

ION TRANSPORT IN THE MIDGUT OF THE COCKROACHES *LEUCOPHAEA MADERAE* AND *BLABERA GIGANTEA*

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The presence of a cation pump on the apical membrane of epithelial cells in insects, responsible for the extrusion of K or Na, has been proposed for Malpighian tubules (Maddrell, 1977) and salivary glands (Berridge, Lindley & Prince, 1976). An essential role of the midgut in K homeostasis has been established only for the phytophagous larvae of Lepidoptera (Harvey & Nedergaard, 1964). The main features of the rheogenic, lumenally-directed potassium pump of this tissue have been extensively studied (Zerahn, 1978; Harvey, Cioffi, Dow & Wolfersberger, 1983). Since the midgut of these animals is also peculiar in that it does not actively absorb Na, the presence of the K pump could be the sole example in insect gut physiology. Na absorption in the midgut has been demonstrated in several species of insects (O'Riordan, 1969; Sauer, Schlenz True & Mills, 1969; Farmer, Maddrell & Spring, 1981; Dow, 1981a; Koefoed & Zerahn, 1982) while K secretion has been suggested for *Periplaneta americana* (Sauer & Mills, 1969), *Leucophaea maderae* (Sacchi & Giordana, 1979) and *Schistocerca gregaria* (Dow, 1981b).

This paper, on the basis of direct measurements of Na, K, Cl and Rb unidirectional fluxes, provides further information on absorptive and secretory processes occurring in the midgut of two cockroaches, *L. maderae* and *Blabera gigantea*.

The experiments were performed on adult *L. maderae* and *B. gigantea*, average weight 2 and 4 g respectively, fed *ad libitum*, unless otherwise specified, with flour, lettuce, fruit and water. Animals were anaesthetized with ether, the dorsal part of the tergites was cut away and the alimentary canal exposed. The midgut (approximately 2 cm long) was excised below the caeca and above the Malpighian tubules, the peritrophic membrane with the enclosed intestinal contents was removed, and the narrow posterior part of the midgut (approximately 0.7 cm) was discarded. The apparatus used for flux measurements was in principle the same as that used by Koefoed & Zerahn (1982) for studies on mealworm guts, with minor changes for the larger guts of cockroaches (Fig. 1). The midgut was tied with cotton threads as a cylinder between two glass capillaries, one of which was connected *via* polythene tubing to a motor-driven Hamilton syringe filled with saline. Luminal perfusion was

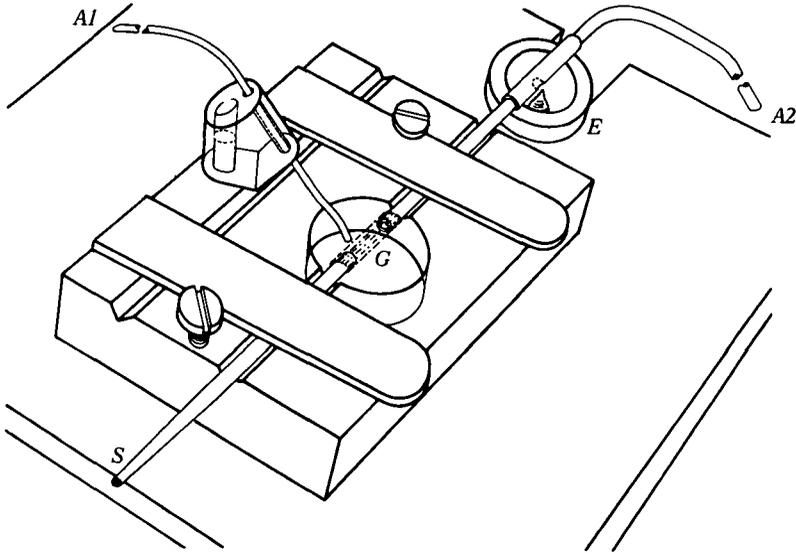


Fig. 1. Apparatus for flux measurements. *G*, isolated midgut. *AI*, *A2*; Ringer agar-agar bridges going to calomel cells and voltmeter; *S*, connection to a precision syringe by plastic tubing; *E*, Eppendorf vial to collect lumen solution dropping down.

accomplished at a constant rate of $20 \mu\text{l min}^{-1}$ and the perfusate was collected in an Eppendorf vial. The haemolymphatic saline was constantly stirred with a magnetic stirrer. Luminal saline always contained 2 mmol l^{-1} amaranth (Merck 1248) to check for possible leakage, and leaky guts were discarded. Unidirectional fluxes were measured by adding appropriate isotopes (^{22}Na , ^{36}Cl , ^{42}K and ^{86}Rb , purchased from The Radiochemical Centre, Amersham) either to the luminal (for lumen to haemolymph flux, $J_{\text{l-h}}$) or to haemolymphatic (for haemolymph to lumen flux, $J_{\text{h-l}}$) saline, with an activity of about $4 \mu\text{C ml}^{-1}$. $200 \mu\text{l}$ of the haemolymph solution or of the perfusate was collected at 10-min intervals for 30 min, and the radioactivity measured by means of a liquid scintillation spectrometer (Tri Carb 300, Packard). Salines had the following composition (mmol l^{-1}): NaCl , 100; KCl , 12; MgSO_4 , 6; CaCl_2 , 3; Na_2HPO_4 , 3.2; NaH_2PO_4 , 2; sucrose, 100; glucose, 30; pH 7 for *L. maderae*; and NaCl , 110; NaHCO_3 , 2; KCl , 25; sucrose, 100; glucose, 30; pH 8.1 for *B. gigantea*. Na and K concentrations in the two salines were similar to those measured in the haemolymph of the two species. For the measurements of Rb unidirectional fluxes all KCl was substituted by equal amounts of RbCl. The trans-epithelial electrical potential difference (PD) was continuously recorded by means of a Keithley 176 microvoltmeter. Flux and PD values used for the means reported in the tables, were the mean between the experimental periods at 20 and 30 min, since they were not significantly different.

The PD recorded during flux measurements showed that a steady value was reached about 10 min after isolation. In *L. maderae* the polarity of the PD was either negative or positive in the lumen, and PD values recorded were constant for the following 20 min. PD recordings ranged between -18 mV (lumen negative to haemolymph) and $+19.3 \text{ mV}$ (lumen positive to haemolymph) in 86 experiments (Fig. 2). As previously pointed out (Sacchi & Giordana, 1979), this range of polarity cannot be

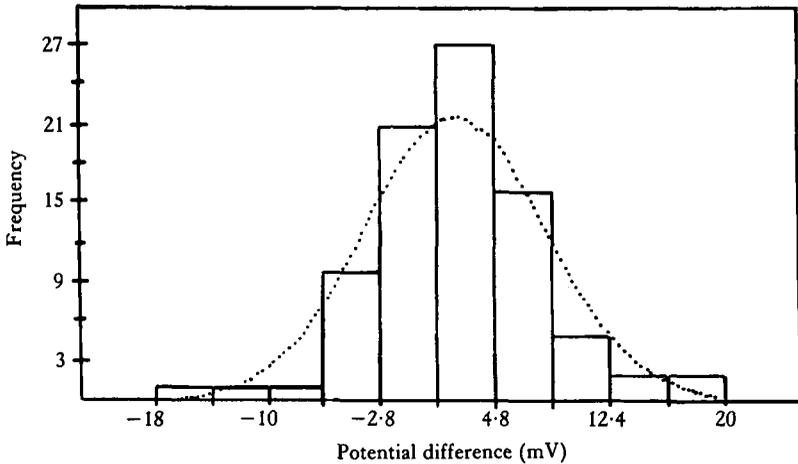


Fig. 2. Frequency distribution of transepithelial electrical potential differences across the midgut of *Leucophaea maderae*, recorded during flux measurements at 10 min.

ascribed to tissue damage during isolation procedure, but rather should be considered a sign of different functional conditions. In *B. gigantea* the PD was -6.1 ± 3.5 mV (mean \pm s.e., 110 experiments), with the lumen negative to the haemolymph. The polarity of the lumen ranged between -19 and $+8$ mV and was positive to the haemolymph in only 19 of the 110 cases.

Unidirectional (J_{l-h} and J_{h-l}) and net ($J_{l-h} - J_{h-l}$) fluxes of Na, K, Rb and Cl measured in *L. maderae* midgut in the absence of chemical gradients (Table 1) were apparently little affected by the range of PD (Fig. 2). A net absorption of Na and a net secretion of K occurs in the midgut. There was no net movement of Cl.

Since ouabain significantly reduced Na J_{l-h} to a value not different from J_{h-l} , it can be concluded that Na absorption is accomplished by a Na,K-ATPase. The ouabain concentration used was rather high (1 mmol l^{-1}), so that the effect of this cardiac glycoside was not inhibited by the relatively high K concentration in the saline. These results are in good agreement with those of O'Riordan (1969) and of Farmer *et al.*

Table 1. *Leucophaea maderae* midgut: ion fluxes and transepithelial electrical potential difference (PD)

Ion	J_{l-h} (nequiv h^{-1} midgut $^{-1}$)	PD (mV)	J_{h-l} (nequiv h^{-1} midgut $^{-1}$)	PD (mV)	J_{net}	<i>P</i>
Na	508.0 ± 51.5 (9)	+0.5	251.9 ± 64.3 (9)	-0.1	256.1	<0.01
Na+ouabain	327.1 ± 54.0 (6)	+4.7	281.9 ± 59.3 (6)	+2.3	—	NS
Cl	345.3 ± 63.4 (12)	+0.2	379.0 ± 54.2 (10)	+1.8	—	NS
K	25.1 ± 5.5 (8)	+2.1	64.0 ± 13.0 (8)	-0.2	-38.9	<0.02
K+ouabain	—	—	110.4 ± 15.4 (8)	+2.5	-85.3	
Rb	37.8 ± 9.9 (13)	-2.0	70.6 ± 10.5 (14)	+2.5	-32.8	<0.05
Rb+ouabain	41.3 ± 15.1 (9)	+2.0	102.8 ± 21.4 (8)	+1.1	-6.5	<0.05

J_{l-h} and J_{h-l} are the unidirectional lumen to haemolymph and haemolymph to lumen fluxes respectively. Mean \pm s.e., number of experiments in parentheses.

J_{net} is the difference between the two unidirectional fluxes.

Differences between J_{l-h} and J_{h-l} have been tested for significance by *t*-test; NS, not significant.

Table 2. *Blaberba gigantea* midgut: ion fluxes and transepithelial electrical potential difference (PD)

Ion	Diet	J_{l-h} (nequiv h ⁻¹ midgut ⁻¹)	PD (mV)	J_{h-l} (nequiv h ⁻¹ midgut ⁻¹)	PD (mV)	J_{net}	<i>P</i>
Na	Mixed	723.4 ± 99.2 (12)	-4.7	434.1 ± 56.3 (14)	-5.2	289.0	<0.02
Cl	Mixed	948.0 ± 173.3 (10)	-4.4	1120.5 ± 195.5 (10)	-2.4	—	NS
Rb	Mixed	111.5 ± 22.5 (11)	-3.2	290.0 ± 41.0 (13)	-1.5	-178.5	<0.01
K	Mixed	250.0 ± 61.0 (10)	-1.6	170.0 ± 98.0 (9)	-3.0	—	NS
Rb	Mixed	115.0 ± 23.0 (6)	-1.1	92.0 ± 14.5 (6)	-1.0	—	NS
Rb	Lettuce	91.0 ± 16.5 (3)	-4.0	361.1 ± 81.9 (5)	-1.3	-270.1	<0.05
Rb	Lettuce	89.1 ± 16.3 (6)	-5.2	150.2 ± 34.1 (8)	-2.7	—	NS

J_{l-h} and J_{h-l} are the unidirectional lumen to haemolymph and haemolymph to lumen fluxes respectively. Mean ± s.e., number of experiments in parentheses.

J_{net} is the difference between the two unidirectional fluxes.

Differences between J_{l-h} and J_{h-l} have been tested for significance by *t*-test; NS, not significant.

(1981), who also found a definite effect of ouabain on Na absorption in the midgut of *P. americana* and *Rhodnius prolixus*. The same concentration of ouabain did not reduce J_{h-l} K flux, therefore the net K transport is not mediated by a Na,K-ATPase. By contrast, O'Riordan (1969) found that ouabain did affect K transport. The results found for *P. americana* showed a decrease of the J_{h-l} of K to 61% of the control in the presence of 10⁻⁴ mol l⁻¹ ouabain. The decrease might however be related to the drop of PD from 12 mV to a lower value.

When K was completely substituted by equal amounts of Rb, a net flux of Rb from haemolymph to lumen (Table 1) took place across the midgut, equal in value to the net K flux, suggesting that in this condition the tissue could not distinguish between K and Rb. This is not surprising since Rb competes more or less favourably with K in K-transporting epithelia (Zerahn, 1980; Berridge *et al.* 1976). As for K transport, ouabain altered neither the J_{l-h} nor the J_{h-l} of Rb. It is noteworthy that the addition of ouabain during flux experiments caused a shift of the PD from an initial negative value of -0.44 ± 0.11 mV to a final positive value of $+2.2 \pm 1.2$ mV (22 experiments).

Flux measurements on midguts of *B. gigantea* were made from animals fed either an uncontrolled diet (varying amounts of flour, fruit and lettuce *ad libitum*) or fed primarily with lettuce, as specified (Table 2). As in *L. maderae*, Na was actively transported from the lumen to the blood, while there was no net movement of Cl. Rb was actively transported in a K-free solution, with the Rb flux from haemolymph to lumen being two to three times larger than from lumen to haemolymph. The entire set of flux experiments was performed in the course of one month. Later experiments performed in 1 week with ⁴²K showed no active transport of the cation across the midgut and the same result was obtained with a new set of Rb experiments (Table 2, lines 4 and 5). The possible explanation of these results seems to be that *B. gigantea* midgut can actively transport K (or Rb) but unlike *L. maderae*, this transport does not always take place. Moreover, the K transport did not seem to be directly dependent on a K-rich diet, as indicated by two sets of experiments performed at different times of the year on midguts isolated from cockroaches fed with lettuce and scanty amounts of flour for 15 days (Table 2, lines 6 and 7). However, if all the Rb fluxes

reported in Table 2 are pooled, a J_{l-h} value of 107.7 ± 11.2 nequiv h^{-1} midgut $^{-1}$ (mean \pm s.e., 26 experiments) and a J_{h-l} value of 225.0 ± 27.2 nequiv h^{-1} midgut $^{-1}$ (mean \pm s.e., 32 experiments) are found. The resulting net flux, from haemolymph to lumen, is equal to 117.3 ± 33.4 nequiv h^{-1} midgut $^{-1}$ (mean \pm s.e., 58 experiments). *L. maderae* does not show the same variation in K transport as does *B. gigantea*. It should be noted that midguts from this species show a lumen positive polarity much more often than do those from *B. gigantea*. Variation in K secretion was also suggested by Sauer & Mills (1969) for *P. americana*.

The suggestion by O'Riordan (1969) of a linked Na-K pump using Na,K-ATPase for the transport of both ions does not apply directly, since in *L. maderae* the K transport is only one-fifth of the active Na transport and it is not affected by ouabain. For *B. gigantea* active Na transport is always present, but the active K transport is sometimes missing, so the ratio K/Na may approach zero. Although the transmural active K flux cannot produce enough K ions to act as counterions for the actively transported Na ions, this will not disagree with the model for a Na,K-ATPase activated Na transport pump. However, in this case only the Na transport is transmural.

In good agreement with these results, the midgut of the mealworm *Tenebrio molitor* shows an active transport of Na from lumen to haemolymph but shows no active K transport (Koefoed & Zerahn, 1982), whereas the American silkworm *Hyalophora cecropia* normally has no Na transport but a large K transport from haemolymph to lumen, which is unaffected by ouabain (Haskell, Clemons & Harvey, 1965). Furthermore, the K secretion in the Malpighian tubules of *R. prolixus* and *S. gregaria* (Maddrell, 1969, 1977) and in the salivary glands of *Calliphora erythrocephala* (Berridge *et al.* 1976) is also insensitive to ouabain.

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