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, luminally-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

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accomplished at a constant rate of 20 μl 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 (²²Na, ³⁶Cl, ⁴²K and ⁸⁶Rb, purchased from The Radiochemical Centre, Amersham) either to the luminal (for lumen to haemolymph flux, Jₗ₋ₕ) or to haemolymphatic (for haemolymph to lumen flux, Jₕ₋ₗ) saline, with an activity of about 4 μCi ml⁻¹. 200 μl 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; MgSO₄, 6; CaCl₂, 3; Na₂HPO₄, 3·2; NaH₂PO₄, 2; sucrose, 100; glucose, 30; pH 7 for L. maderae; and NaCl, 110; NaHCO₃, 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 transepithelial 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 mV (lumen positive to haemolymph) in 86 experiments (Fig. 2). As previously pointed out (Sacchi & Giordana, 1979), this range of polarity cannot be
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In B. gigantea the PD was $-6.1 \pm 3.5$ 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)

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

$J_{l-h}$ 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. Blaberoida gigantea midgut: ion fluxes and transepithelial electrical potential difference (PD)

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

\( J_{\text{h}^{-\text{midgut}}} \) and \( J_{\text{b}^{-\text{midgut}}} \) are the unidirectional lumen to haemolymph and haemolymph to lumen fluxes respectively. Mean ± s.e., number of experiments in parentheses. \( J_{\text{int}} \) is the difference between the two unidirectional fluxes. Differences between \( J_{\text{h}^{-\text{midgut}}} \) and \( J_{\text{b}^{-\text{midgut}}} \) 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 \textit{P. americana} and \textit{Rhodnius prolixus}. The same concentration of ouabain did not reduce \( J_{\text{b}^{-\text{midgut}}} \) 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 \textit{P. americana} showed a decrease of the \( J_{\text{b}^{-\text{midgut}}} \) 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 \textit{et al.} 1976). As for K transport, ouabain altered neither the \( J_{\text{h}^{-\text{midgut}}} \) nor the \( J_{\text{b}^{-\text{midgut}}} \) 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 ± 1.2 mV (22 experiments).

Flux measurements on midguts of \textit{B. gigantea} were made from animals fed either an uncontrolled diet (varying amounts of flour, fruit and lettuce \textit{ad libitum}) or fed primarily with lettuce, as specified (Table 2). As in \textit{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 \(^{42}\text{K}\) 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 \textit{B. gigantea} midgut can actively transport K (or Rb) but unlike \textit{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
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Reported in Table 2 are pooled, a $J_{\text{Na}}$ value of $107.7 \pm 11.2$ nequiv h$^{-1}$ midgut$^{-1}$ (mean ± s.e., 26 experiments) and a $J_{\text{K}}$ value of $225.0 \pm 27.2$ nequiv h$^{-1}$ midgut$^{-1}$ (mean ± s.e., 32 experiments) are found. The resulting net flux, from haemolymph to lumen, is equal to $117.3 \pm 33.4$ nequiv h$^{-1}$ midgut$^{-1}$ (mean ± 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. prolixius* 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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**REFERENCES**


