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POTASSIUM ACTIVITY MEASUREMENTS IN THE MICRO-ENVIRONMENT OF THE CENTRAL NERVOUS SYSTEM OF AN INSECT

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SUMMARY

The activity of K^+ and the control of influx of K^+ into the extracellular space (micro-environment) of the central nervous system of the cockroach, *Periplaneta americana*, were measured directly with K^+-sensitive microelectrodes. Using an *in vivo* preparation, it was possible to follow the effects of changes in K^+ concentration in the medium bathing the nervous system on extracellular K^+ and spontaneous and evoked neuronal activity. For bath K^+ levels less than 31 mmol l^{-1}, roughly corresponding to maximal haemolymph level in natural physiological conditions, the blood–brain barrier was found to be suitably efficient in restricting the influx of K^+ and thereby allowing normal neural activity. At an external K^+ concentration of 100 mmol l^{-1}, however, the system was unable to maintain a sufficiently low extracellular K^+ concentration and neuronal activity was suppressed. Influx of K^+ from the external medium into the micro-environment occurred mainly in two phases. The early phase had a fast time course and probably reflects the physical aspects of the blood–brain barrier. The later, second phase was a slower process possibly corresponding to activation of metabolic ion pumps. The time courses of the functioning of these two systems and their control of the extra-axonal K^+ activity are also discussed.

INTRODUCTION

In order to function effectively, it is important that neurones maintain steep ionic gradients across their axonal membranes. Thus, the ionic composition of the fluid immediately surrounding a neurone as well as that of the intracellular medium must normally be efficiently regulated. As with all invertebrates, the insect central nervous system (CNS) is bathed by the haemocoelomic fluid, which has markedly different ion compositions, among Orders and also among individuals of the same Order and given species during their life cycles, including diurnally (Bishop, Briggs & Ronzoni, 1925; Bone, 1946; Tobias, 1948; Hoyle, 1952, 1953, 1954; van Asperen & van Esch, 1956; Pichon & Boistel, 1963, 1968; Sutcliffe, 1963; Brady, 1967; Pichon, 1970; Lettau, Foster, Harker & Treherne, 1977). In the insect nervous system a

Key words: blood–brain barrier, extracellular potassium, ion-sensitive microelectrodes, *Periplaneta americana*.

blood–brain barrier is involved in regulation of extracellular ion concentrations within the CNS and major peripheral nerves and control of movements of ions into and out of the extracellular space in the face of changing haemolymph ion concentrations. Present evidence from the cockroach suggests that the blood–brain barrier is located in the layer of superficial neuroglia (perineurium) (Treherne & Schofield, 1981; Schofield, Swales & Treherne, 1984), and that the largest diffusion potentials are generated across the outer membranes of the perineurial cells when the ionic composition of the bathing solution is altered (Pichon & Treherne, 1970; Schofield *et al.* 1984). There is also diffusion of ions through gap junctions between perineurial and underlying glial cells. The immediate, truly extracellular space of the CNS is a micro-environment of much less than 1 μm in width (Treherne, 1985). The ionic composition of the micro-environment is maintained finally by ion pumps on glial and neuronal cell membranes (Treherne, Lane, Moreton & Pichon, 1970; Pichon & Treherne, 1974; Treherne & Schofield, 1979, 1981; Treherne, 1974, 1985).

Although a great deal is known concerning mechanisms of ion transport between the cellular elements of the cockroach CNS (neurones and glia) and the bathing medium (see Treherne, 1974, 1985 for reviews), ionic fluxes taking place within the micro-environment itself have not been studied in any detail. In the present study, using guidelines established by Schofield *et al.* (1984), we have succeeded in measuring and characterizing K^+ activities within the micro-environment (extra-axonal spaces) of the cockroach CNS. K^+-sensitive microelectrodes and an intact *in vivo* preparation involving minimal disturbance of the CNS were used.

MATERIALS AND METHODS

All experiments were performed on adult male *Periplaneta americana* (L.) cultured at 27 °C in a 16 h:8 h dark:light regime and fed on rat pellets and tap water. They were given only water for 24 h prior to experimentation. After anaesthesia with carbon dioxide, the legs and wings were removed and the cockroach was dissected dorsally so as to expose the ventral nerve cord (VNC). A staple-pin was positioned over the VNC anterior to ganglion A_2 to aid stability. This procedure did not affect diffusion of solutions to the recording sites in the VNC (Fig. 1 inset). Great care was taken not to stretch or allow the VNC to come into contact with air, both of which have been shown to have adverse affects on neuronal functioning (Pichon & Treherne, 1970). The body cavity was flooded with regularly changed Ringer solution modified from that of Yarom, Grossman, Gutnick & Spira (1982) (in mmol l^{-1}): NaCl, 211·5; KCl, 3·1; $CaCl_2$, 7·0; $NaHCO_3$, 2·5; 2-amino-2-(hydroxymethyl)propane-1,3-diol (Tris-base), 1·0. Ringer solutions were prepared daily and equilibrated with atmospheric CO_2; pH 7·2–7·4, checked daily. We have specifically included HCO_3^- in our saline as this ion has been shown to be important for intracellular pH regulation in a variety of cells (see Thomas, 1984, for a review; Schlue & Thomas, 1985). In high potassium solutions, sodium ions were used as the substitute to maintain isotonicity. Rapid changes in the bathing medium could be

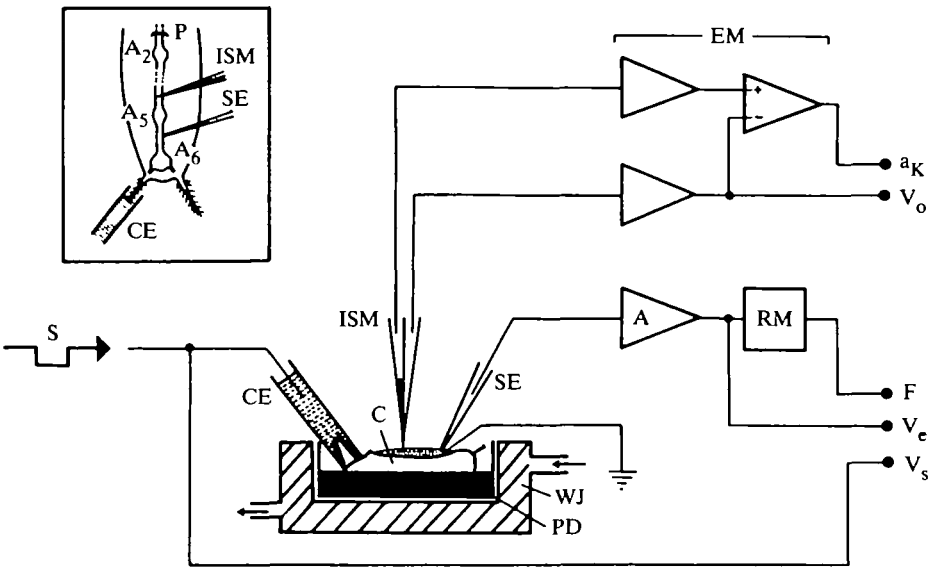

Fig. 1. A diagrammatic representation of the recording chamber and equipment. The cockroach (C) is dissected on a wax-bottomed Petri dish (PD) that is set in a water circulated chamber (WJ) to control temperature. Evoked activity is generated by a square electrical pulse (S) applied to a cercus by means of a capillary electrode (CE) and recorded by a suction macroelectrode (SE) placed between ganglia A_4 and A_5. Both the stimulating pulse (V_s) and the evoked action potential (V_e) are recorded on a storage oscilloscope. Spontaneous activity is continuously monitored through a rate meter (RM) and recorded as frequency (F) on a chart recorder. K^+ activity (a_K) and field potential V_o are recorded with a double-barrelled K^+-sensitive microelectrode (ISM) on the pen chart recorder. EM, electrometer; A, amplifier. Inset: positioning of the electrodes in the cockroach CNS. A_2, A_5, A_6, second, fifth and sixth abdominal ganglia. P is a staple-pin which is used to secure the VNC anterior to A_2. Other symbols, as in main figure.

made *via* a manual double pipette system. All experiments were carried out at a bath temperature in the range 18–22°C.

The recording circuitry is shown in Fig. 1. Both spontaneous and evoked spike activity were recorded using a glass suction macroelectrode (tip diameter 0·2 mm) placed between ganglia A_4 and A_5. Spontaneous activity was channelled through a rate meter for continuous frequency recording. Evoked activity was elicited by stimulating a cercal organ with small, 1 ms electrical pulses from a physiological stimulator (Grass SD9) *via* a Ringer-filled capillary. The evoked compound action potential was recorded on an Entec storage oscilloscope. Two parameters were measured: (1) the delay between stimulus and response, and (2) the amplitude of the negative phase of the compound action potential. Under control conditions, all measured parameters were found to be extremely stable (Hendy & Djamgoz, 1985; Gregory, Djamgoz & Irving, 1986). Spontaneously occurring spikes in the ventral nerve cord of the cockroach have been suggested to represent a combination of ascending and descending activity in the CNS (Roeder, Tozian & Weiant, 1960; Narahashi, 1963). Evoked activity represented mainly the responses of the giant interneurones (see Hendy & Djamgoz, 1985, for further details).

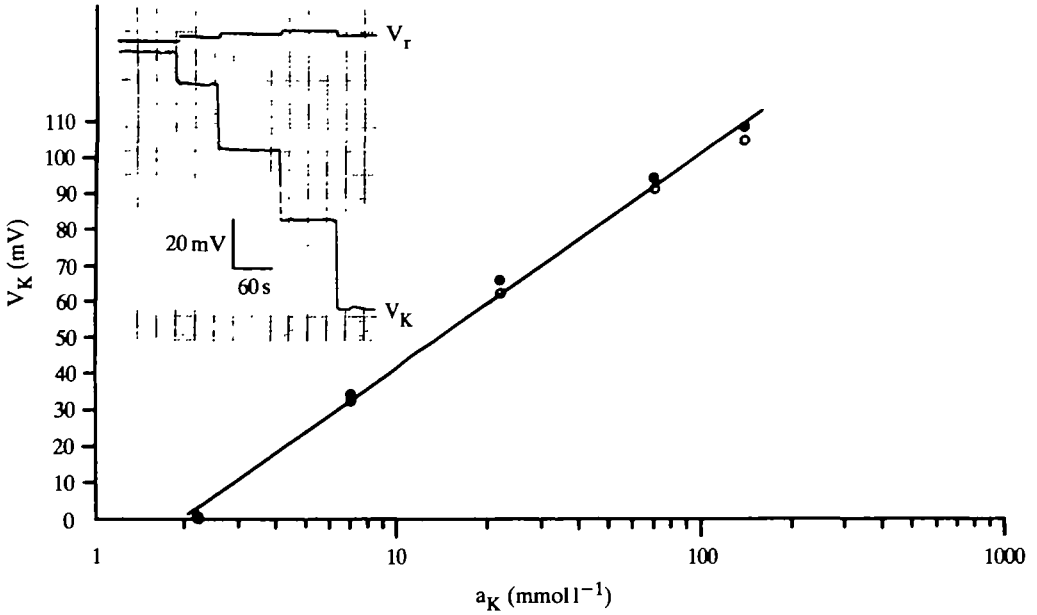

Fig. 2. Calibration of a double-barrelled K^+-sensitive microelectrode employing the neutral ion carrier (NIC). V_K is the differential voltage response of the two barrels and represents K^+ activity. V_K is arbitrarily fixed as 0 mV for the lowest value of K^+ in the Ringer-based calibrating solutions i.e. 3·1 mmol l^{-1}; a_K is the K^+ activity in the various calibrating solutions; (●) calibration before an experiment; (○) after an experiment. *Inset is a chart recording showing the variation of* V_K *and the reference barrel potential* (V_r) with K^+ activity.

Double-barrelled, potassium-sensitive microelectrodes (K^+-SMs) were made using procedures described previously (Djamgoz & Laming, 1981; Djamgoz & Dawson, 1986). The valinomycin-based neutral ion carrier (NIC) (Oehme & Simon, 1976) was used instead of the 'classical' liquid ion exchanger (LIX: Corning 477317) since the latter has been shown to be greatly sensitive to acetylcholine (Oehme & Simon, 1976) which is present in substantial concentrations in the cockroach CNS (Twarog & Roeder, 1957). The reference barrel was filled with 5 mol l^{-1} LiCl. The K^+-SM was connected to a high input impedance, dual/differential electrometer (WPI F223A) and traces were recorded on a pen chart recorder (WW 314; Fig. 1). For a typical K^+-SM, the resistance of the active barrel was 20–30 MΩ and the tip size was 1 μm or less. K^+-SMs had response times of around 10 s and were used only if they had calibrations giving in excess of 50 mV for a 10-fold change in $[K^+]$ (Fig. 2). The calibration solutions were based on the Ringer with K^+ and Na^+ interchanged. The preparations were earthed *via* a Ag–AgCl wire embedded in 3 % agar/Ringer in a capillary. The same earth electrode was used for the calibrations of the K^+-SMs. Junction potentials at the earth electrode did not vary by more than ±3 mV during solution changes in the range 3·1–100 mmol l^{-1} K^+. Junction potential changes were not systematic and could only account for an error in K^+ measurements of 11 % or less. In fact, only those results where the K^+-SM calibrated to within ±5 mV at all concentrations before and after use were retained. The voltage

response of a K^+-SM in a preparation was converted to ionic activity by direct reference to its calibration graph. These ionic activities have been converted to concentrations in the text, for comparison with published data, by assuming that the K^+ activity coefficient in the micro-environment is the same as in the normal Ringer, i.e. 0·71. In some experiments, control measurements were carried out using conventional, ultrafine microelectrodes. These were drawn from 1 mm o.d. borosilicate tubing on a Livingstone-type puller, filled with 2·5 mol l^{-1} KCl and had d.c. resistances of 50 MΩ or greater. Where multiple measurements are mentioned, these were taken from different insects. Data are given in the text as means ± standard errors.

To assist in the penetration of the K^+-SM, the nerve sheath was pre-treated with 1% collagenase (Sigma Type I) for 10 min (Schofield *et al.* 1984). Published evidence suggests that this neural lamella does not represent an appreciable barrier to the diffusion of inorganic cations and plays no significant role in maintaining extracellular ionic balance (Treherne *et al.* 1970; Pichon, 1970).

RESULTS

Measurement of the extracellular K^+ concentration, $[K^+]_e$

To record from the extracellular space, the K^+-SM was advanced onto the surface of the VNC and the micromanipulator gently tapped. This was sufficient for the tip of the microelectrode to penetrate the neural lamella and attain an extracellular position with a positive field potential (V_o). In some cases, following the initial penetration, the microelectrode was advanced deeper into the nerve cord until a negative resting potential, presumably of a giant axon, was recorded; if the microelectrode was then carefully withdrawn a few μm, an extracellular region was again found. Pichon & Boistel (1967) and more extensively Schofield *et al.* (1984) have provided compelling evidence that this position corresponds to the micro-environment of the CNS. A K^+-SM could be maintained in this position for about 2 h giving stable readings of $[K^+]_e$ and V_o. The stability of the recordings suggested that steady states were achieved within seconds and that there was insignificant disruption of the CNS. The resting value of V_o measured within seconds of penetrating the neural lamella was 6·0 ± 0·2 mV ($N = 19$). V_o values obtained using conventional microelectrodes were not statistically significantly different (Student's *t*-test), again indicating little, if any, damage to the CNS by K^+-SMs. In all cases, $[K^+]_e$ was found to be higher than the concentration of K^+ in the bathing medium (3·1 mmol l^{-1}), with a mean value of 4·70 ± 0·16 mmol l^{-1} ($N = 19$). A histogram of the measured $[K^+]_e$ values is shown in Fig. 3.

Effects of changing $[K^+]_o$ on $[K^+]_e$

By rapidly changing the bath K^+ concentration, $[K^+]_o$, it was possible to follow the corresponding changes in $[K^+]_e$. Fig. 4 shows a sample recording from such an experiment in which $[K^+]_o$ was increased 10-fold from the normal value of 3·1 to 31 mmol l^{-1}. There is a gradual rise in $[K^+]_e$ over a period of 45 min when $[K^+]_e$

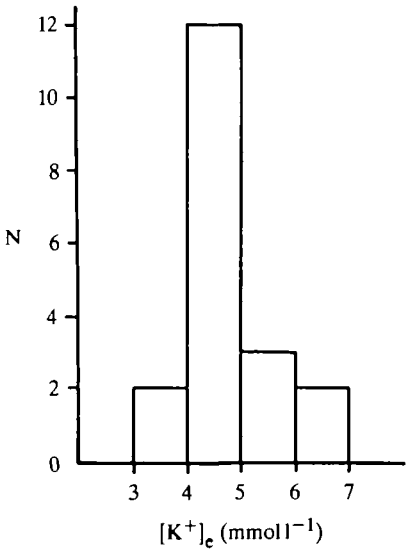

Fig. 3. Histogram of values (N) of extracellular K^+ concentration $[K^+]_e$ (mmol l^{-1}) recorded using K^+-sensitive microelectrodes in the cockroach ventral nerve cord. The nerve was bathed in 'normal' (3·1 mmol l^{-1} K^+) Ringer solution. The mean value of $[K^+]_e$ is 4·70 mmol l^{-1}.

appears to reach a more or less steady level at around 14 mmol l^{-1}. On return to the normal Ringer, $[K^+]_e$ declines to near its original value over approximately the same time course. The changes in $[K^+]_e$ in response to changing $[K^+]_o$ to three different values (10, 31 and 100 mmol l^{-1}) and back were thus studied. When $[K^+]_o$ was increased from the normal value of 3·1 to 10 mmol l^{-1}, $[K^+]_e$ increased gradually and

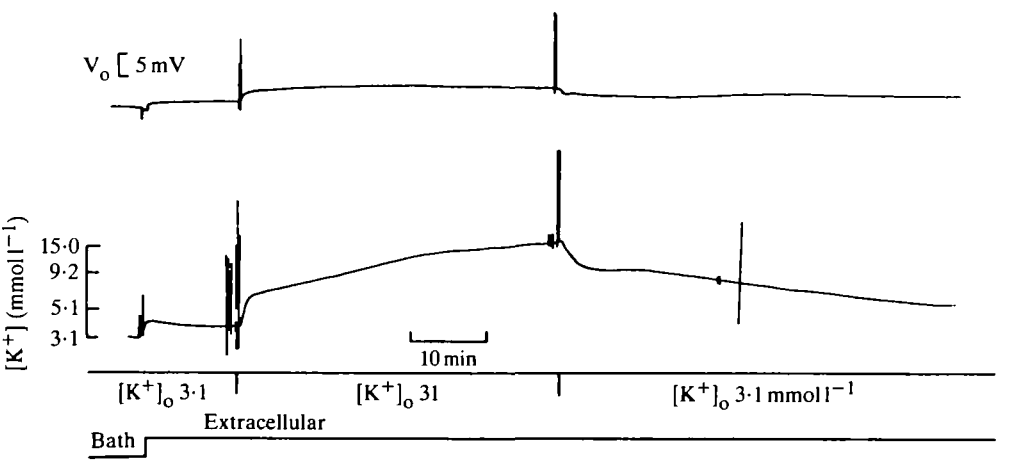

Fig. 4. A chart recorder trace showing the effect of changing the concentration of K^+ in the bathing medium $[K^+]_o$, from 3·1 to 31 mmol l^{-1}. The lower trace indicates K^+ concentration $[K^+]$, including the change in the extracellular value, $[K^+]_e$. The upper trace indicates the field potential, V_o.

levelled off at $6{\cdot}4 \pm 0{\cdot}5$ mmol l^{-1} ($N = 7$) over some 15 min. This indicates that the blood–brain barrier was effective in restricting the influx of K^+ from the bath into the micro-environment such that $[K^+]_e$ increased by only $1{\cdot}7$ mmol l^{-1} as a result of increasing $[K^+]_o$ by $6{\cdot}9$ mmol l^{-1}. In the other two sets of experiments involving increasing $[K^+]_o$ to 31 and 100 mmol l^{-1}, $[K^+]_e$ could be monitored for 30 and 45 min, respectively, and during these periods, final, steady values of $[K^+]_e$ were not quite achieved. The relationship between $[K^+]_o$ and the final recorded $[K^+]_e$ values obtained in these recordings is shown in Fig. 5. Importantly, in all cases, the greatest $[K^+]_e$ changes observed were still considerably less than the corresponding changes in $[K^+]_o$.

When $[K^+]_o$ was increased, an immediate depolarization of V_o was also recorded (Fig. 4). The relationship between these two parameters obtained with the reference barrels of K^+-SMs is shown in Fig. 6 (filled circles). Essentially identical V_o changes were recorded using conventional, ultrafine microelectrodes (Fig. 6, open circles). These data are very similar to the values given by Pichon, Moreton & Treherne (1971), plotted for immediate comparison (see dotted line Fig. 6).

The time courses of $[K^+]_e$ increase resulting from raising $[K^+]_o$, plotted on a semilogarithmic scale in Fig. 7, suggest that K^+ influx into the CNS micro-environment occurs in two distinct, successive phases. In each case, an early phase during which a relatively fast K^+ influx occurs is followed by a later phase during which K^+ influx is considerably slower (Fig. 7A–C). As $[K^+]_o$ is increased, the time taken for $[K^+]_e$ to double gets significantly shorter (Fig. 7D). In other words, the greater the gradient between $[K^+]_o$ and $[K^+]_e$, the faster the rate of the early influx. On average, the switch from the first to the second phase seems to occur rapidly for a $[K^+]_o$ increase to 10 or 31 mmol l^{-1}, whilst for 100 mmol l^{-1} it is more gradual.

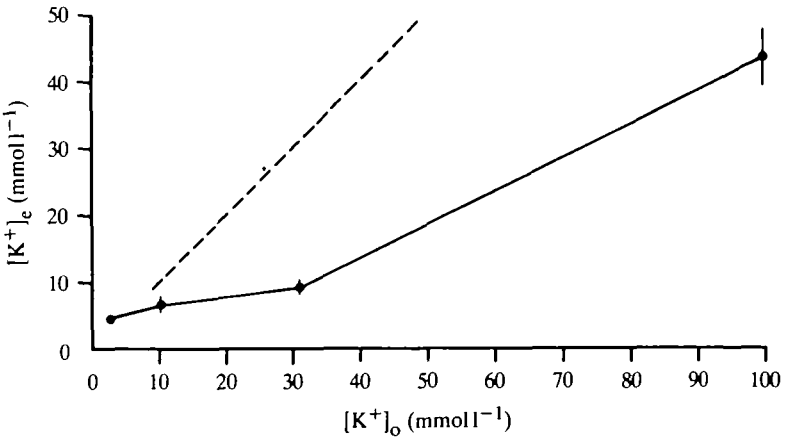

Fig. 5. The relationship between the bath K^+ concentration, $[K^+]_o$, and the maximal K^+ concentration measured in the CNS micro-environment, $[K^+]_e$. Each point is the mean of at least six measurements. Error bars denote standard errors. Dashed line shows equality i.e. $[K^+]_e = [K^+]_o$.

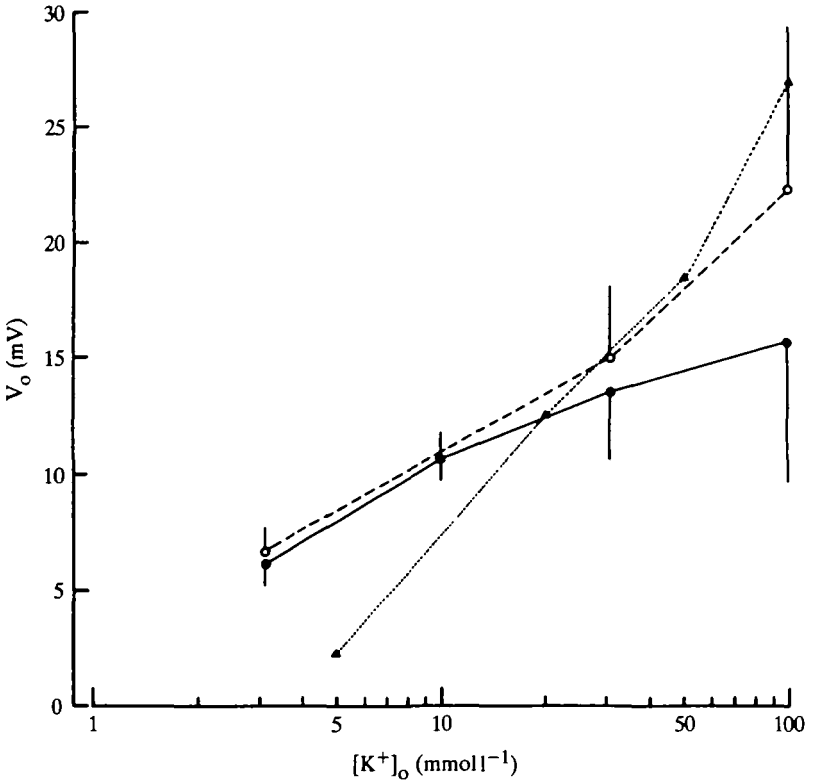

Fig. 6. Three relationships between V_o (extracellular field potential) and $[K^+]_o$ (bath K^+ concentration). Filled circles (solid line) and open circles (dashed line), data obtained with the reference barrels of K^+-sensitive microelectrodes and ordinary microelectrodes, respectively ($N = 7$, minimum). Triangles (dotted line), data from Pichon, Moreton & Treherne (1971), plotted for comparison.

These data suggest that the initial phase of K^+ influx is a first-order diffusion process.

The time courses of the influx and the efflux of K^+ in these experiments have been compared (Fig. 8). It would appear that, within the limits of the standard errors, corresponding pairs of time courses are essentially identical, i.e. for a given stepwise change in $[K^+]_o$, the influx and efflux of K^+ follow the same time course.

Effects of changing $[K^+]_o$ on nervous activity

An experiment demonstrating effects of increasing $[K^+]_o$ from 3·1 to 31 mmol l^{-1} on $[K^+]_e$ and spontaneous activity measured simultaneously is illustrated in Fig. 9. The change in $[K^+]_e$ is essentially as described in the preceding section. The frequency of spontaneous activity, on the other hand, does not alter significantly. Average data showing the effects of raising $[K^+]_o$ up to 100 mmol l^{-1} on the amplitude and latency of evoked activity and the frequency of spontaneous activity are given in Fig. 10. It appears that raising $[K^+]_o$ has little effect on the delay or amplitude of the evoked action potential (Fig. 10A,B). With spontaneous activity, no

effect is seen at 10 and 31 mmol l^{-1}, but there is a gradual decline in frequency 5 min after introducing 100 mmol l^{-1} $[K^+]_o$ into the bathing medium, leading to complete suppression (Fig. 10C). Noticeable onset of decline in activity corresponds to a critical $[K^+]_e$ level of 30 ± 6 mmol l^{-1} ($N = 7$). The effect was found to be reversible when normal Ringer was substituted for the 100 mmol l^{-1} $[K^+]_o$. No increase in the frequency of the spontaneous activity was seen, even transiently, in any of the experiments.

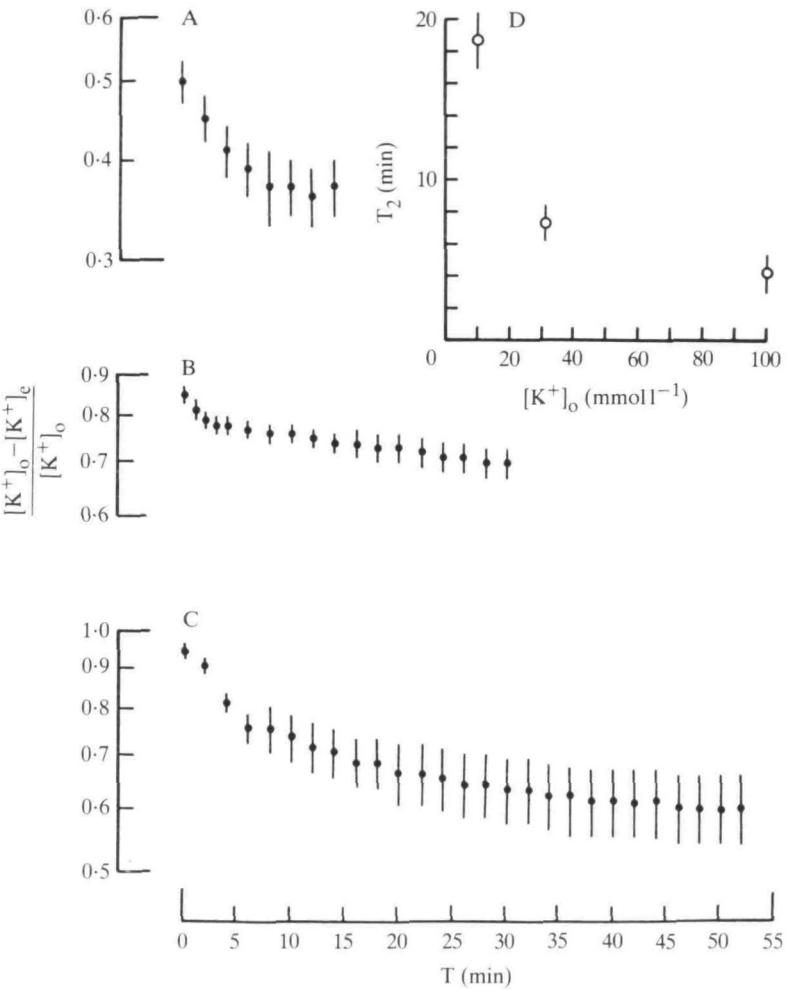

Fig. 7. Average time course of rise in the extracellular K^+ concentration, $[K^+]_e$, resulting from the introduction of the following bath K^+ concentrations, $[K^+]_o$ values: (A) 10 mmol l^{-1}; (B) 31 mmol l^{-1}; (C) 100 mmol l^{-1}. In each case, the $[K^+]_o$ increase occurs at $t = 0$. Data points are the means of at least six experiments, and typical error bars are shown. In each set, all data points can be fitted by two separate straight lines each with a correlation coefficient of at least 0·90. (D) The relationship between time taken for $[K^+]_e$ to double (T_2) (in min) and $[K^+]_o$. T_2 was estimated from individual experiments included in A–C, and then means and standard errors were calculated.

DISCUSSION

The extracellular K^+ concentration within the micro-environment of the intact cockroach CNS has been measured extensively *in vivo* for the first time. An average value of 4·7 mmol l^{-1} was obtained when the CNS was bathed in the normal saline containing 3·1 mmol l^{-1} K^+. Grossman & Gutnick (1981) studied $[K^+]_e$ changes in the cockroach CNS induced by electrical stimulation of the giant axons. They did not give a mean value of resting $[K^+]_e$, but their data indicate that they obtained values in the range 4·6–5·6 mmol l^{-1} (deduced from the value of basal K^+ activity apparent

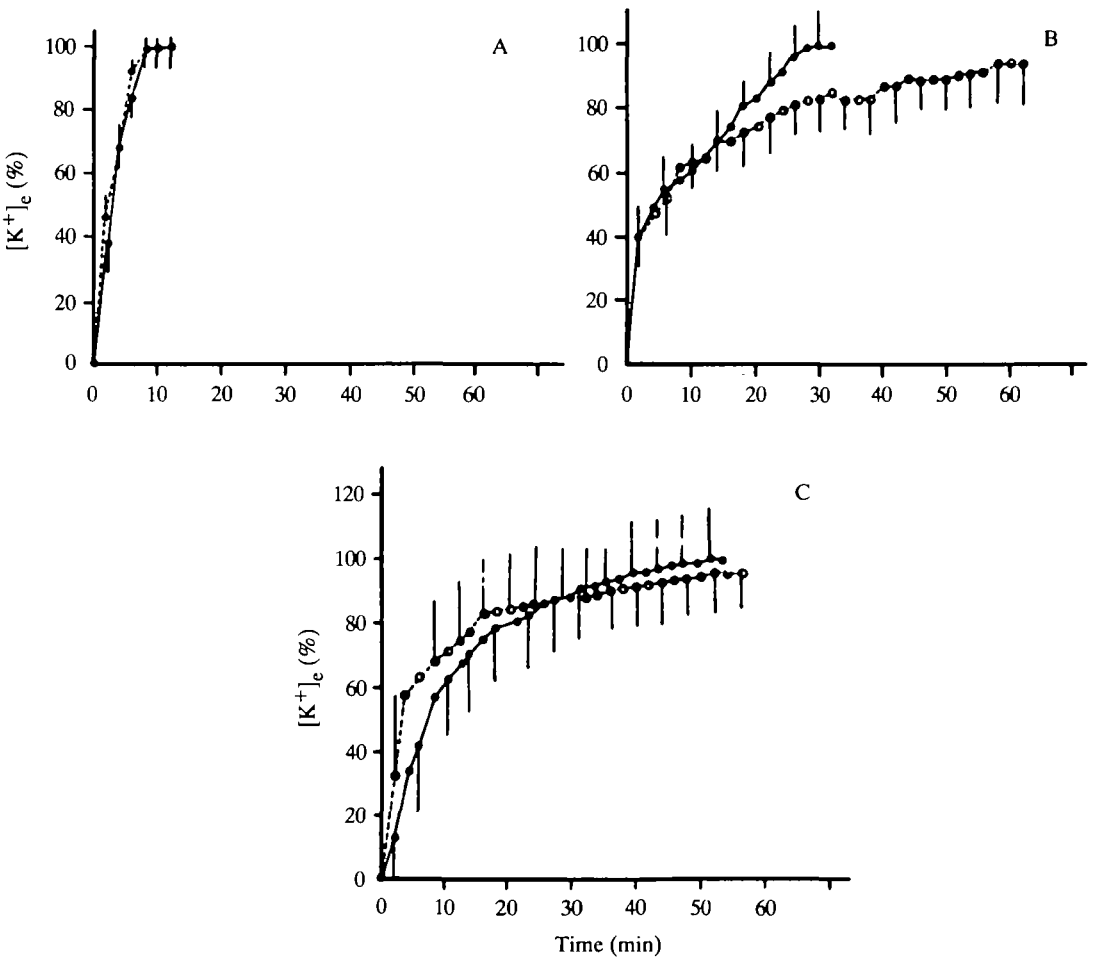

Fig. 8. Comparisons of time courses of K^+ influx (●) and efflux (○); extracellular potassium concentrations, $[K^+]_e$, are expressed as percentages for three bath K^+ concentrations; (A) 10 mmol l^{-1}; (B) 31 mmol l^{-1}; (C) 100 mmol l^{-1}. Data points are given as means ± S.E., from a minimum of five experiments for each set. Error bars are shown only on alternate data points for clarity. In each case the range used is between original (minimum; 0%) and final (maximum; 100%) values measured; t = 0 is the moment of change from normal to high K^+ (influx) and from high K^+ back to normal (efflux).

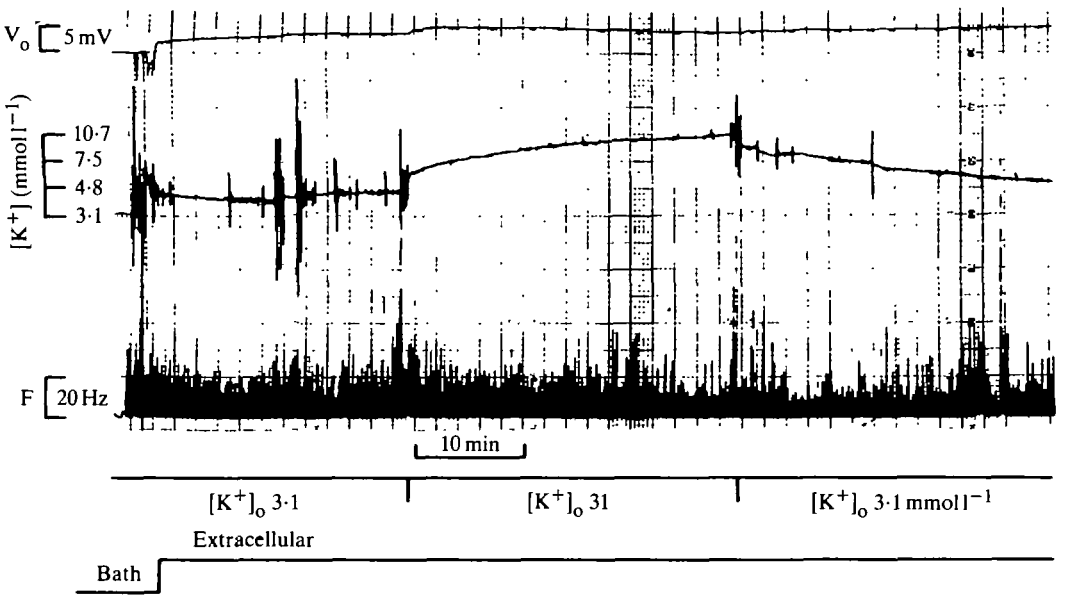

Fig. 9. As in the experiment described in Fig. 4 legend, but additionally including a recording of the frequency (F) of spontaneous activity.

in their figures and converting these to concentrations for comparison by assuming an activity coefficient of 0·71; they used a Ringer almost identical to ours). The data of Grossman & Gutnick are well within the range of $[K^+]_e$ values that we have obtained (Fig. 3). Earlier, Thomas & Treherne (1975) found in the cockroach that resting potentials of giant axons in intact preparations were similar to those recorded in desheathed axons bathed in 3·1 mmol l^{-1} K^+, and deduced that the *in situ* value of the extracellular K^+ concentration in a preparation bathed in 25 mmol l^{-1} K^+ Ringer must be close to this value.

The K^+-SMs employed in the present study had tip diameters of around 1 μm. The CNS micro-environment, on the other hand can be considerably narrower than this. We believe that when a microelectrode was positioned extracellularly, a 'dead space' was formed around the tip and measurements were taken within this space. It seems possible that K^+ leaked into the recording site from damaged cells, thereby causing a slight exaggeration in the measured value of $[K^+]_e$. However, since $[K^+]_e$ measurements were found to be relatively steady, the ionic composition of the 'dead space' must have reached an equilibrium within seconds of impalement (see Figs 4, 9). The resting values of V_o and changes in V_o resulting from increasing $[K^+]_o$ are very similar to the data of Pichon *et al.* (1971), but smaller than comparable data reported by Schofield *et al.* (1984). Importantly, in our experiments consistent results were obtained using conventional, ultrafine microelectrodes, suggesting minimal damage of the blood–brain barrier. There is one consideration, however, which indicates that the efficiency of the blood–brain barrier may have been somewhat underestimated in our experiments. Thomas & Treherne (1975) and Grossman & Gutnick (1981) have shown that axonal conduction is abolished by

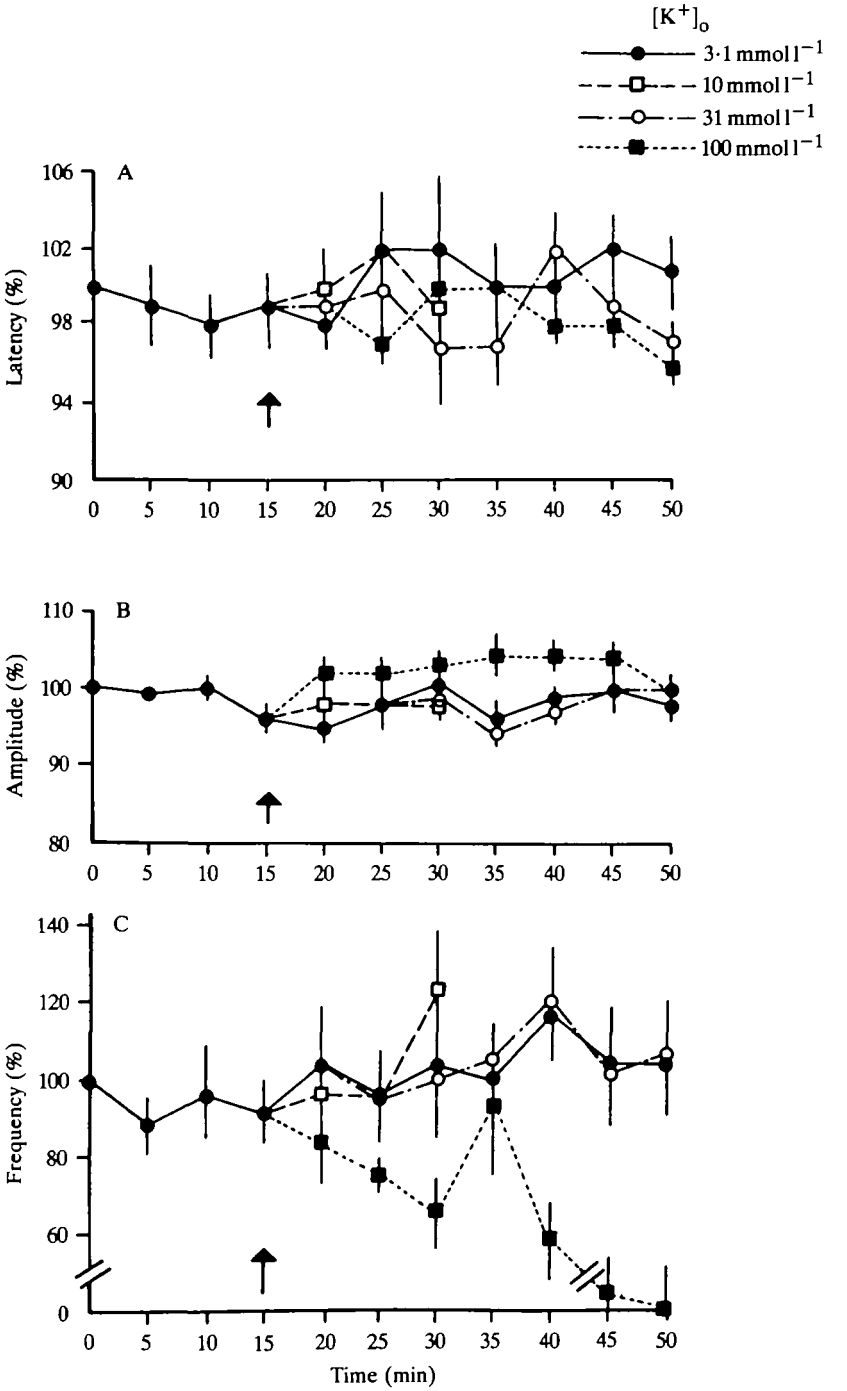

Fig. 10. The time courses of percentage changes in some parameters of evoked and spontaneous activity recorded by a suction electrode placed on the cockroach ventral nerve cord, following exposure (indicated by arrows) to different bath K^+ concentrations; 3·1 mmol l^{-1} K^+, control (●); 10 mmol l^{-1} (□); 31 mmol l^{-1} (○); 100 mmol l^{-1} (■). (A) latency; (B) amplitude of evoked compound action potential; (C) frequency of spontaneous activity.

depolarizing the axons in high-K^+ Ringers. Grossman & Gutnick (1981) estimated a $[K^+]_e$ value of 14 mmol l^{-1} at which conduction block would occur. In our experiments, spontaneous activity started to decline when $[K^+]_e$ reached 30 mmol l^{-1} and even at 42 mmol l^{-1} (the highest level recorded when $[K^+]_o$ was increased to 100 mmol l^{-1}) the evoked compound action potential persisted (Fig. 10B). It seems possible, therefore, that the 'dead space' in which the K^+ activity measurements were made was somewhat separated from the axonal surface. Interestingly, Grossman & Gutnick (1981), who carried out the only other available study of the cockroach CNS micro-environment with double-barrelled K^+-SMs, experienced a similar situation. This may not be so surprising remembering that the micro-environment is significantly narrower than the tip size of a functional, double-barrelled K^+-SM and the cellular geometry of the micro-environment is extremely complex (Treherne, 1985).

Extracellular K^+ has been measured in the CNS of a few other invertebrates using K^+-SMs. In extracellular spaces of the retina of the drone *Apis mellifera*, $[K^+]_e$ was 9·0 mmol l^{-1} and this value was not significantly different when the preparation was bathed in a superfusate containing 3·2 mmol l^{-1} K^+. Increasing $[K^+]_o$ to 10 mmol l^{-1} had no effect on $[K^+]_e$ at recording depths greater than 20 μm (Coles & Tsacopoulos, 1979). In a comparable study on the CNS of the leech *Hirudo medicinalis*, Schlue & Deitmer (1980) have shown that the K^+ concentration in the extracellular space of the ganglia and nerve cell body region was also some 50% higher than the corresponding bath concentration. In response to a change in $[K^+]_o$, however, there was eventually an almost identical change in $[K^+]_e$, presumably due to the fact that the blood–brain barrier in annelids is not as well developed as in insects.

The K^+ concentration in the cockroach haemolymph falls in the range of 10–30 mmol l^{-1} (Pichon & Boistel, 1963; Pichon, 1970; Lettau *et al.* 1977). The values of $[K^+]_e$ expected from these concentrations would therefore be within the range 6·5–9·0 mmol l^{-1} (Fig. 5). The present study suggests that $[K^+]_o$ in the cockroach CNS is well regulated. Thus, up to 31 mmol l^{-1} $[K^+]_o$, the maximum measured value of $[K^+]_e$ is less than 10 mmol l^{-1} and nervous activity was unaffected. In intact stretched connectives, which are considerably more permeable to changes in $[K^+]_o$, however, 30 mmol l^{-1} $[K^+]_o$ was found to cause a decline in the amplitudes of action potentials of about 25% in 10 min (Treherne *et al.* 1970). At $[K^+]_o$ values higher than 31 mmol l^{-1}, it is clear that there is a rapid rise in $[K^+]_e$ and this causes a gradual reduction of spontaneous activity. We have estimated the critical value of $[K^+]_e$ at which nervous activity starts to decline to be 30 mmol l^{-1}. Interestingly, Hoyle (1954) has also shown that 40 mmol l^{-1} or greater K^+ injected directly beneath the neural lamella of the locust was sufficient to cause conduction block in the crural nerve. The adverse effects of K^+ are much more pronounced in stretched or desheathed preparations (Yamasaki & Narahashi, 1959; Pichon & Treherne, 1970; Treherne *et al.* 1970; Pichon *et al.* 1971; Pichon, Sattelle & Lane, 1972; Thomas & Treherne, 1975; Schofield & Treherne, 1984). Behaviourally, it has been documented that increasing the $[K^+]$ in the haemolymph of active

cockroaches causes a decline in locomotive activity (van Asperen & van Esch, 1956; Pichon & Boistel, 1963) and similarly with the locust (Hoyle, 1954).

Another interesting aspect of the current work is what would appear to be possible sub-systems involved in the control of K^+ influx into the extracellular space. Due to the complex geometrical network of perineurial and glial cells that form the barrier between the haemolymph and the extracellular space, multiple diffusion phases could be expected. In our results, two distinct phases were observed. For a given increase in $[K^+]_o$, the activity of the early phase (system I) would be the first one to become apparent, although its efficiency is relatively low. The later phase (system II) comes into operation with a delay but its efficiency is greater than that of system I. It is clear from the morphology of the nervous tissue that the initial restriction to any change in $[K^+]_o$ would be the tightly coupled perineurial cells and/or an extracellular anion matrix comprising a physical diffusion barrier (Treherne, 1974; Treherne, Schofield & Lane, 1982). It is only later that the activities of the inner glial and/or axonal Na^+/K^+ pumps may be enhanced sufficiently to play a significant role. Thus, system I may correspond to 'physical' aspects, essentially comprising a first-order diffusion mechanism in accordance with Fick's Law, whilst system II may represent the 'metabolic' component of the blood–brain barrier. Treherne & Schofield (1981) also found that ionic regulation in the micro-environment of the cockroach CNS involves diffusional and metabolic components, although a kinetic difference between the two was not suggested. Quantitatively, a kinetic difference between the two sub-systems may serve the following situations. (i) During influx of K^+ into the micro-environment, the greatly enhanced pumping rate (apparent as the second phase) will clearly resist the detrimental rise in $[K^+]_e$. (ii) During the efflux of K^+, however, the metabolic component appears to function as a fine tuning mechanism, eventually resisting the drop in $[K^+]_e$ to ensure smooth return to baseline. The combined effect of systems I and II appears to be sufficient to maintain the level of $[K^+]_e$ within physiologically acceptable limits for bath/haemolymph K^+ concentrations up to 31 mmol l^{-1}. At 100 mmol l^{-1} $[K^+]_o$, however, the blood–brain barrier cannot prevent excessive entry of K^+ into the extracellular space, thereby giving rise to adverse effects on neuronal functioning, leading to some loss of neuronal activity. It is worth noting that the 'decelerating' effect of the blood–brain barrier in opposing the influx of K^+ from 100 mmol l^{-1} $[K^+]_o$ during the late phase is more than four times that for 31 mmol l^{-1} $[K^+]_o$, indicating an increase in the efficiency of system II when $[K^+]_o$ is increased (see Appendix). The activity of the Na^+/K^+ pump in mammalian heart muscle has indeed been shown to be increased by raising extracellular $[K^+]$ (Glitsch, Grabowski & Thielen, 1978), and Erlij & Grinstein (1976) estimated that the maximum turnover capacity of the pump can be as much as 75 times the turnover rate at rest.

Finally, a surprising finding of the present study concerns the identical time courses for the K^+ influx into the micro-environment and for the K^+ efflux, resulting from a given increase and corresponding decrease in $[K^+]_o$, respectively (Fig. 8). One might expect that a physiologically better design for the insect blood–brain barrier would have incorporated a transport rate for K^+ much more efficient in the

outward than in the inward direction. Such a design would serve to restrict any adverse effect(s) of haemolymph K^+ fluctuations on the CNS, but should $[K^+]_e$ rise from inside the CNS (e.g. following hyperactivity and/or metabolic inhibition), then the excess K^+ would be lost rapidly to the haemolymph thereby minimizing secondary effects on neuronal functioning. Indeed, Schofield & Treherne (1975, 1978) showed that Na^+ influx and efflux in some experiments could have dissimilar time courses. However, K^+ movements recorded directly by K^+-SMs (this study) and inferred from time-courses of K^+-induced membrane potential changes (Treherne *et al.* 1970; Schofield *et al.* 1984) appear to be more symmetrical. The cause(s) of this difference between Na^+ and K^+ movements in the cockroach CNS is not clear at present. Schofield & Treherne (1975, 1978) suggested that the apparent asymmetry in Na^+ movements, which is seen only during the first application of a Na^+-free saline, might be due to irreversible loss of Na^+ from CNS tissues. In our experiments, the K^+ fluxes measured within the micro-environment were due to increases of external K^+ from the normal level of 3·1 mmol l^{-1} and back, and would be unlikely to involve net movement of cellular K^+. We conclude, therefore, that the cockroach blood–brain barrier could act as a potentiator of neurotoxic effects originating in the CNS and involving seizures such as the action of some pyrethroid insecticides (Grossman & Gutnick, 1981; Casida, Gammon, Glickman & Lawrence, 1983).

APPENDIX

We present an approximate comparison of the efficiency of the presumed metabolic component of the blood–brain barrier during K^+ influx into the micro-environment when $[K^+]_o$ is increased from 3·1 to 31 mmol l^{-1} and 3·1 to 100 mmol l^{-1}.

Assume that in the absence of the metabolic component of the blood–brain barrier, K^+ influx would occur at the rate J_1 (mmol min^{-1}). If metabolic pumping simply resists the influx (or decelerates it) by p mmol min^{-2}, then at time t (min) following the onset of pumping, the rate of influx J_2 would be given by

$$J_2 = J_1 - pt.$$

According to our model, the metabolic phase of the blood–brain barrier becomes apparent at the point of inflexion in the curve relating $[K^+]_e$ to t. If we call this time t = 0, then J_1 and J_2 can be measured from the curves at given times and p calculated for each case, thus giving the values:

$[K^+]_o$ change (mmol l^{-1})	J_1 (mmol min^{-1})	J_2 (mmol min^{-1})	t (min)	p (mmol min^{-2})
3·1 to 31	0·16	0·02	28	0·005
3·1 to 100	0·72	0·08	28	0·023

From these values metabolic pumping appears to be 4·6 times greater during the latter change, i.e. the efficiency of the metabolic component of the blood–brain barrier increases as the gradient between $[K^+]_e$ and $[K^+]_o$ is increased.

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