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ACTIVE TRANSPORT OF SODIUM BY THE ISOLATED MIDGUT OF *HYALOPHORA CECROPIA*

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INTRODUCTION

Potassium is actively transported from blood-side to lumen of the isolated silkworm midgut (Harvey & Nedergaard, 1964). Rubidium is also actively transported and, when present in equal amounts, potassium and rubidium compete on an almost equal basis (Nedergaard & Harvey, 1968). Recently, Zerahn (1970) has found that caesium as well is actively transported, but the competition with potassium is unusual.

We have now found that sodium and lithium are also actively transported by the midgut when potassium is not available. The competition between sodium and potassium is unusual and the active sodium transport is not inhibited by ouabain.

METHODS

Larvae of *Hyalophora cecropia* (L) were reared on artificial diet by the techniques of Riddiford (1968). Mature fifth-instar caterpillars were chilled in ice for at least an hour. The midgut was excised and mounted in the apparatus described by Harvey, Haskell & Zerahn (1967). It was bathed on both sides with an aqueous medium (K-32) of 30 mM-KCl, 2 mM-$KHCO_3$ and 166 mM sucrose. A Na-32 and a Li-32 medium each contained the same amounts of the corresponding sodium or lithium salts. A solution, Na-16, K-16, was made up of 50% of Na-32 and 50% of K-32.

The potential difference across the midgut was measured with either a Radiometer pH meter (PHM4) or a Keithley electrometer (602). The instrument was connected to two calomel cells which were connected via two 30 mM-KCl-agar bridges to the bathing solutions. The short-circuiting techniques of Ussing & Zerahn (1951) were adapted for the midgut (see Zerahn, 1970) and were used in these experiments.

For changing the bathing solutions to an experimental solution, for example Na-32 all but the last 4 ml of lumen solution was removed and the remaining solution was diluted with 25 ml of Na-32. This dilution was repeated five times. The lumen chamber was then closed and the 25 ml blood-side chamber was flushed gently with 200 ml of Na-32. This procedure keeps the shape of the midgut sphere undisturbed throughout the experiment.

^{22}Na was obtained from New England Nuclear as carrier-free solution in 0·5 N-HCl. The solution was diluted 20 times with distilled water and 2–5 μCi was added to either the blood-side or the lumen solution. This small amount of solution will not change the pH or sodium concentration significantly. Samples from the lumen-side

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were taken with a 1 ml Carlsberg micropipette and those from the blood-side with a 1 ml syringe. They were counted on a Packard liquid scintillation counter or on a Nuclear Chicago gamma spectrometer. The standard error for counting the samples was below 2% except in one efflux determination. The fluxes were calculated by comparing the radioactivity in the samples with that in the solution to which the isotope had been added.

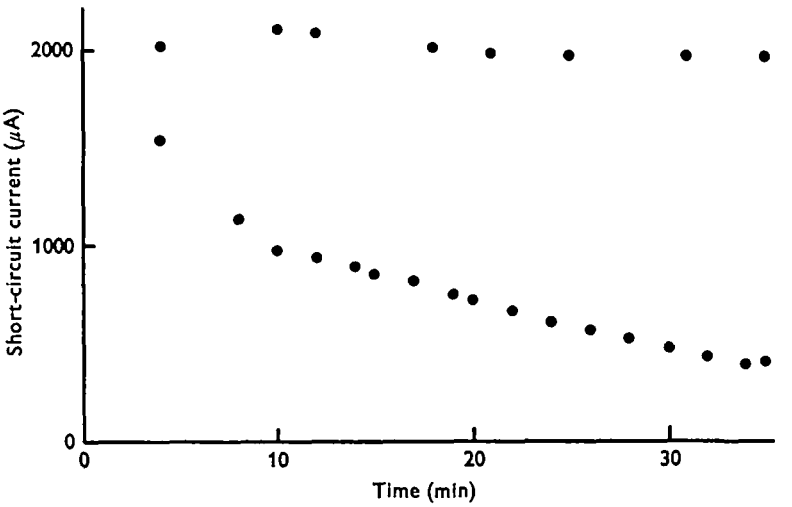

Fig. 1. Representative curves for change of short-circuit current with time. At zero time the blood-side was flushed with Na-16, K-16 (upper curve) or Na-32 (lower curve).

RESULTS

The ability of a potassium-free solution to sustain a large short-circuit current is demonstrated in Fig. 1 (lower curve). At zero time the K-32 solutions on both sides of the isolated midgut were replaced by Na-32 solutions. The current drops rapidly to about 50% of its value in potassium solution and thereafter decays as shown in Fig. 1. In ten similar experiments the current dropped on the average to about 50% of its value in K-32. These determinations were not corrected for the decay of current with time so that the mean value for short-circuit current remaining is probably too low.

The flux of ^{22}Na from blood-side to lumen was determined in ten measurements from five isolated midguts and found to have an average value of 22·5 μ-equiv./h, a value very close to the mean short-circuit current of 22·9 μ-equiv./h (Table 1). In a series of eight determinations from four isolated midguts with a similar mean short-circuit current of 18·2 μ-equiv./h, the average flux from lumen to blood-side was but 1·2 μ-equiv./h, amounting to about 5% of the flux from blood-side to lumen (Table 2).

The inability of sodium to compete with potassium when both are present in equal amounts is shown by the data in Table 3. The sodium flux from blood-side to lumen in Na-16, K-16 was but 3·4% of the short-circuit current in that solution and equal in size to the flux from lumen to blood-side in the Na-32 solution.

Ouabain had no effect on the short-circuit current in the Na-32 solution when present at a concentration of 10^{-4} M on either side of the isolated midgut.

Table 1. *Sodium flux from blood-side to lumen of the short-circuited, isolated midgut of* Hyalophora cecropia

(The bathing solutions on both sides of the midgut contained 30 mM-NaCl, 2 mM-$NaHCO_3$ and 166 mM sucrose and were oxygenated.)

Date	Period (min)	Sodium-flux (μ-equiv./h)	Current (μ-equiv./h)
22 June (A)	10–15	18·7	21·2
	15–30	18·7	16·5
22 June (B)	10–15	19·6	19·0
	15–30	16·7	15·3
23 June (A)	10–15	20·9	28·0
	15–30	17·0	17·5
23 June (B)	10–20	35·7	42·0
	20–30	29·4	30·0
23 June (C)	10–20	26·8	21·5
	20–30	21.1	18·3
Mean	—	22·5	22·9

Table 2. *Sodium flux from lumen to blood-side of the short-circuited, isolated midgut of* Hyalophora cecropia

(The bathing solutions on both sides of the midgut contained 30 mM-NaCl, 2 mM-$NaHCO_3$ and 166 mM sucrose and were oxygenated.)

Date	Period (min)	Sodium-flux (μ-equiv./h)	Current (μ-equiv./h)
24 June (A)	10–20	0·67	22·0
	20–30	0·79	19·0
24 June (B)	10–20	1·40	28·0
	20–30	3·40	17·0
24 June (C)	10–20	1·00	10·1
	20–30	1·06	9·2
24 June (D)	10–21	0·50	22·4
	21–30	0·74	17·5
Mean	—	1·2	18·2

DISCUSSION

Sodium flux ratios and amount of sodium transported. The sodium flux from blood-side to lumen during short-circuiting is found to be 22 μ-equiv./h (Table 1) and the sodium flux from lumen to blood-side is found to be only 1·2 μ-equiv./h (Table 2). This flux ratio for sodium is the same as that found previously for transport of potassium. It is quite clear that sodium is actively transported and, under the experimental conditions given, that the sodium ion is the only ion transported. This active transport of sodium is large—half that of the potassium transport by the gut and five times that of the sodium transport for a similar area of frog skin or toad bladder. Furthermore, the rate of sodium transport is large compared to the amount of ions present in the midgut; the sodium transport obviously is not disturbed by small losses of other ions from the midgut tissue as seen already by the agreement of sodium flux and current in Table 1.

Influence of ions in bathing solutions on sodium transport. The time-course of sodium current with the midgut bathed in Na-32 solutions is not the same as that found earlier when the bathing solutions contained 5 mM-$MgCl_2$ and 5 mM-$CaCl_2$ in addition to

the constituents present in Na-32. With magnesium and calcium added to Na-32 the midgut potential drops rapidly with time and often becomes negative (lumen negative to blood-side). Similarly, the short-circuit current is small, decays rapidly, and often reaches negative values.

In their original studies Harvey & Nedergaard (1964) included magnesium and calcium in their insect Ringer solution because these ions are present in the haemolymph, midgut lumen, and midgut tissue. It is rather surprising that whereas these divalent cations do not affect the potassium transport, they depress the potential and short-circuit current when the potassium is substituted by sodium.

This phenomenon is probably the reason why the earlier studies of the behaviour of the midgut towards the sodium ion mostly have considered the sodium ion to be an inert ion. As shown in this paper this assumption is justified with a sufficiently high concentration of potassium in the bathing solution (Table 3). Only when sodium is present in high concentration in relation to potassium do the earlier results have to be re-evaluated with some care to details.

Table 3. *Sodium flux from blood-side to lumen of the short-circuited, isolated midgut of* Hyalophora cecropia

(The bathing solutions on both sides of the midgut contained 16 mM-Na^+, 16 mM-K^+, 30 mM-Cl^-, 2 mM-HCO_3^- and 166 mM sucrose and were oxygenated.)

Date	Period (min)	Sodium-flux (μ-equiv./h)	Current (μ-equiv./h)
26 June (A)	20–30	0·61	21
26 June (B)	10–20	0·20	32
	20–30	0·25	32
26 June (C)	10–20	1·95	69
	20–30	3·56	69
27 June (A)	10–20	1·43	37
	20–30	2·50	35
27 June (B)	11–20	1·73	74
	20–30	2·70	74
Mean	—	1·7	49

The active transport of potassium reflected in the short-circuit current is not very dependent on osmotic pressure; it therefore seems unlikely that the effects of calcium and magnesium on the current in Na-32 are due to an increase in osmotic pressure.

Specificity of the transport mechanism. It had been found earlier that rubidium as well as potassium is actively transported by the midgut (Nedergaard & Harvey, 1968). When the potassium in the bathing solutions was replaced by rubidium there was no effect on the current. Moreover, when potassium and rubidium were present in equal amounts both were transported equally. Similarly, when all of the potassium is replaced by sodium about half of the current remains. By comparison with rubidium one might expect that approximately one-fourth of the current would be carried by sodium when the concentrations of sodium and potassium are equal. However, we see from Table 3 that no sodium at all is actively transported in 16 mM potassium, 16 mM sodium solutions.

Lithium, like sodium, does not interfere with potassium transport when potassium and lithium are present in equal concentrations (Nedergaard & Harvey, 1968). We were not able to deal as decisively with lithium as with sodium in this paper because

radioisotopes of lithium are not usable. However, we were able to demonstrate a short-circuit current in Li-32 which is fair proof of the active transport of lithium. Moreover, the lithium transport in Li-32 was about 50% of the potassium transport in K-32. The lithium transport and its relationship to potassium transport are in these respects almost identical to the sodium transport and its relationship to potassium transport.

Comparison with results from Malpighian tubules. The secretion of salt solutions by the Malpighian tubules of *Rhodnius*, *Calliphora* and *Calpodes* is thought to be an index of sodium and potassium transport under appropriate conditions. Malpighian tubules apparently are like the midgut in their ability to transport a variety of ions, in particular potassium and sodium. When sodium and potassium are present in equal amounts the *Rhodnius* tubules secrete only a little more potassium than sodium (Maddrell, 1969). By contrast the transport in *Calliphora* is predominantly potassium transport (Berridge, 1968) as it is in the midgut. An intermediate condition is found in *Calpodes* where the secretion of the rectal lead portion of the tubules is a fluid with a potassium/sodium concentration ratio of 4·15/1 when potassium and sodium are present in equal concentration (Irvine, 1969).

Conclusion. It was found earlier that the isolated midgut can actively transport potassium and rubidium and more recently that it also can transport caesium. Now it is shown that the midgut is able to transport both sodium and lithium under appropriate conditions. We conclude that the midgut can transport all stable alkali metal ions. We do not really know whether other ions may also be transported.

Parsimony requires that we assume that there is just one mechanism for the transport of potassium and sodium, and for the movements of other ions as well.

Rubidium competes almost equally with potassium, but in relation to the other ions potassium is always the preferred ion. Therefore the mechanism is specific for potassium when the potassium concentration is sufficiently high. When the potassium concentration is sufficiently low the mechanism changes its specificity for potassium and will now transport the other alkali metal ions.

Unlike the transport mechanism for sodium in most of the other known tissues, the transport mechanism in the midgut is not sensitive to ouabain, not even when the midgut is transporting sodium. The ability of the mechanism to transport several ions and the completeness with which it changes from the exclusive transport of one ion to the exclusive transport of a different ion may be valuable tools in obtaining more precise knowledge of transport mechanisms in general.

SUMMARY

1. Sodium and lithium are actively transported by the isolated midgut of *Hyalophora cecropia*.

2. The short-circuit current in 32 mM sodium solution is about half of that in 32 mM potassium solution.

3. The sodium flux measured with ^{22}Na from blood-side to lumen accounts for all of the short-circuit current and is 19 times the flux from lumen to blood-side.

4. In a solution containing 16 mM potassium and 16 mM sodium there is no transport of sodium, although a large current remains.

5. The sodium transport mechanism is not sensitive to ouabain.

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