

## 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.

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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- $\text{CaCl}_2$  and 5 mM- $\text{MgCl}_2$  ('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  $950 \pm 65$  (s.e.)  $\mu\text{A cm}^{-2}$  for 13 experiments in standard medium and  $1141 \pm 101$  (s.e.)  $\mu\text{A cm}^{-2}$  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:  $\text{K}^+$  transport, divalent ions, lepidopteran midgut.

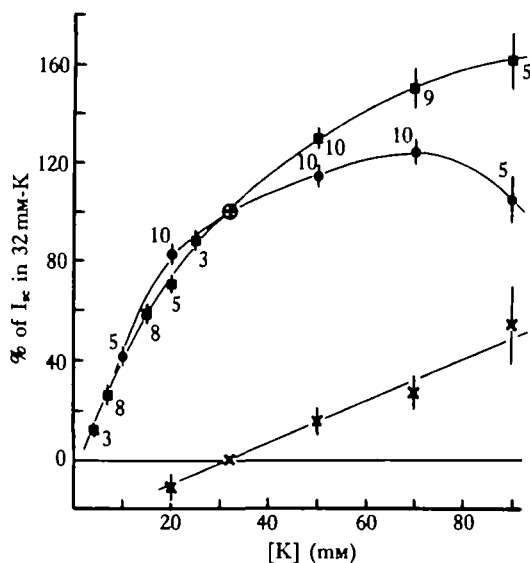


Fig. 1. Dependence of  $I_{sc}$  on  $K$  in standard medium (squares) and in divalent-free medium (circles). The two upper curves are drawn by eye. The numbers indicate number of experiments for each data point. Lower curve (crosses) represents difference between the two upper curves. For all curves, vertical bars indicate  $\pm 1$  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  $48 \text{ nequiv cm}^{-2} \text{ min}^{-1}$  (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 Wicczorek (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 component

(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 abscissa at 32 mM-K. The relation so derived has a slope of  $0.85 \pm 0.03$  (s.e.) %/mM K (weighted regression). This is the equivalent of  $8.2 \mu A/mM K$ ; 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).

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