BRIEF COMMUNICATION

DEMONSTRATION OF A PUMP-MEDIATED EFFLUX IN THE EPITHELIAL POTASSIUM ACTIVE TRANSPORT SYSTEM OF INSECT MIDGUT

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ABSTRACT The larval midgut epithelium of lepidopteran insects (e.g., *Hyalophora cecropia* and *Manduca sexta*) actively transports potassium from hemolymph to lumen when mounted in a chamber. The potassium active transport is rheogenic and does not require the presence of other alkali ions. The transepithelial potential difference, short-circuit current, and electromotive force of active transport are rapidly diminished by anoxia. The efflux of potassium, opposite in direction to potassium active transport, dramatically increased in anoxia, whereas the effluxes of sodium, cesium, and chloride did not increase in anoxia. The increase in efflux was found to have an alkali selectivity similar to that of potassium active transport. It is concluded that the rise of efflux in anoxia is due to the changed characteristics of the epithelial potassium active transport mechanism in anoxia.

INTRODUCTION

Plant-eating insects such as *Hyalophora cecropia* must contend with a dietary potassium concentration of up to 250 mM and maintain a hemolymph potassium concentration of 26 mM (Harvey et al., 1975). To accomplish this formidable task, insects have evolved a fascinating array of structures and active transport mechanisms, quite different from those found in vertebrate systems. A structure that may be involved in in vivo potassium and osmotic regulation is the insect midgut. The midgut of *H. cecropia* is composed of three cell types: large, numerous columnar cells, with apical microvilli and basal infoldings lined with mitochondria; smaller, less numerous goblet cells, with an apical cavity lined with cytoplasmic projections, each containing a mitochondrion; replacement cells (rare in *H. cecropia*) with a spherical shape and large nucleus. The cells are arranged in a single layer resting on a basement lamina associated with tracheae, tracheoles, and two discontinuous muscle layers (Anderson and Harvéy, 1966). The epithelium is ca 100 μm from basement lamina to brush border of the columnar cells.

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The in vitro midgut of phytophagous insects actively transports potassium and, to a lesser degree, other alkali cations across the epithelium from the hemolymph to the lumen (see Fig. 1). Active transport of potassium is found in the absence of other alkali ions which, under low-calcium conditions, compete with potassium for active transport (for review, see Harvey and Zerahn, 1972). This transport is rheogenic (current-generating) and requires no specific co-ions or counter-ions under open or short-circuit conditions. The transepithelial potential difference (PD) and short-circuit current (I_{sc}) are rapidly diminished by anoxia and are responsive to changes in the potassium concentration of the hemolymph-side bathing solution (Harvey and Zerahn, 1972). It will be shown in this paper that the efflux of potassium (lumen to hemolymph) rises when potassium active transport ceases in anoxia and that the rise shows selectivity characteristics which strongly suggest that the rise in efflux is mediated by the potassium active transport mechanism or pump.

MATERIALS AND METHODS

The isolated insect midgut was mounted as a flat-sheet in an Ussing-type chamber (0.5-cm^2 chamber opening, 10-ml vol/half; Wood, 1978). The midgut was secured by a loop of surgical thread onto a plastic lip (Wood, 1978). This technique was adopted to avoid edge damage to the midgut. The bathing solution contained 32 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Tris, and 166 mM sucrose, and was adjusted to pH 8.3 (high calcium). The sucrose is present as an osmotic agent and an energy source. In one set of experiments (see Table II), all of the potassium was replaced by cesium and no calcium was added (low calcium). The PD was measured with 3% agar-bathing solution bridges, calomel half-cells, and a Keithley 602 electrometer (Keithley Instruments, Inc., Cleveland, Ohio). Current was applied through silver-silver chloride electrodes by the three-bridge method of Wood (1978) to correct for solution resistance. An automatic voltage clamp was used to maintain the PD at zero. Experiments were performed at room temperature (20°–27°C).

Isotopes were obtained from New England Nuclear (Boston, Mass.). Rubidium-86 and potassium-42 were counted in H_2O by Cerenkov effect, and cesium-137 and chloride-36 were counted in scintillation fluid (Packard Insta-Gel) using a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co. Inc., Downers Grove, Ill.). Sodium-22 was counted in a deep well gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Ion fluxes were measured by taking 1-ml samples from the hemolymph-side bathing solution at 5-min intervals. Fluxes were calculated with a standard formula that accounted for bathing solution dilution and decay of the potassium-42 isotope during the experiment and during counting (Blankemeyer, 1976).

Insects used as sources for the experimental midguts were either *H. cecropia* (American silkworm) or *Manduca sexta* (tobacco hornworm). *H. cecropia* were raised outdoors on wild cherry leaves, and *M. sexta* were raised indoors on an artificial diet (modified from Yamamoto, 1969). *M. sexta* were used when *H. cecropia* were unavailable. The midguts are very similar in structure and nearly identical in electrical and kinetic characteristics (Blankemeyer, 1976).

RESULTS

Fig. 1 is a schematic diagram of the insect midgut that illustrates the direction of active transport of potassium and of influx and efflux. An example of a double-label efflux experiment is plotted in Fig. 2. The protocol for this experiment, using 32 mM K bathing solution on both sides of the midgut, was to insert a period of nitrogen between

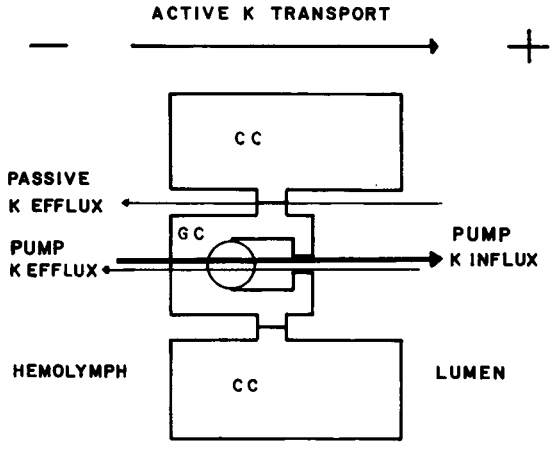

FIGURE 1 Schematic diagram of the structure and function of insect midgut. The two major cell types in the midgut are the columnar cells (CC) and the smaller, less frequent goblet cells (GC). The transepithelial potential difference is oriented positive lumen to hemolymph. The large arrow at the top of the figure represents the direction of active potassium transport. The site of active potassium transport is identified at the apical membrane of the goblet cell by the large circle. The probable routes for the passive efflux (thin line), and pump-mediated influx (thick line) and pump-mediated efflux (thin line) are indicated on the figure. Representative values are: transepithelial potential difference (PD), 100 mV; short-circuit (I_{sc}, 600 $\mu A/cm^2$; transepithelial resistance in oxygen, 180 Ω-cm^2, in nitrogen, 400 Ω-cm^2. Data from Blankemeyer (1976).

periods of oxygen as the stirring gas. Tracer fluxes of potassium and cesium were measured from the lumen to the hemolymph. It can be seen directly from Fig. 2 that as the I_{sc} fell to zero in nitrogen, the potassium efflux more than quadrupled and the cesium efflux remained relatively constant. Results from experiments in which the anoxia lasted more than 15 min showed that the efflux plateaued after 15 min. When

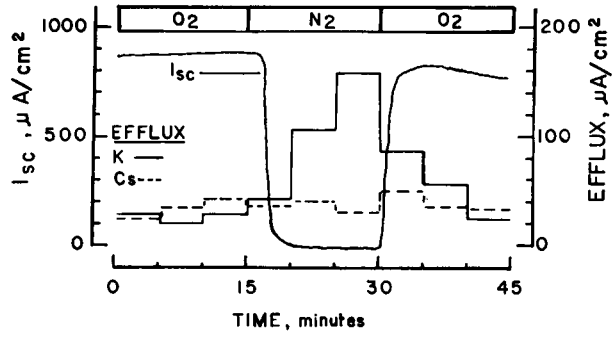

FIGURE 2 Representation of a double isotope efflux experiment using a midgut from *H. cecropia* during which the effluxes of both potassium (solid bars) and cesium as a tracer for potassium (dashed bars) were measured as was the short-circuit current (I_{sc}). Both bathing solutions contained 32 mM KCl as described in the text. The protocol was to insert a 15 minute period of nitrogen between the periods of oxygen and to measure the isotopic efflux by sampling the hemolymph-side bathing solution (5-min intervals) during all the periods. The efflux is plotted for every 5-min period.

TABLE I

EFFLUX EXPERIMENTS INVOLVING ALKALI IONS AND CHLORIDE

Ion	Ratio of nitrogen to oxygen efflux
K	2.19 ± 0.254 (7 expts., 3 tissues)
Rb	2.15 ± 0.33 (4 expts., 2 tissues)
Cs	0.95 ± 0.1 (4 expts., 2 tissues)
Na	0.93 ± 0.03 (2 expts., 2 tissues)
Cl	0.98 ± 0.16 (7 expts., 4 tissues)

All experiments were performed with 32 mM potassium bathing solution as described in the text. The protocol for these experiments was to follow a period of efflux measurement in oxygen with a period of efflux measurement in nitrogen, then return to oxygen. The experiments were done under short-circuit conditions. The ratio presented in this table is the average value of the efflux in nitrogen to the average value of the efflux in oxygen ± SEM. The only alkali cation added (other than K) in these experiments was the carrier in the aliquot of isotope added to the chamber. This resulted in high ratios of K to the cation. The typical K:Rb ratio was 200, the K:Na was 4,700, and the K:Cs was 2,000. The K:Cl ratio was 0.76.

oxygen was returned as the stirring gas (in Fig. 2), the I_{sc} and potassium efflux returned to near their previous levels of oxygen.

The results of Table I, from experiments with the same protocol as Fig. 2, demonstrate that the effluxes of rubidium and potassium increase in anoxia, whereas the effluxes of sodium, cesium, and chloride show no significant change in anoxia. Because potassium and rubidium are about equally favored by the "pump" over sodium and cesium (Zerahn, 1973), it is possible that the rise in efflux is associated with the pump. This possibility was further tested in the following experiment.

The rationale for this experiment was to use the cation competition sequence of the potassium pump (K $\cong$ Rb > Cs > Na) to determine whether the anoxic rise in efflux was mediated by the pump. The bathing solution on the hemolymph-side contained potassium as the only alkali ion and that on the lumen-side contained cesium as the only alkali ion. The low calcium lumen-side solution favors cation competition, whereas the high calcium hemolymph-side favors potassium selectivity (see Harvey and Zerahn, 1972). If the efflux increase in anoxia is pump-mediated, then the cesium efflux should show an increase in anoxia similar to the rise of potassium efflux observed in Fig. 2 because there is no potassium (on the lumen-side) to compete with cesium. Table II shows that the cesium efflux rose significantly in anoxia (in low or high calcium bathing solutions), whereas the potassium efflux, as mimicked by rubidium, was constant. The alkali ion ratios in the lumen-side bathing solution determine whether the efflux of a particular alkali ion increases in anoxia. That is, high ratios of cesium to potassium produce an anoxic increase in cesium efflux (Table II); high ratios of potassium to cesium produce an anoxic rise in potassium (or rubidium) efflux (Table I). The com-

TABLE II

EFFLUX EXPERIMENTS WITH CESIUM BATHING SOLUTION

Ion	Ratio of nitrogen to oxygen efflux
Cs	1.38 ± 0.06 (4 expts., 3 tissues) (no added calcium)
Cs	1.85 ± 0.42 (4 expts., 2 tissues) (1 mM calcium)
Rb	1.03 (1 expt., 1 tissue) (no added calcium)

The results of experiments in which the lumen-side solution contained cesium as the only alkali ion and the hemolymph-side solution contained potassium as the only alkali ion (both 32 mM). The PD was maintained at zero during the experiment. The protocol otherwise was identical to Table I. The results are presented as the ratio of the average cesium efflux in nitrogen to the average cesium efflux in oxygen ± SEM. The Rb experiment was performed by adding rubidium tracer to the cesium-only lumen-side solution. The ratio of Cs to Rb in the rubidium experiment was 200:1.

petition sequence is K $\cong$ Rb > Cs $\cong$ Na. Inasmuch as this ranking is similar to that of the influx, I can now reasonably suggest that the epithelial K "pump" is involved in the rise of cation efflux in anoxia.

DISCUSSION

The results presented in Fig. 2. and Table I demonstrate that when the K concentration is much greater than Rb, Na, and Cs, the K and Rb effluxes increase dramatically in anoxia, whereas Na, Cs, and Cl effluxes do not increase. The simplest explanation for the data is that a general increase of midgut ion permeability occurs in anoxia. If this hypothesis is correct, the transepithelial resistance should decrease in anoxia and all of the ions in Table I should show an increased efflux in anoxia. However, the transepithelial resistance increases in anoxia (Blankemeyer, 1976) and examination of Table I shows that there is not a general increase of efflux, but rather a cation-selective increase. We must then find an explanation for efflux cation selectivity similar to that of active potassium transport in the midgut (i.e., K $\cong$ Rb > Cs > Na).

One explanation for the results of Table I and Fig. 2 is that an unstirred layer exists near the hemolymph-side of the pump. Because this unstirred layer would have a lower potassium concentration than the bathing solution, the efflux of labeled K would have a chance of being pumped back into the lumen-side bathing solution and not entering the hemolymph-side bathing solution as an efflux sample. Cessation of active transport in anoxia would then cause an apparent increase in efflux. The observed cation selectivity (in Table I) would be explained because tracer amounts of Cs and Na would have a low probability of being pumped back into the lumen-side (due to competition from K), and thus Cs and Na would have an unchanged efflux in anoxia. The unstirred layer model was tested by performing the experiments reported in Table II. In these experiments the hemolymph-side of the K pump had a K-only (with Ca) bathing solution, whereas the lumen-side of the pump had a cesium-only

solution (with cesium isotope). The cesium efflux arriving in the hemolymph-side would have a low probability of being actively transported back to the lumen (due to the high K, high Ca bathing solution). I would predict, based on the unstirred layer model, that the cesium efflux should not increase in anoxia. Because Table II demonstrates that the cesium efflux increases in anoxia, a different explanation is needed for the data of both Tables I and II.

The simplest remaining explanation is that the anoxic efflux of potassium and rubidium in 32 mM potassium lumen-side solution (Fig. 2 and Table I) and the anoxic efflux of cesium in 32 mM cesium lumen-side solution (Table II) are mainly through the epithelial potassium pump. The difference between Tables I and II in the effect of anoxia on cesium efflux is due to competition for the lumen-side of the pump, with selectivity in the same order as that for influx.

The pump-mediated K efflux in oxygen (in high K solutions as in Fig. 2 and Table I) can be estimated from the open circuit PD (using the PD as the minimum electromotive force) and the K influx. Using values from experiments with similar I_{sc} and PD to that of Fig. 2, the pump-mediated K efflux would be $<10\ \mu A/cm^2$ (calculated by the flux ratio equation; Ussing and Zerahn, 1951). Then the K efflux in oxygen (see Fig. 2) is primarily through nonpump pathways, probably via a paracellular shunt, although edge damage cannot be ruled out. In anoxia, when the apical voltage and conductance of the goblet cell fall to zero (Blankemeyer, 1976), the K efflux rises, whereas the Na, Cs, and Cl effluxes are constant. Results presented in this paper make it probable that this increased K efflux is pump mediated.

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