

## IONIC PERMEABILITY OF THE BLOOD–BRAIN BARRIER SYSTEM OF AN INSECT *CARAUSIUS MOROSUS*

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(Received 26 February 1979)

Large ionic gradients must be maintained across the blood–brain barrier system of *Carausius morosus*, for the neurones are apparently conventional in their ionic requirements (Treherne & Maddrell, 1967; Weidler & Diecke, 1969) while the haemolymph has been found to contain ionic concentrations as adverse as 9 mM-Na, 28 mM-K and 73 mM-Mg (Duchateau, Florkin & Leclercq, 1953). The ionic permeability of the system is here examined in the manner of Pichon, Moreton & Treherne (1971), by observing changes in extraneuronal potential produced by an alteration in the ionic composition of the saline bathing the first pair of thoracic connectives (from adults) from which the fat-body sheath had been removed. (The fat body has been shown to be a relatively leaky structure: Lane & Treherne, 1971; Treherne, 1972.)

The extraneuronal potential changes that were observed upon substitution of sodium in the bathing saline (Fig. 1) show that the blood–brain barrier system of *Carausius morosus* has a permeability to sodium that is higher than the permeability to the other cations tested. With substitution by tris there was a mean change of  $-4$  mV (S.D. 0.5,  $n = 6$ ); with lithium,  $-3$  mV (S.D. 1,  $n = 3$ ); with potassium,  $-3$  mV (S.D. 1,  $n = 6$ ); the calcium,  $-5$  mV (S.D. 3,  $n = 8$ ); with magnesium  $-5$  mV (S.D. 2,  $n = 7$ ). In the C.N.S. of other insects – *Periplaneta americana* (Pichon *et al.* 1971) and *Manduca sexta* (Pichon, Sattelle & Lane, 1972) – a relatively high potassium permeability has been found. Since *C. morosus* has levels of sodium and potassium in the haemolymph (Duchateau *et al.* 1953; Wood, 1957) and the neuronal environment (Treherne & Maddrell, 1967) that are similar to those in *M. sexta* (Pichon *et al.* 1972), the difference may reflect a difference in the mechanism by which these insects maintain the ionic environment of their neurones. Likewise, the similarity between lithium and tris permeabilities in *C. morosus*, compared with a similarity between lithium and sodium permeabilities in *P. americana*, indicates that the nature of the sodium permeability of the blood–brain barrier system is different between the two species, and hence a mechanism of sodium regulation may be different.

Permeability of the barrier to chloride appears low by comparison with permeability to sodium: substitution of 137 mM chloride by a relatively impermeant anion, methyl sulphate (Fig. 2a), produced no potential change (S.D. 1,  $n = 3$ ) compared to the  $-3$  mV produced upon substitution of only 15 mM sodium with the relatively impermeant tris ion. This indirect comparison of course rests on the assumption that the permeability of the barrier to methyl sulphate is comparable to the permeability to tris.

When nitrate was substituted for chloride, there was a negative shift in extraneuronal

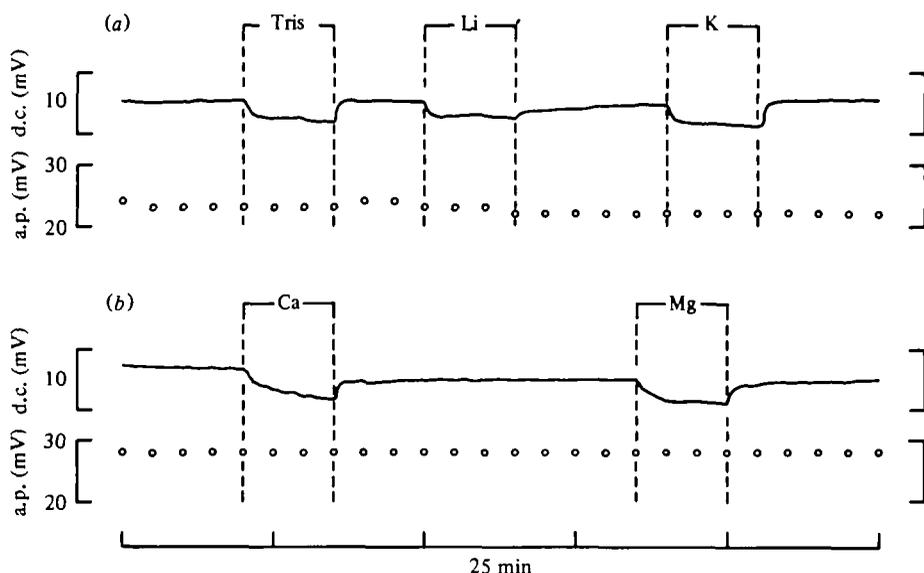


Fig. 1. Changes in the extraneuronal potential recorded from connectives of *C. morosus* with the sucrose-gap technique; produced by replacement of sodium in the bathing saline with (a) tris, lithium or potassium (b) calcium or magnesium. Extraneuronal potential changes are those changes in d.c. potential that occur with no corresponding change in the amplitude of the compound action potential (a.p.). The basic saline was derived from that of Wood (1957) by replacing the phosphate buffer with PIPES/NaOH, and had the following composition (mM): Na, 15; K, 18; Ca, 7.5; Mg, 50; PIPES, 7.5; Cl, 137; OH, 11; sucrose, 185. Salines were derived from the basic type by the complete substitution of one ion by another. The high-magnesium and high-calcium salines each contained an extra 15 mM-Cl.

potential (Fig. 2*b*) of 32 mV (S.D. 4,  $n = 15$ ). The negative shift was completed within about 10 s and followed by a slower, positive shift (Fig. 2*b*). There was usually a decline in action potential amplitude of  $c 1$  mV within 20 s. When nitrate was removed, the original extraneuronal potential was rapidly recovered and then there was a transient positive shift in potential. The amplitude and time course of this transient were proportional to the length of exposure to nitrate. These potential changes were unlikely to have been generated at the junction of saline and the KCl/agar electrode since chloride and nitrate have similar ionic conductances (Spiegler & Wyllie, 1968). To check this, a nitrate potential recorded using an agar electrode was compared with one recorded using a broken-tip microelectrode and found to be very similar.

The negative change in potential that was observed upon exposure to nitrate appears to indicate that the blood-brain barrier system of *C. morosus* has a greater permeability to nitrate than to chloride. The positivation that occurred during the exposure could hardly have been a chloride potential (since little chloride permeability was indicated by the experiment with methyl sulphate) or an axonal depolarization (since a depolarization of such magnitude would result in a greater decline in action potential amplitude). It seems more likely that the positivation represents permeation of the barrier by nitrate. The potential is in this way similar to the transient potential change induced by application of potassium to the crustacean nervous system (Abbott, Moreton & Pichon 1975). Such a build up of nitrate at the inner surface of the barrier would explain the

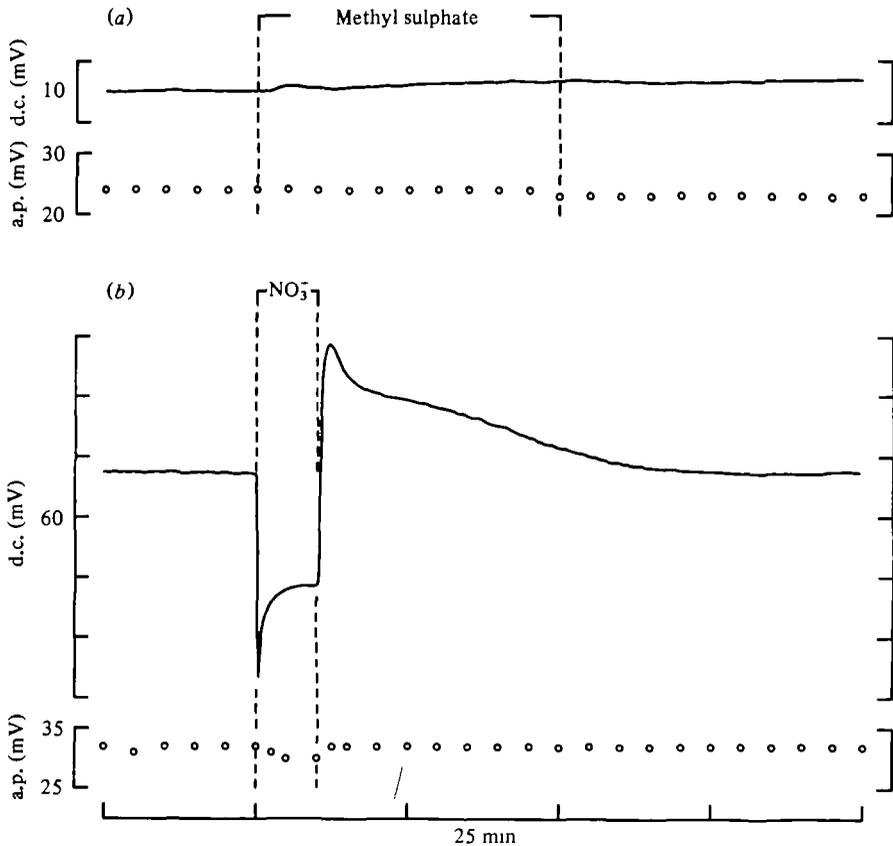


Fig. 2. Extraneuronal potential changes – indicated as in Fig. 1 – produced by replacement of chloride in the bathing saline with (a) methyl sulphate, (b) nitrate.

positivation observed upon removal of nitrate. The decay of the positivation would represent the exit of nitrate from the system. However, an equally plausible explanation is that nitrate reversibly alters the permeability of the barrier to some other ion.

The apparent permeability of the barrier to nitrate may indicate that nitrate is a metabolite of the nervous system of *C. morosus*. Alternatively nitrate could be following a pathway intended for some other ion, such as an amino acid. This could be a fruitful topic of research, and the nitrate potential itself might also be used as an indicator of the integrity of the blood-brain barrier system, in similar fashion to the use of potassium in an insect where the potassium permeability is relatively large (Treherne, Schofield & Lane, 1973).

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