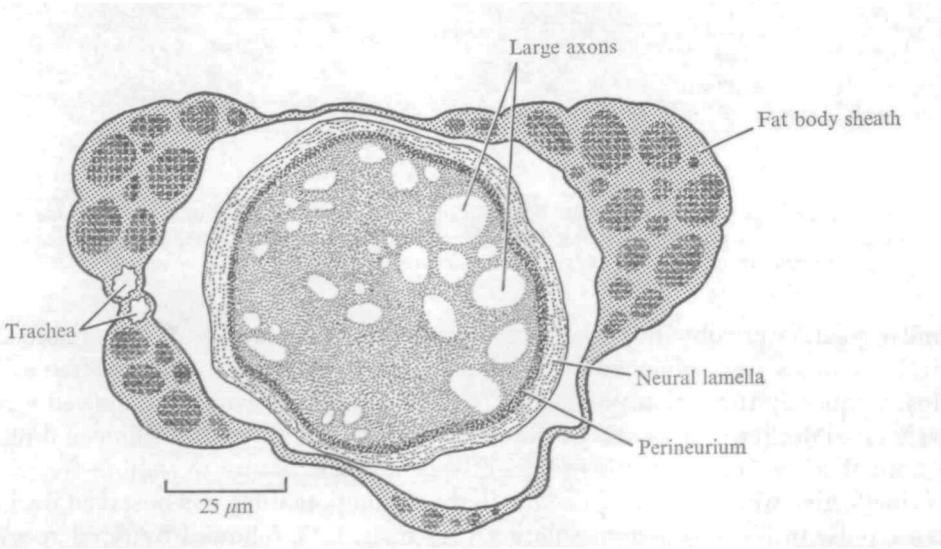

Fig. 5. Diagram of a transverse section through the crural nerve of the locust, showing the cellular fat body sheath, or 'tracheolated' layer of Hoyle (1952), that loosely surrounds the nerve. Note that the nerve itself possesses a neural lamella which ensheaths a perineurial layer beneath which lie glial-ensheathed axons.

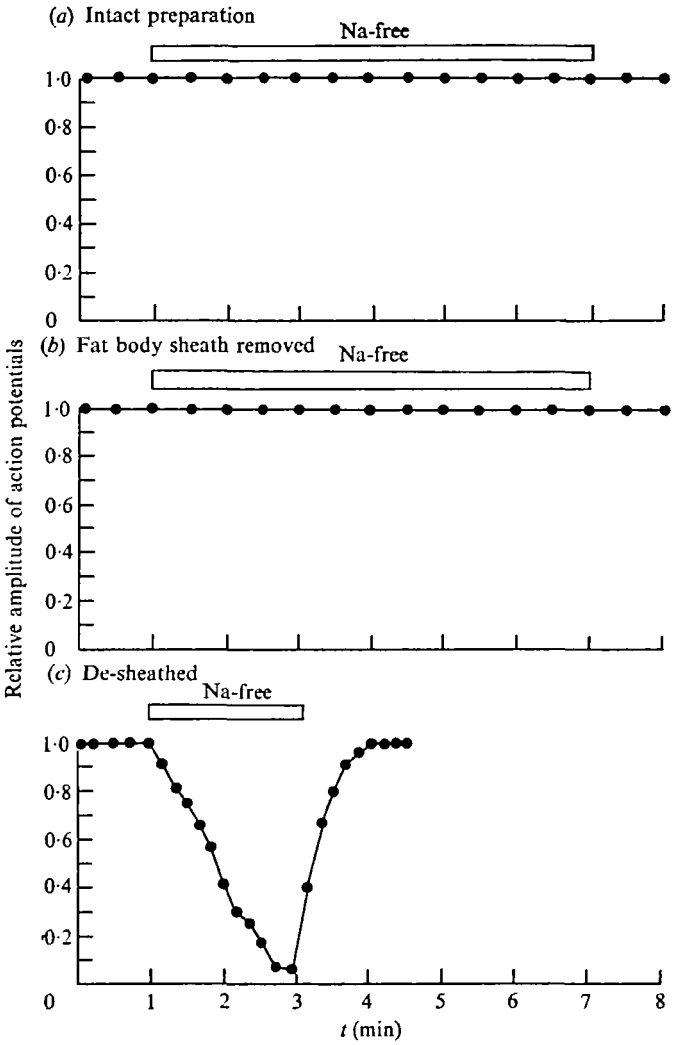

Fig. 6. Effects of removal of the fat body sheath (*b*) and stretching (*c*) on the amplitude of compound action potentials during exposure to sodium-free, tris, saline. Recorded across a 'sucrose-gap' from the crural nerve of the locust.

similar results were obtained when this sheath was removed (Fig. 6*b*). Variable electrical responses were obtained with stretched nerves bathed in sodium-free saline. Most frequently the action potentials recorded in such preparations showed a relatively rapid decline and recovery following exposure to sodium-free saline and return to normal saline (Fig. 6*c*). Less frequently an initial exposure to sodium-free saline was ineffective whereas a rapid decline in the action potentials was observed during a 30 sec pulse of high-potassium solution (217 mM/l K^+), followed by rapid recovery on return to normal saline. Of interest is the fact that, following this treatment, further exposure to sodium-free conditions resulted in a relatively rapid collapse of the action potentials with recovery on return to the normal sodium concentration in the bathing medium (Fig. 7). These results suggest that, at a critical degree of applied tension,

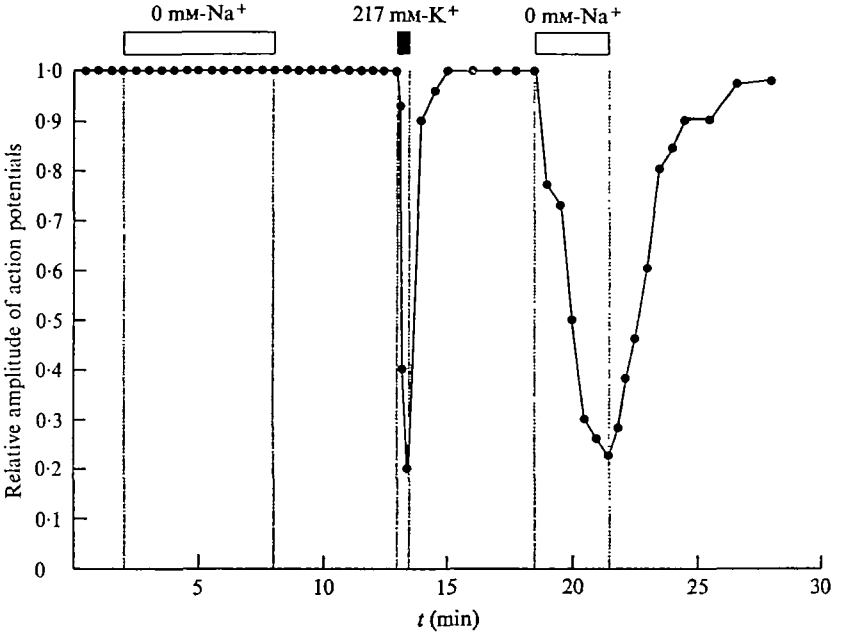

Fig. 7. The effect of a 30 sec pulse of high potassium (217 mM) on the electrical behaviour of a stretched crural nerve of the locust on exposure to sodium-free, tris, conditions. Sucrose-gap recording.

a brief exposure to high potassium saline alters the state of the preparation so that the movement of sodium ions away from the axon surfaces now occurs more rapidly.

Effects of sodium-free, high-potassium, saline on locust crural nerve

In de-sheathed preparations exposure to solutions of elevated potassium concentration resulted in relatively rapid electrical responses. Fig. 8 shows the d.c. potential change recorded under these conditions. This apparent depolarization, recorded with the sucrose-gap technique, was accompanied by an equivalent reduction in the amplitude of the action potentials. These results can be interpreted in terms of a relatively rapid access of potassium ions to the axon surfaces, the d.c. potential change corresponding to axonal depolarization.

The electrical response of more intact nerves to exposure to elevated potassium concentrations differed markedly from that of de-sheathed preparations. Fig. 9 illustrates the effects of high external potassium concentration in a crural nerve from which the overlying fat body sheath had been removed, the nerve sheath being left intact. It will be seen that there is an extremely rapid change in the recorded d.c. potential (approx. 78 mV sec^{-1}). This change was not accompanied by an appreciable decline in the amplitude of the recorded action potentials, which, as will be seen from Fig. 9, showed only a potential shift equivalent to a depolarization. By analogy with the situation in cockroach central connectives (Treherne *et al.* 1970) it can be reasonably supposed that the rapid apparent depolarizations, recorded in the absence of any appreciable decline in axonal responses, are extraneuronal in origin.

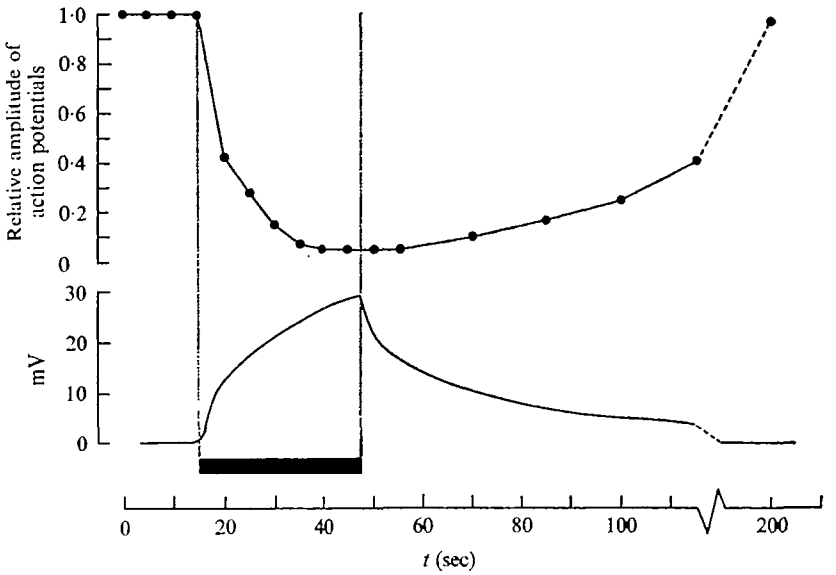

Fig. 8. Sucrose-gap recording showing the effect of high-potassium, sodium-free saline on action potentials and d.c. potential in the de-sheathed crural nerve of the locust.

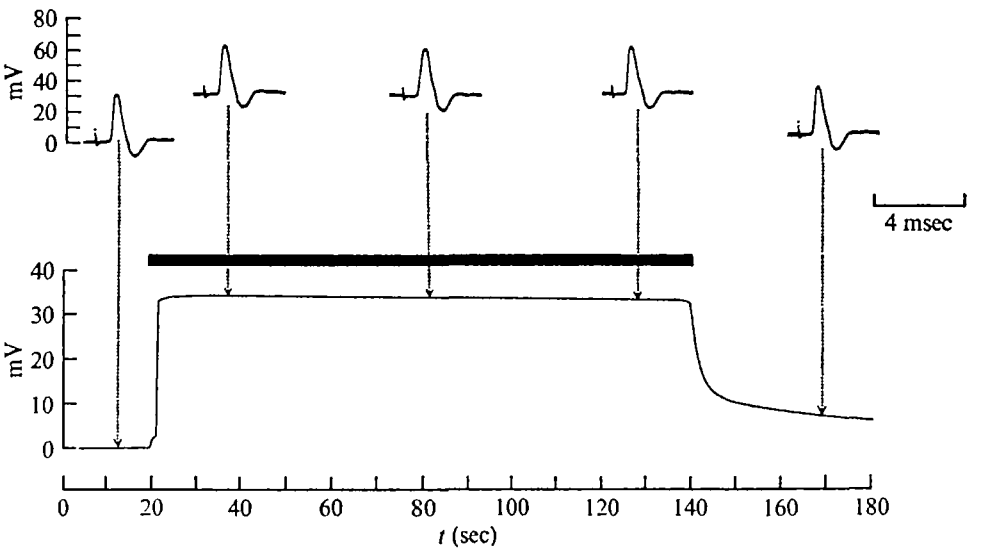

Fig. 9. Sucrose-gap recording of action potentials and d.c. potential of locust crural nerve during exposure to high-potassium, sodium-free, saline. Fat body sheath removed.

The form of the potassium-induced potential change differed in some respects from the changes observed in the absence of the neural fat body sheath (Fig. 10). A consistent feature of the extra-neuronal potentials recorded in intact connectives with the fat body sheath intact was the presence of an appreciable slow phase of depolarization immediately following exposure to high potassium. On initial exposure to high-potassium solutions the slow phase lasted for a period of up to 43 sec before the onset of the rapid phase of apparent depolarization.

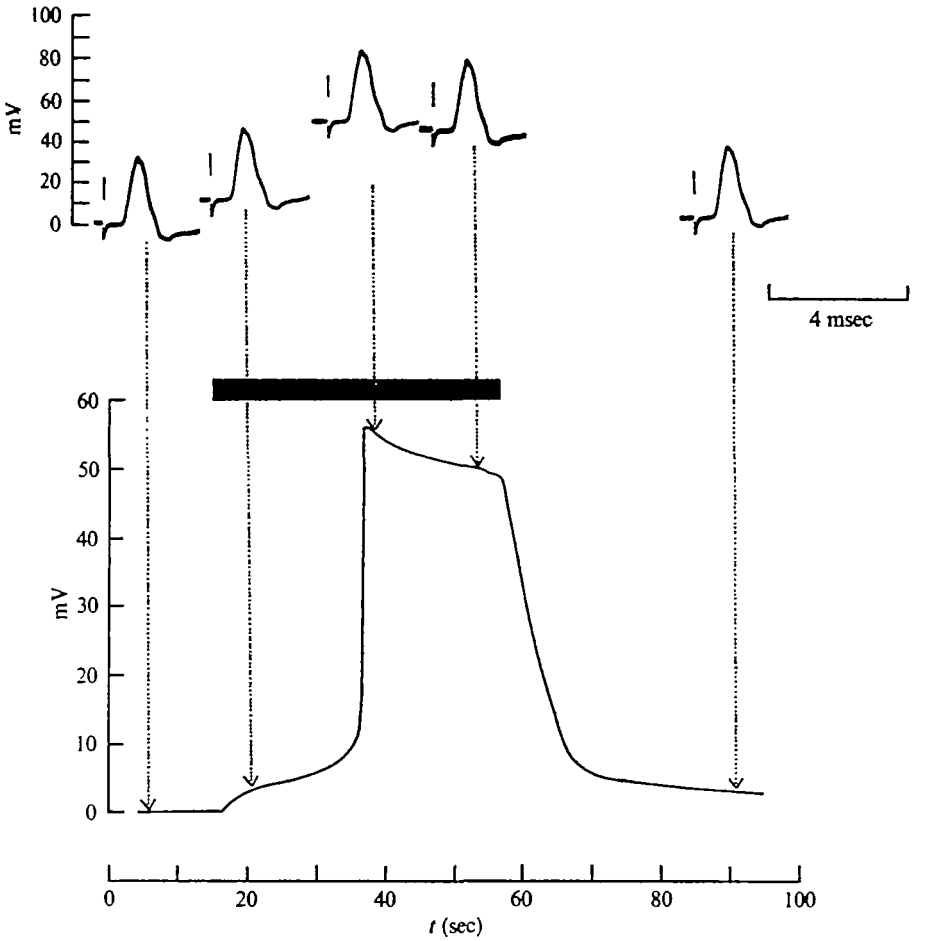

Fig. 10. Effects of exposure to high-potassium, sodium-free, saline on the d.c. potential and action potentials recorded across a 'sucrose-gap' in an intact crural nerve of the locust.

The duration of the initial slow phase of the potassium-induced extraneuronal potentials (in presence of the fat body sheath) depended upon the time which had elapsed since previous exposure to high-potassium conditions (Fig. 11). The slow phase was always maximal on first exposure and was reduced during a subsequent exposure to high potassium, the reduction being inversely proportional to the interval between successive exposures. This relation appears to be roughly linear for a given preparation (Fig. 12).

The mid-point of the slow initial potential change corresponded to a 3–5 mV depolarization. The tangent to the curve at this point had a slope of the order of 1 mV min^{-1} (i.e. 500 times slower than the rising phase of the fast extraneuronal depolarization). Its intersection with the straight line extrapolated from the fast-rising phase corresponded to a depolarization of 6–8 mV.

An additional feature associated with the extraneuronal potentials recorded in the presence of the fat body sheath was the slight decay following attainment of maximal

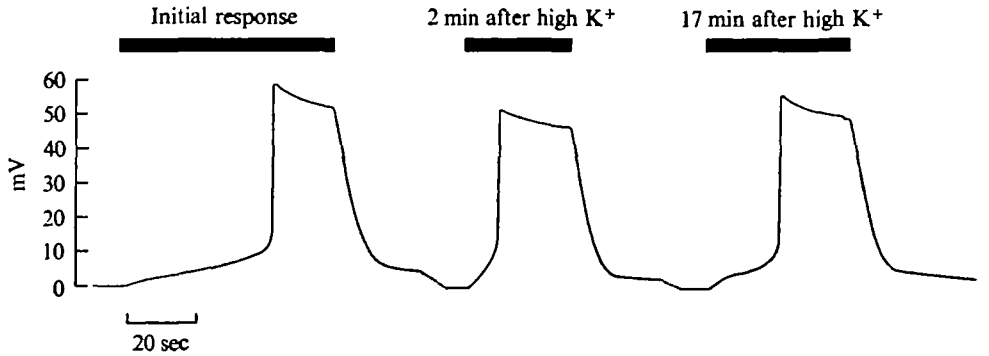

Fig. 11. Effects of successive exposures to high-potassium, sodium-free, saline on the time course of the d.c. potential changes recorded in the intact crural nerve of the locust. 'Sucrose-gap' recording.

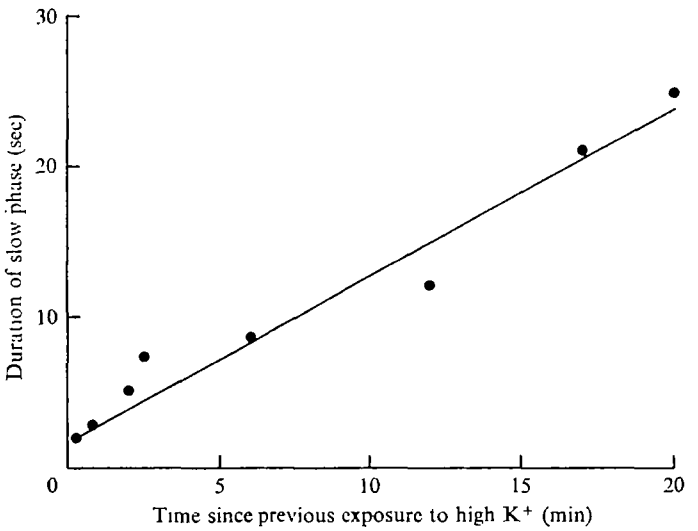

Fig. 12. Relationship between the duration of the slow phase of potassium-induced depolarization and time since previous exposure to high-potassium saline. Intact crural nerve of the locust. 'Sucrose-gap' recording.

potential. This slight decay, which was not observed in preparations from which the fat body sheath was removed, corresponded to 5–8 mV exponential repolarization with a $t_{0.5}$ between 5 and 8 sec in the preparation illustrated in Fig. 10.

The slow phases elicted by successive short-duration exposures to high potassium could summate (Fig. 13). When a critical level of depolarization was reached an all-or-none fast extraneuronal depolarization was triggered followed by a slower return to a slightly depolarized level. This extraneuronal 'spike' always appeared when the critical level was reached even though the potassium concentration was quickly switched back to its original low level.

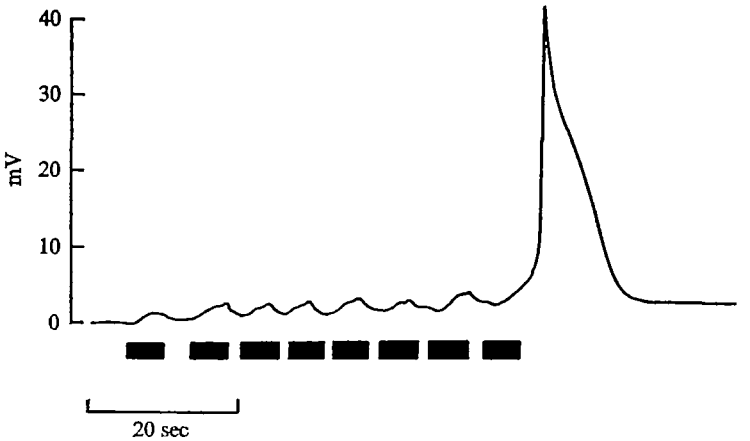

Fig. 13. Effects of successive short exposures to high-potassium, sodium-free saline, on the d.c. potential changes recorded in an intact crural nerve of the locust. 'Sucrose-gap' recording. Note appearance of terminal 'all-or-none' response.

DISCUSSION

The above results amply confirm the conclusions of Hoyle (1952, 1953) who postulated a restricted access of potassium ions to the axons in the crural nerve of the locust *Locusta migratoria*. This restriction is manifested in the delayed effects of sodium-deficient and elevated-potassium solutions on the amplitude of the recorded action potentials in intact nerves, as compared with the rapid collapse observed in de-sheathed preparations.

Stretching of both locust and cockroach peripheral nerves was found to result in an apparent disruption of the peripheral intercellular diffusion barrier, as has been previously shown in cockroach central connectives (Pichon & Treherne, 1970). Of particular interest in this respect was the observation of a lack of effect of sodium-free solution on the action potentials recorded in some stretched preparations of the locust crural nerve. A 30 sec exposure to high-potassium solution followed by a return to normal solution, however, was found to render the axons accessible following subsequent exposures to sodium-free solution. This phenomenon can be presumed to result from the depolarizing effect of high-potassium conditions, which could produce an uncoupling of perineurial tight junctions (see Lane & Treherne, 1972) in preparations maintained under a critical degree of applied tension.

The restricted access of water-soluble cations to the axon surfaces in large leg nerves of the cockroach and locust apparently differs from the situation in the small peripheral nerves of the cockroach described by Lane & Treherne (1972) in which extraneously applied lanthanum was found to penetrate to the axon surfaces. The apparent lack of restriction in the smaller peripheral nerves of this species can presumably be correlated with ultrastructural organization of these small peripheral nerves in which the perineurium is not recognizable as a discrete cellular layer (Lane & Treherne, 1973). The peripheral restriction to cation movements in the large leg nerves of the locust and cockroach also contrasts with the situation in the leg nerves of *Carcinus* and *Procambarus* despite the demonstrated perineurial restriction to cation movements

associated with central nervous connectives of these crustacean species (Abbott & Pichon, 1973). In peripheral nerves of these crustaceans, for example, the potential changes recorded in response to exposure to isotonic KCl were interpreted in terms of a progressive depolarization of the axon membranes as the potassium ions diffused into the intercellular spaces (Abbott & Pichon, 1972). Quantitative considerations showed that the rapidity of these electrical responses could be interpreted in terms of a ready movement of small water-soluble ions and molecules between the blood and the nerve interstitial space (see also Baker, 1965).

As in cockroach central connectives (Treherne *et al.* 1970; Pichon & Treherne, 1970; Pichon *et al.* 1971) substitution of potassium ions for those of sodium in the bathing medium induced rapid depolarizing potential changes in intact locust crural nerve from which the fat body sheath had been removed. As the recorded action potentials exhibited no equivalent reduction in amplitude it can be concluded that the apparent depolarizations did not occur at the axonal level. The rapidity of the potassium-induced extraneuronal potential change clearly suggests that the depolarization of a peripheral cellular structure is involved. By analogy with the cockroach central nervous system it can be reasonably supposed that the extraneuronal potential changes associated with locust crural nerve result from the effects of alteration of the external cation concentration on the outwardly directed membrane of the peripheral cellular layer which immediately underlies the connective tissue neural lamella. This analogy implies that the penetration of small water-soluble cations is restricted as in cockroach central connectives (Lane & Treherne, 1972) by occlusions of the intercellular channels which limit the access of cations to the inwardly directed membrane of the peripheral cellular layer underlying the neural lamella.

The potassium-induced extraneuronal potential change of locust crural nerve occurred with greater rapidity than the equivalent response observed in cockroach central connectives. In the former preparation depolarization occurred at approximately 78 mV sec^{-1} as compared with the maximal value of around 12 mV sec^{-1} measured in cockroach central connectives. This suggests either that in the absence of the fat body sheath access of potassium ions to the outwardly directed perineurial surface occurs more rapidly in locust crural nerve as compared with cockroach central connectives or that the slope of the curve relating to the extraneuronal potential to the external potassium concentration is steeper in locust nerve.

The results indicate that the presence of the neural fat body sheath has a noticeable effect on the form of the extraneuronal potentials recorded in locust crural nerve when bathed with high-potassium saline. This is particularly apparent in the appearance of the initial slow phase which preceded the rapid phase of depolarization and which was absent in preparations from which the fat body sheath had been removed. It was further demonstrated that the duration of the initial slow phase depended upon the extent of previous exposure to high-potassium conditions.

It is conceivable that the diffusion of potassium ions through the fat-body sheath involves some degree of restriction, thus giving rise to a relatively slow initial perineurial depolarization. Such a restriction would imply the development across this sheath of a potential difference whose value would be proportional to the relative permeability of the sheath to potassium and to the concentration gradient for these ions. If the permeability remained constant, the potential would be maximal at $t = 0$, then decrease

with time and be equal to zero at equilibrium. The rapid extraneuronal potential would then be preceded by an early potential exhibiting a sharp rising phase. The amplitude of this early potential change would depend on the relative permeabilities of the fat-body sheath to different ions.

As the major ions which are involved are K^+, Na^+ and Cl^- the value of this potential would approach V_m, where

$$V_m = \frac{RT}{F} \log \frac{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o},$$

P_K, P_{Na} and P_{Cl} are the relative permeabilities of K^+, Na^+ and Cl^- ions and $(Na)_o$, $[K]_o$, $[Cl]_o$, $[Na]_i$, $[K]_i$ and $[Cl]_i$ the activites of the ions respectively at the outer and the inner side of the fat body sheath. R, T and F have their usual significance. This simple model is not sufficient to explain the experimental results. First, the rising phase of the early potential recorded in an experiment was always relatively slow. Secondly, there is no obvious reason why the initial potential should be different after the first exposure to high potassium. Thirdly, the rising phase of the theoretical extraneuronal potentials should be slower in fully intact preparations than in preparations from which the fat body sheath has been removed. A tentative explanation might be that the permeability could increase after a period of exposure to high concentration of potassium. The mechanism whereby such an increase could occur remains speculative. It cannot be excluded, for example, that K^+ ions crossing the fat body sheath down their concentration gradient could increase the permeability of this sheath to potassium. This explanation would imply that an extremely large change in K^+ permeability must take place in order to account for the observed rate of rise of the extraneuronal potential.

Alternatively, it could be postulated that virtually no potential is created across the fat body sheath, which has been so far shown to be very leaky in other preparations (Lane & Treherne, 1971; Treherne, 1971) but that the slow initial potential could result from a peripheral reservoir effect imposed by the presence of the fat body sheath. Such a reservoir could be associated with the uptake of this cation by the fat body cells or with the presence of anionic molecules maintained at the periphery of the nerves by the fat body sheath and washed out when this last structure is removed. The observation that the duration of the initial slow phase of depolarization is dependent upon the extent of previous exposure to potassium ions would accord with the latter hypothesis. Similarly, the summation and the threshold affects demonstrated in the initiation of the rapid phase of depolarization, recorded following successive short pulses of high potassium, accords with the concept of a peripheral cation reservoir associated with the fat body sheath. This interpretation implies that the potassium concentration at the outer perineurial surface changes at maximal rate only after the cations have come into equilibrium with the peripheral reservoir. The data, applied to this model also suggests that the uptake of potassium ions into the reservoir occurs more rapidly than the reverse process on return to normal conditions. The slow initial phase of depolarization, for example, only shows a substantial return after relatively long periods of exposure to normal ionic conditions in the bathing medium.

The slight decay which always follows the fast rise in intact preparations cannot,

unfortunately, be interpreted in terms of a reservoir effect. A superficial analysis could lead to the conclusion that it is due to the depolarization of the inner perineurial membrane by K^+ ions moving through the nerve sheath as predicted from the model erected by Pichon *et al.* (1971) for the connectives of the central nervous system of the cockroach, and extended by Abbott and Pichon (1972) to the central nervous systems of the crab and the crayfish. It would then imply that intact nerves are more leaky than those from which the fat body sheath has been removed—an assumption which is hardly tenable. It is more likely that this decay is in some way related to the existence of the fat body sheath and is superimposed on a stable extraneuronal depolarization. Such a repolarization might, for instance, correspond to the falling phase, of the early potential originating across the fat body sheath. However, a more careful analysis of the experimental findings does not directly support this hypothesis. There is thus some evidence that the time course of this phase is correlated with the size of the extraneuronal potential at the peak; the higher this peak the faster the decay. Furthermore, when the slow depolarizing phase has been abolished by a recent exposure to high potassium, the decay phase is reduced but not suppressed.

The nature of the transient electrical responses elicited from the perineurium in the presence of the fat body sheath does not suggest that the latter structure acts as a significant diffusion barrier to potassium ions. For example, the rapid phase of potassium depolarization occurs at a rate similar to and even faster then that observed in the absence of the fat body sheath. Furthermore, it should be borne in mind that the initial slow phase of potassium depolarization is accomplished at a rate which is nevertheless rapid when compared with the effects of elevated potassium concentrations on the axonal responses in preparations from which the fat body sheath was removed. This conclusion accords with the electrophysiological and ultrastructural observations carried out on the neural fat body sheath associated with the central nervous system of *Carausius morosus* (Lane & Treherne, 1971; Treherne, 1972).

Further studies on both quantitative and ultrastructural aspects of this work are in progress in this laboratory.

SUMMARY

1. Experiments carried out *in situ*, using suction electrodes, and *in vitro*, using the 'sucrose-gap', have demonstrated a restricted access of sodium and potassium ions to the axon surfaces in crural nerves of the cockroach *Periplaneta americana* and the locust *Schistocerca gregaria*.

2. Elevation of the external potassium concentration produced appreciable extraneuronal potential changes in intact crural nerves of the locust.

3. In the locust the presence of the over lying fat body sheath was found to alter the time course of potassium-induced d.c. potential changes.

4. In particular, an initial lag period in the d.c. response is described and tentatively interpreted in terms of a cation reservoir effect.

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