J. exp. Biol. 105, 403–405 (1983)
Printed in Great Britain © The Company of Biologists Limited 1983

THE KINETICS OF ACTIVE K TRANSPORT BY THE MIDGUT OF LEPIDOPTERAN LARVAE: EFFECTS OF DIVALENT IONS

BY DAVID F. MOFFETT AND ALAN R. KOCH

Department of Zoology, Washington State University, Pullman, WA 99164, U.S.A.

(*Received 30 November 1982 – Accepted 8 March 1983*)

The isolated midgut of lepidopteran larvae actively transports K from haemolymph to lumen, providing a model for K transport in insect secretory and excretory tissues (reviewed by Harvey, 1980). The dependence of this transport on extracellular K has been studied by several investigators. Recently, Zerahn (1982) estimated a value of 10 mM for K_m in *Hyalophora cecropia* from his data and also from previous observations (Harvey & Zerahn, 1972), and obtained a value of 40 mM from data for *Manduca sexta* (Moffett, 1979). In the studies upon *H. cecropia*, the only cation present in the bathing solution was K; in the study upon *M. sexta*, 5 mM-Ca and 5 mM-Mg were also present as well as sufficient NaCl to maintain constant osmolarity. Although there may be slight innate differences in the transport kinetics of the two species, we show here that the reported differences can be accounted for by the presence or absence of divalent cations.

The morphologically distinct posterior midguts (Cioffi, 1979; Cioffi & Harvey, 1981) of 5th instar larvae of *M. sexta* reared on artificial diet (Yamamoto, 1969) were mounted and equilibrated as in previous studies (Moffett, 1979). The dependence of the short-circuit current (I_{sc}) on K concentration was measured by a rapid method in which I_{sc} was determined at quasiequilibrium within 3 min after a change to a new K concentration. In one set of experiments, the bathing solution contained 166 mM-sucrose, 5 mM-Tris buffer (pH = 8·0) and KCl as needed ('divalent-free medium'). In a second set of experiments, bathing solutions also contained 5 mM-$CaCl_2$ and 5 mM-$MgCl_2$ ('standard medium'). Values of I_{sc} were determined for K concentrations from 4 mM to 90 mM in the standard medium and from 10 mM to 90 mM in the divalent-free medium. No more than five different K concentrations were presented in any single experiment, and each experiment included a measurement of I_{sc} at 32 mM-K; the latter value is generally used as the standard in work with this tissue, and was used for normalization of results. The mean I_{sc} in 32 mM-K was 950 ± 65 (S.E.) $\mu A\,cm^{-2}$ for 13 experiments in standard medium and 1141 ± 101 (S.E.) $\mu A\,cm^{-2}$ for 15 experiments in divalent-free medium. Although the choice of 32 mM-K for normalization was arbitrary, these values are not different at the 0·1 level of probability. Bathing solution resistance was fully compensated for in the measurements of I_{sc}.

In the standard medium, I_{sc} rose over the whole range of measurement (Fig. 1).

Key words: K^+ transport, divalent ions, lepidopteran midgut.

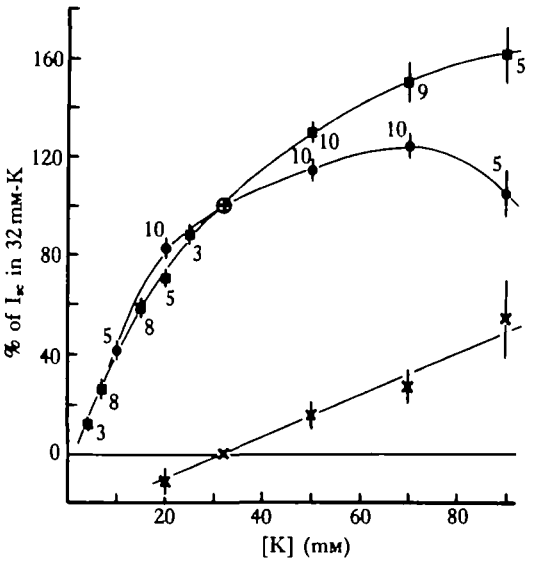

Fig. 1. Dependence of I_{sc} on K in standard medium (squares) and in divalent-free medium (circles). The two upper curves are drawn by eye. The numbers indicate number of experiments for each data point. Lower curve (crosses) represents difference between the two upper curves. For all curves, vertical bars indicate ±1 S.E. The slope of the difference curve was determined by weighted regression.

The results are very similar to those reported by Moffett (1979). The experiments in divalent-free medium showed a different pattern. I_{sc} rose somewhat more rapidly at low K concentrations. When K was between 50 and 70 mM, I_{sc} was nearly constant. Elevation of K to 90 mM produced a distinct inhibition of I_{sc}. Potassium-independent active transport of Ca (Wood & Harvey, 1976) and Mg (Wood, Jungreis & Harvey, 1975) produces underestimates of net K flux from I_{sc} in the standard medium. The error can be calculated to be large for the lowest K concentrations; it is about 70% in 4 mM-K, assuming a net divalent flux of 48 nequiv $cm^{-2} min^{-1}$ (Wood *et al.* 1975; Wood & Harvey, 1976). It falls to less than 10% for K concentrations of 20 mM or greater, so that active divalent transport appears unlikely to account for the large difference in kinetics of I_{sc} between the two media at higher K concentrations.

In the range where comparisons can be made (up to 32 mM-K), the results in divalent-free medium are very similar to those previously reported for *H. cecropia* (Zerahn, 1982; Harvey & Zerahn, 1972). For the present results, if only the points within this range are used, K_m could be calculated as approximately 15 mM in the divalent-free medium and approximately 50 mM in the standard medium. However, in neither series do the results follow Michaelis-Menten kinetics. The most obvious deviation in the divalent-free series is the depression of I_{sc} at high K concentration. Indeed, the shape of this curve is very similar to that found by Wieczorek (1982) for K-activated ATPase of fly labellum, a tissue believed to possess a similar electrogenic K pump. The relation seen in standard medium is most easily explained as the sum of two separate processes. One is the relationship seen in the divalent-free medium. The other, which is induced in the presence of divalent ions, produces a component of I_{sc} which increases linearly with external K. The slope of the linear compone

(lowest curve in Fig. 1) is obtained by subtracting normalized values of I_{sc} in divalent-free medium from corresponding values in standard medium. Normalization of the data constrains the difference curve to intersect the abcissa at 32 mM-K. The relation so derived has a slope of $0{\cdot}85 \pm 0{\cdot}03$ (S.E.) %/mM K (weighted regression). This is the equivalent of $8{\cdot}2\,\mu A/mM$ K; a similar slope is obtained from direct subtraction of unnormalized I_{sc} values. If there is a single apical K pump mechanism in posterior midgut, as suggested by Harvey, Cioffi & Wolfersberger (1981), the parallel components might correspond to separate mechanisms of entry of K into the transport pool. In this regard we note that, in the presence of 2 mM-Ba on the haemolymph side, I_{sc} is proportional to K concentration over the range 4 mM to 90 mM (Moffett & Koch, 1982).

REFERENCES

Cioffi, M. (1979). The morphology and fine structure of the larvae midgut of a moth (*Manduca sexta*) in relation to active ion transport. *Tissue and Cell* **11**, 467–479.

Cioffi, M. & Harvey, W. R. (1981). Comparison of potassium transport in three structurally distinct regions of the insect midgut. *J. exp. Biol.* **91**, 103–116.

Harvey, W. R. (1980). Water and ions in the gut. In *Insect Biology in the Future*, (eds M. Locke & D. S. Smith). New York: Academic Press.

Harvey, W. R., Cioffi, M. & Wolfersberger, M. G. (1981). Portasomes as coupling factors in active ion transport and oxidative phosphorylation. *Am. Zool.* **21**, 775–791.

Harvey, W. R. & Zerahn, K. (1972). Active transport of potassium and other alkali metals by the isolated midgut of the silkworm. In *Current Topics in Membranes and Transport*, Vol. 3, (eds F. Bronner & A. Kleinzeller). pp. 367–410.

Moffett, D. F. (1979). Bathing solution tonicity and potassium transport by the midgut of the tobacco hornworm *Manduca sexta*. *J. exp. Biol.* **78**, 213–223.

Moffett, D. F. & Koch, A. R. (1982). Ba^{++} as a probe of K^+ uptake mechanism of insect midgut. *Am. Zool.* **22**, 891.

Wieczorek, H. (1982). A biochemical approach to the electrogenic potassium pump of insect sensilla: potassium sensitive ATPases in the labellum of the fly. *J. comp. Physiol.* **148**, 303–311.

Wood, J. L. & Harvey, W. R. (1976). Active transport of Ca^{2+} across the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **65**, 347–360.

Wood, J. L., Jungreis, A. M. & Harvey, W. R. (1975). Active transport of magnesium across the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **63**, 313–320.

Yamamoto, R. T. (1969). Mass rearing of the tobacco hornworm II. Larval rearing and pupation. *J. econ. Ent.* **62**, 1427–1431.

Zerahn, K. (1982). Inhibition of active K transport in the isolated midgut of *Hyalophora cecropia* by Tl^+. *J. exp. Biol.* **96**, 307–313.