J. exp. Biol. 172, 403-415 (1992)
Printed in Great Britain © *The Company of Biologists Limited 1992*

DRIVING FORCES AND PATHWAYS FOR H^+ AND K^+ TRANSPORT IN INSECT MIDGUT GOBLET CELLS

BY DAVID F. MOFFETT AND ALAN KOCH

Laboratory of Molecular Physiology, Program in Zoophysiology, Washington State University, Pullman, WA 99164–4236, USA

Summary

In the midgut of larval lepidopteran insects, goblet cells are believed to secrete K^+; the proposed mechanism involves an electrogenic K^+/nH^+ ($n>1$) antiporter coupled to primary active transport of H^+ by a vacuolar-type ATPase. Goblet cells have a prominent apical cavity isolated from the gut lumen by a valve-like structure.

Using H^+- and K^+-selective microelectrodes, we showed that electrochemical gradients of H^+ and K^+ across the apical membrane and valve are consistent with active secretion of both ions into the cavity and that the transapical H^+ electrochemical gradient, but not the transapical pH gradient, is competent to drive K^+ secretion by a K^+/nH^+ antiporter.

We used 10 mmol l^{-1} tetramethylammonium ion (TMA^+) as a marker for the ability of small cations to pass from the gut lumen through the valve to the goblet cavity, exploiting the high TMA^+ sensitivity of 'K^+-sensitive' microelectrodes. These studies showed that more than half of the cavities were inaccessible to TMA^+. For those cavities that were accessible to TMA^+, both entry and exit rates were too slow to be consistent with direct entry through the valves. One or more mixing compartments appear to lie between the lumen bathing solution and the goblet cavity. The lateral intercellular spaces and goblet cell cytoplasm are the most likely compartments. The results are not consistent with free diffusion of ions in a macroscopic valve passage; mechanisms that would allow K^+ secreted into the goblet cavity to exit to the gut lumen, while preventing H^+ from exiting, remain unclear.

Introduction

Active electrogenic transport of K^+ is an important primary mechanism of salt and fluid secretion in insect epithelial organs such as Malpighian tubules (Maddrell, 1977), salivary glands (Berridge, 1977), molting integument (Jungreis and Harvey, 1975) and alimentary organs, including the midgut of lepidopteran larvae (Harvey and Nedergaard, 1964, reviewed by Dow, 1986). A similar mechanism appears to be responsible for electrogenesis in insect sensilla (Thurm and Küppers, 1980; Wieczorek, 1982) and muscle cells (Rheuben, 1972). The K^+ pump has been characterized as being Na^+-

Key words: tobacco hornworm, *Manduca sexta*, ion-selective microelectrodes, active potassium transport, intracellular pH, intracellular potassium.

independent, relatively nonselective among alkali metal ions, and rapidly inhibited by anoxia (Harvey and Zerahn, 1972; Keynes, 1973).

The large size and relatively simple organization of the lepidopteran midgut make it an especially favorable model for study of the mechanism of K^+ transport and associated processes (Anderson and Harvey, 1966; Cioffi, 1979). When isolated in a minimal saline (32KS) containing 32 mmol l^{-1} KCl, 166 mmol l^{-1} sucrose, 5 mmol l^{-1} each of $CaCl_2$, $MgCl_2$ and Tris buffer (pH 8.0) and equilibrated with 100% O_2, the midgut maintains a lumen-positive transepithelial voltage (V_t) as large as 100 mV and a hemolymph-to-lumen short-circuit current (I_{sc}) as high as 1 μequiv cm^{-2} min^{-1}. The net isotopic K^+ flux across the isolated tissue closely approximates I_{sc} (Cioffi and Harvey, 1981; Wood and Moreton, 1978).

The midgut is a straight tube consisting of a single layer of epithelial cells of two types: columnar cells and goblet cells which possess a large apical cavity. The goblet cells have long been implicated in K^+ transport (reviewed by Dow, 1986). Anterior, middle and posterior regions of the midgut can be distinguished on the basis of gross appearance and cellular morphology (Cioffi, 1979), although all regions transport K^+ (Cioffi and Harvey, 1981).

The goblet cavity contains a matrix material into which numerous microvilli of the goblet cell apical membrane (GCAM) project. The cavity is guarded at its apical end by a complex, valve-like structure with an aperture reduced in places to about 10 nm in diameter, and apparently closed in some electron micrographs (Smith *et al.* 1969; Flower and Filshie, 1976). While several secretory and sensory epithelial cell types have prominent apical crypts or cavities, the midgut seems to be unique among transporting epithelia in the extent to which the apical membrane of the putative transporting cells is isolated from the luminal surface. Dow and Peacock (1989) have presented evidence that the goblet valve is not readily penetrated by fluorescent dyes.

The model for active K^+ secretion by the isolated midgut, developed by Harvey, Zerahn, and their associates more than 20 years ago (reviewed by Harvey and Zerahn, 1972) and referred to hereafter as the standard model, incorporates the following three major features. (1) Passive, Na^+-independent uptake of K^+ across the basal membrane. (2) An electrogenic Na^+-independent pump in the GCAM that extrudes K^+ into the goblet cavity. Recently, Wieczorek *et al.* (1991) have presented evidence that the molecular mechanism of the K^+ pump involves an amiloride-sensitive alkali metal–proton antiporter driven by the primary active H^+ secretion of a vacuolar-type H^+-ATPase (V-ATPase). This mechanism is readily incorporated into the original model. (3) Passive exit of K^+ from the goblet cavity through the goblet valve. In subsequent sections of this paper, we will proceed by subjecting each of the three major features of the standard model to the Cartesian dictum (Doubt everything).

Measurements of intracellular ionic activities and electrochemical gradients using double-barrelled ion-selective microelectrodes

Much of this review rests on measurements of the electrochemical gradients for K^+ and

H^+ across the cell membranes of the isolated, superfused midgut tissue, made with double-barrelled ion-sensitive microelectrodes. This technique provides a simultaneous measure of the electrical and chemical components of the ionic gradient between the extracellular solution and the electrode tip in the interior of the epithelial cells.

The microelectrode consists of fused parallel tubes of capillary glass, pulled to a tip diameter of less than 1 μm in a standard microelectrode puller. One barrel is filled with electrolyte and serves as a reference for measurement of membrane potentials. The other barrel is first made hydrophobic by exposure to organosilane vapor. Its tip is then filled with liquid ion-selective resin, and its shank filled with a standard solution of the ion to be sensed. Measurements of TMA^+ were made using K^+-selective electrodes. Ion-selective electrodes measure ionic activity. Each electrode is calibrated in standard solutions for which the activities are determined from their concentrations using standard tables. Electrodes were calibrated for TMA^+ in the presence of 100 mmol l^{-1} KCl.

Impalements showing high $[K^+]$ and positive potential were identified as from goblet cavities and those showing high $[K^+]$ and negative potential were identified as from cytoplasm of either goblet or columnar cells (Moffett *et al.* 1982; Moffett and Koch, 1988*b*). Ionic activity of cytoplasm or goblet cavities is calculated from the difference between the output of the ion-specific and reference barrels, using either the Nernst equation or the Nicolsky equation, as appropriate.

An overview of the K^+ and H^+ electrochemical gradients

Fig. 1 shows idealized intraepithelial voltage measurements in short-circuited and open-circuited midguts, including abbreviations to be used in the subsequent discussion. Fig. 2 summarizes the measurements of the ionic activities (upper part) and the electrochemical gradients (lower part) for K^+, H^+ and Cl^- in short-circuited posterior midgut bathed in 32KS (Moffett and Koch, 1988*a*,*b*; Chao *et al.* 1989, 1991). In the case of K^+, the electrochemical gradient is favorable for K^+ entry into the cell from the basal side, unfavorable for movement from cytoplasm to goblet cavity across the GCAM, and favorable for K^+ exit from the goblet cavity to the luminal solution. These results are consistent with passive K^+ entry from basal solution to cytoplasm, active K^+ transport across the GCAM from cytoplasm to goblet cavity, and passive exit from the goblet cavity through a patent goblet valve.

Both the cytoplasmic pH and the goblet cavity pH are lower than the equilibrium values calculated with respect to the transbasal potential (V_b), the transapical potential (V_{gc}) and the transvalve potential (V_g). These results are consistent with metabolic production of H^+ accompanied by active H^+ secretion into the goblet cavity.

The picture presented by these results is, at least at first glance, consistent with the original model. We can now examine it further by means of the following questions. (1) Is the transbasal K^+ gradient both necessary and sufficient to account for the rapid rate of K^+ entry the gut maintains? (2) Is the transapical H^+ gradient sufficient to drive K^+ extrusion? (3) Is the way clear for K^+ to leave the goblet cavity once it has been deposited there? And if so, why does H^+ not leave the cavity by the same route?

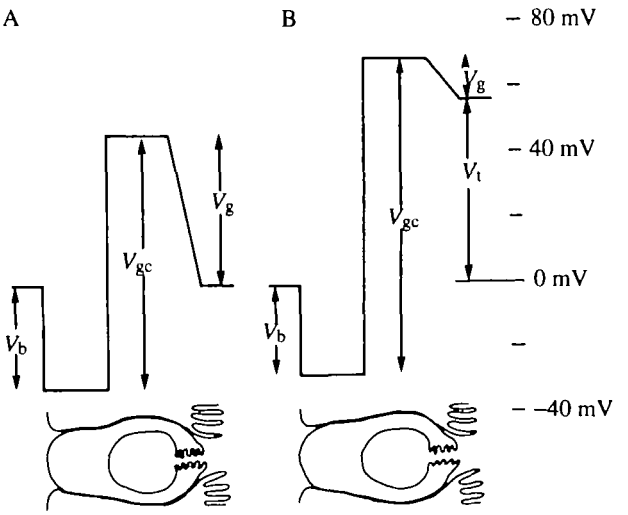

Fig. 1. The electrical potential profiles of short-circuited (A) and open-circuited (B) midgut epithelial cells recorded by advancing the electrode from the reference solution on the hemolymph (left-hand) side to the luminal solution on the right-hand side. In each case, the horizontal axis reflects the position of the electrode in the epithelium as indicated by the goblet cell diagram below each trace. V_b, transbasal voltage step; V_{gc}, voltage step between cytoplasm and goblet cavity; V_g, voltage step between goblet cavity and luminal solution; V_t, transepithelial potential. Under short- circuit, V_t is zero. Traces are idealized but values shown are actual means (from Chao *et al.* 1991).

The transbasal electrochemical gradients and mechanisms of K^+ uptake

Conductive pathways for K^+ uptake

Passive, conductive K^+ uptake is supported by three pieces of evidence: the favorable electrochemical gradient (Fig. 2), the presence of K^+ channels in the basal membrane (Zeiske *et al.* 1986; Moffett and Lewis, 1990) and the finding that the K^+ channel blockers Ba^{2+} and lidocaine, applied to the basal surface, hyperpolarize V_b and inhibit I_{sc} (Moffett and Koch, 1988a, 1991). The latter result led us to propose that V_b consists of two components, a Nernstian K^+ diffusion potential and a potential associated with the current of net entry through the K^+ channels. In the standard model, the current of net entry arises from the activity of the apical K^+ pump. There is no other cation abundant enough to balance the intracellular negative charge, so the driving force of the apical pump is electrostatically coupled to V_b by the current of K^+ flowing through the transport route. Because of the current-dependent component of V_b, an increase in the resistance of the basal membrane will hyperpolarize the cell and cause the transapical step to be larger.

Active pathway for K^+ uptake

Lepidopteran hemolymph is characteristically high in K^+ and low in Na^+, and midgut cells lack ouabain-sensitive Na^+/K^+-ATPase (Jungreis and Vaughn, 1977). Therefore, there would be no reason to expect that midgut cell membranes should contain any innate active mechanism for concentrating K^+ in the cytoplasm. We were surprised to find that

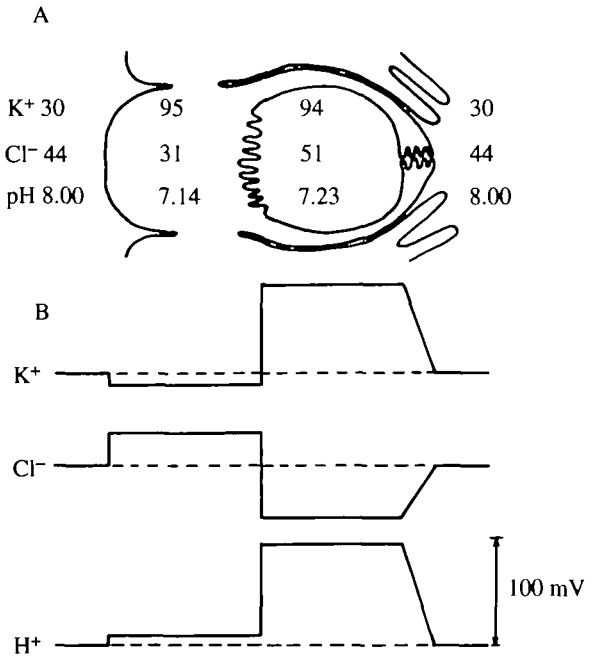

Fig. 2. (A) Mean extracellular, cytoplasmic and goblet cavity pH and activities of K^+ and Cl^- (in mmol l^{-1}) for short-circuited tissues bathed in 32KS. (B) Electrochemical gradients of the three ions across the basal membrane, goblet cell apical membrane and goblet valve (from Moffett and Koch, 1988*a*,*b*; Chao *et al.* 1989, 1991).

hypoxia (induced by gassing superfused tissues with 5% O_2/95% N_2), low extracellular [K^+], or both factors combined, resulted in a low, steady I_{sc} with an uphill, rather than downhill, K^+ electrochemical gradient across the basal membrane (Chao *et al.* 1990). The current could be sustained for periods much longer than the computed turnover time for total tissue K^+ and without a significant change in intracellular K^+ activity. This finding suggests that there is a parallel, active mechanism for K^+ uptake that is not O_2-dependent and not apparent under standard conditions. From an estimate of I_{sc} when the driving force for K^+ across the basal membrane is zero, about 70% of basal K^+ uptake is accomplished by the active process. Despite the difficulty of its detection, the data suggest that the active pathway is normally responsible for most of the basal K^+ uptake.

Electrochemical gradients between cytoplasm and goblet cavity and the problem of K^+/H^+

Acceptance of feature 2 of the original model requires that the following points be demonstrated. (1) The V-ATPase and antiporter must both be present in the GCAM. For the V-ATPase at least, evidence for this has been provided by immunohistochemistry (Klein *et al.* 1991). (2) The V-ATPase and antiporter must mediate active K^+ transport when both are present in membrane vesicles. Evidence for this has also been presented (Wieczorek *et al.* 1991). (3) The driving forces for K^+ and H^+ across the GCAM of the

intact, transporting tissue must be in accord with the hypothesis that H^+ is the driver ion and K^+ the driven ion. There is essentially no difference in either K^+ or H^+ activity across the GCAM in 32KS bathing solution. Hence neither ion can drive movement of the other in a 1/1 antiport (see Figs 2 and 3). However, an antiport that accepted more than one H^+ in exchange for each K^+ would have the voltage step of V_{gc} as an energy source. An advantage to this type of voltage-driven antiport is that, initially, the pump need only transfer enough charge to polarize the GCAM capacitor and need not measurably modify concentrations in either goblet cavity or cytoplasm.

Concentration-dependence of the K^+ transport system

In bathing solutions containing Ca^{2+} and Mg^{2+}, the rate of K^+ transport by the short-circuited midgut increases almost linearly and by about fourfold with increases of bathing solution $[K^+]$ over the range of 10 mequiv l^{-1} to 70 mequiv l^{-1} (Moffett, 1979; Moffett and Koch, 1983). The response to altered extracellular K^+ concentration is immediate and is complete within several minutes. Measurements of the intracellular K^+ activity in bathing solutions containing 10 mmol l^{-1}, 32 mmol l^{-1} and 70 mmol l^{-1} KCl showed that, over this range, intracellular K^+ activity changes by about 10% (see Fig. 3; Moffett and Koch, 1988*b*). The same studies showed that as the extracellular $[K^+]$ of the short-circuited preparation is increased there is a corresponding decrease in both V_{gc} and the K^+ electrochemical gradient across the GCAM. Unfortunately, the effect of changing extracellular $[K^+]$ on the pH gradient across the GCAM is not yet known. However, measurements of the cytoplasmic pH show a modest alkalization (0.04 ± 0.004 pH units) upon changing extracellular $[K^+]$ from 5 mmol l^{-1} to 32 mmol l^{-1}. Assuming that there are

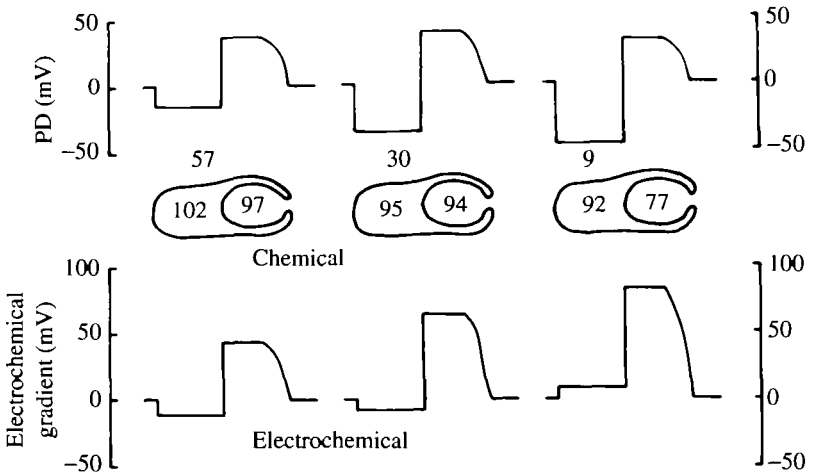

Fig. 3. The electrical potential profiles (top), K^+ activities (measured in mmol l^{-1}) (center) and K^+ electrochemical gradients (bottom) of midguts bathed in solution containing 70 mmol l^{-1} KCl (left), 32 mmol l^{-1} KCl (middle) and 10 mmol l^{-1} KCl (right). The activities of the extracellular solutions are given above each cell diagram (from Moffett and Koch, 1988*b*).

no large changes in the pH gradient across the GCAM, this result suggests that, in the context of the original hypothesis, the kinetics of the transport system with respect to K^+ may be determined primarily by the sensitivity of the H^+ pump to V_{gc}.

Adaptive significance of the goblet cavity and matrix

The goblet cavity matrix accounts for perhaps 40% of the mass of posterior midgut cells, and has been reported to stain with Alcian Blue at low pH (Schultz *et al.* 1981). Ion-microprobe studies of the elemental composition of goblet matrix report a concentration of elemental sulphur of about 58 mmol kg^{-1} wet mass (Dow *et al.* 1984; Gupta *et al.* 1985). These results are consistent with the presence of sulphated acidic mucosubstance. At the pH of goblet cavities (7.2–7.3; Chao *et al.* 1991), such a substance would constitute a large store of fixed negative charges.

In the absence of any other cation, much of the fixed charge of the matrix must be matched by K^+. This expectation is supported by experiments in which the apical pump was inhibited with N_2 hypoxia while the K^+ activity of impaled goblet cavities was measured. In these studies (Moffett and Koch, 1988*b*), a drop in I_{sc} to 30% of the initial value was accompanied by a decrease in V_g to about 50% of its initial value. Over the same time, the goblet cavity K^+ activity actually increased slightly. This result suggests that the fixed negative charge of the goblet cavity, rather than pump activity, is the major determinant of the K^+ activity of the cavity. Although it is difficult to maintain an electrode in place continuously during hypoxic transitions that go to full inhibition of I_{sc}, multiple penetrations before, during and at the end of such transitions show that V_g reaches values approaching the Donnan potential calculated for the goblet cavity (−18 mV).

Why is there a goblet cavity? In the context of the original hypothesis, the goblet cavity would protect and amplify the driving force for K^+ secretion. First, in its absence, the H^+ pump would be exposed to the high pH of the gut lumen (pH 8 or higher *in vivo*; Dow, 1984), and secreted protons would be likely to be accepted by buffers in the gut contents and thus not be available to the antiporter. Moreover, the presence of the cavity allows the voltage across the GCAM to reflect the full driving force of the H^+ pump, undiminished by the effect of paracellular conductance. This 'extra' voltage might make the K^+/nH^+ antiporter energetically feasible *in vivo*.

The original hypothesis does not require the presence of a goblet matrix; but since a matrix is clearly present, it is necessary to consider its chemistry. If the bulk of the fixed negative charges are anions of strong acids such as sulphate or sulphonate, and protons are continuously secreted into the cavity, we do not see how the pH of the goblet cavity matrix can fail to be lower than that in cytoplasm.

The transvalve electrochemical gradients and the problem of the goblet valve

Feature 3 of the original model – passive exit of K^+ through a patent valve – is not proved simply by demonstrating that there is an electrochemical driving force favoring

K^+ exit. It is necessary to show that the goblet valve is also patent to K^+, and preferably to show that a current, the single-cell correlate of I_{sc}, actually flows through the valve.

We then asked whether valves are open. For the purposes of this question, an 'open' valve is one in which ions diffuse in free solution. For these experiments we exploited the fact that the resin used for K^+-sensitive electrodes is far more selective for quaternary amines than for K^+. In the case of tetramethylammonium (TMA^+), the selectivity factor is at least 1000 for TMA^+ over K^+. Furthermore, in studies in which TMA^+ was introduced into cells as a marker for cell volume changes (Reuss, 1985), it was found not to cross cell membranes. The procedure in these experiments was to penetrate a goblet cavity with a double-barrelled K^+ electrode and then switch the luminal superfusate from standard solution to standard solution plus 10 mmol l^{-1} TMACl. Concentrations of TMA^+ as low as 2 μmol l^{-1} could be detected in the goblet cavity using this method (Figs 4, 5). The most important finding of these studies was that TMA^+ entry was undetectable for more than half of the goblet cavities, even when the measurement was prolonged for up to 90 min.

The uptake curves of those cavities to which TMA^+ did gain measurable access were characteristically concave-up (Fig. 5). This result is inconsistent with direct entry of TMA^+ to the cavity from the lumen and requires the presence of at least one mixing compartment between the lumen and the goblet cavity. Further, under open-circuit conditions, wherein the concentrations of TMA^+ reach relatively high values, the turnover time for this first compartment averaged more than 30 min, a time much too long to be accounted for by the combination of the time constants of the chamber (about 0.5 min) and any reasonable apical unstirred layer. An unstirred layer was further ruled out by control experiments in which the electrode was advanced through the tissue until it just crossed into the gut lumen and then the luminal superfusate was changed to one containing TMA^+. In these control experiments, the lag time was only a few seconds (Fig.

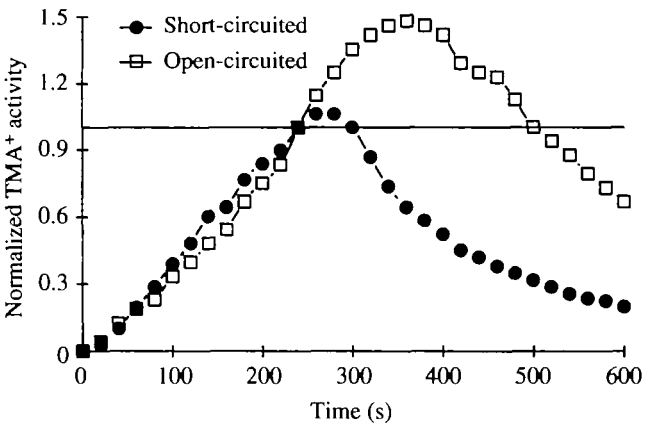

Fig. 4. TMA^+ influx and washout curves for goblet cavities from a short-circuited tissue (no symbols) and an open-circuited tissue (squares). The experiments chosen had unusually high turnover times. The values are normalized to the highest activity reached during the influx period, indicated by the horizontal line.

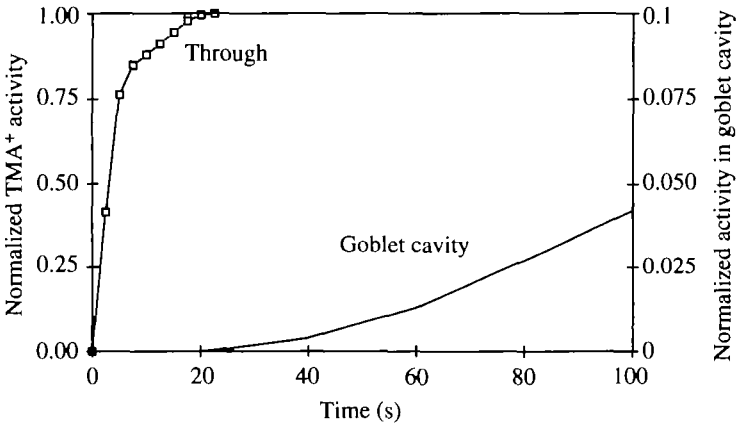

Fig. 5. Representative TMA^+ influx curve (squares, Through) from a control experiment in which the microelectrode was advanced just through to the luminal side of the epithelium. A typical influx curve for a goblet cavity (no symbols) is shown for comparison; note that the scale for the goblet cavity influx curve is one-tenth that of the control.

5). Finally, the TMA^+ washout curves also depart from first-order kinetics, and are characteristically different between short-circuited and open-circuited guts (Fig. 4).

These discrepancies from the behavior expected from the direct entry model led us to formulate a model in which TMA^+ entered the goblet cavity only indirectly (Fig. 6). The presence of one or more compartments between the lumen and the goblet cavity is in accord with the concave-up shape of the early uptake curves (Fig. 5). The fact that these compartments could retain TMA^+ after luminal TMA^+ had been washed out explains the overshoot of open-circuited preparations shown in Fig. 4. Quantitative fits of the TMA^+ data suggest that there are probably two compartments between the gut lumen and the goblet cavity. As shown in Fig. 6, the second of these is presumably the goblet cell cytoplasm; the first could be either columnar cell cytoplasm or lateral interstitial space. Quantitative fits of the model to the experimental data require that the volume of the first compartment constitute more than 10% of the tissue volume. This value is far higher than those found for interstitial fluid (Koch and Moffett, 1977). Accordingly, we believe that columnar cell cytoplasm is the first intratissue compartment for TMA^+. The model predicts that TMA^+ would enter cells at about the same frequency that it was found in goblet cavities.

In four of eight cell impalements, TMA^+ was detected entering the cytoplasm. This result invalidated our original assumption that TMA^+ would not cross cell membranes. In the washout measurements (Fig. 4), the TMA^+ activity of the goblet cavity continued to rise long after the luminal TMA^+ had been removed. Only a tissue compartment could have continued to feed TMA^+ to the goblet cavity compartment. The greater overshoot in open-circuited tissues suggests that the presence of the lumen-positive transepithelial potential causes the first compartment to load a greater amount of TMA^+. The results of these experiments lead us to conclude that, in the isolated tissue, entry of TMA^+ into some goblet cavities does not reflect direct access through 'open' goblet valves.

Once in the columnar cell cytoplasm, TMA^+ would be expected to move quickly into

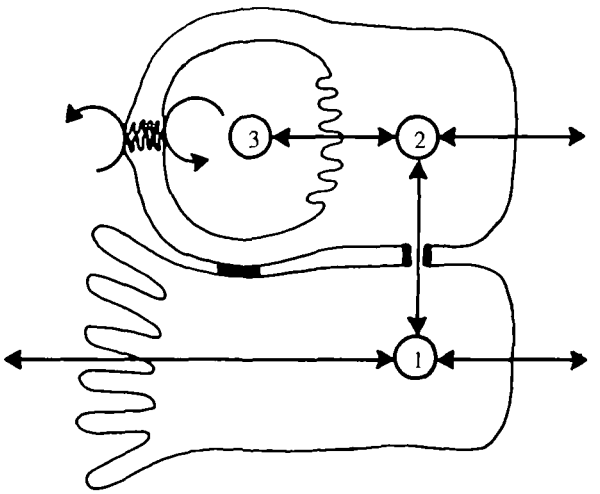

Fig. 6. Model used to explain entry and exit kinetics of TMA^+. The three compartments are 1, columnar cell cytoplasm; 2, goblet cell cytoplasm; and 3, goblet cavity; the arrows are paths of entry or exit. Each movement is taken as proportional to the net electrochemical difference between compartments. Neither entry nor exit is allowed through the goblet valve in this model.

goblet cell cytoplasm, for these cells seem to be locally coupled (Moffett *et al.* 1982). From there, we believe that it is accepted by the K^+/nH^+ antiporter. Amiloride-sensitive antiporters are relatively nonselective among alkali metal ions in a number of systems (Soleiman *et al.* 1991), and have been reported to accept NH_4^+ (Thomson *et al.* 1988). In the absence of Ca^{2+} and Mg^{2+} the midgut can actively transport all alkali metals and NH_4^+ (Harvey and Zerahn, 1972; Zerahn, 1971, 1977).

Do the valves ever open? In the isolated preparation, it appears that they never do. In

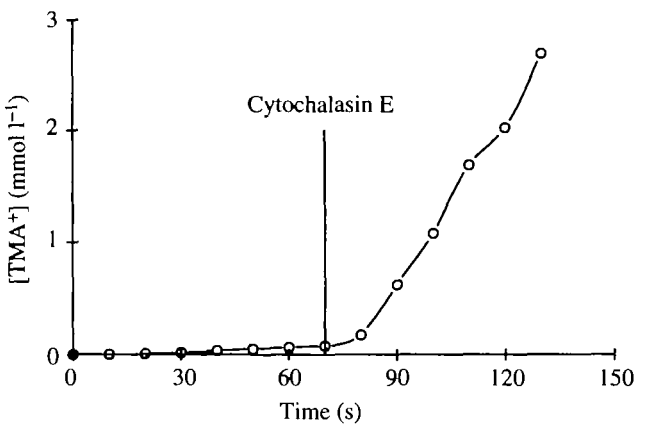

Fig. 7. Data from an experiment in which a goblet cavity was penetrated and TMA^+ influx started at time zero. At the vertical line, the superfusate was switched to one containing TMA^+ and cytochalasin E (10^{-4} mol l^{-1}).

more than 100 TMA^+ influx measurements, some lasting for more than 1 h, we never observed a sudden change in entry rate that might indicate a change between open and closed states. However, the valve is surrounded by a network of actin-containing microfilaments, as revealed by Rhodamine–Phalloidin binding (U. Klein, personal communication), suggesting that there is a cellular mechanism that regulates opening. To test this hypothesis, we applied cytochalasin E ($10^{-5}-10^{-4}$ mol l^{-1}), an inhibitor of actin-based cellular motility, during TMA^+ influx measurements. Cytochalasin E dramatically increased the rate of TMA^+ entry into cavities (Fig. 7). The effect was dose-dependent, with the higher concentration resulting in rates of TMA^+ uptake of the order of those expected for open valves. Such high rates were never observed in the absence of cytochalasin E.

Conclusions

The studies reviewed here have shown that the ionic driving forces across the goblet cell apical membrane of the intact posterior midgut goblet cell are consistent with energization of K^+ secretion by primary H^+ secretion, as long as the coupling between these processes is electrical. Furthermore, these studies suggest that the electrogenic H^+ pump itself is quite sensitive to the voltage across the GCAM, since increases in V_{gc} result in decreases in K^+ transport that are independent of changes in intracellular K^+ activity. These findings are consistent with the central aspect of the accepted model for midgut K^+ transport: that K^+ is actively transported across the GCAM by an H^+-coupled mechanism.

However, the accepted model, while remarkably robust, cannot be taken for granted. The following major questions remain open. (1) If K^+ is secreted into the goblet cavity, how does it get from goblet cavity to gut lumen? (2) Why does the secreted H^+ not leak out into the lumen? First, we can advance no hypothesis under which TMA^+ would absolutely fail to enter an 'open' valve. If this result is taken at face value, it means either that there is some mechanism other than free diffusion that permits K^+ to leave the goblet cavity no matter whether the valve is open or closed, or that on average more than half of the goblet cells simply do not contribute to I_{sc}. The latter possibility is difficult to accept, because it would place a heavy metabolic burden on the small number of active cells. Furthermore, in a large number of measurements of intracellular $[K^+]$ and V_{gc} we were not able to distinguish two populations of values that might correspond to cavities with open and closed valves.

Second, the failure of TMA^+ to pass directly from the gut lumen into goblet cavities and the apparent inability of H^+ to exit the goblet cavity to the lumen could be reconciled with the standard hypothesis if the goblet cavity matrix acted as an ion-exchange resin highly selective for K^+ over H^+ and TMA^+. A valve filled with such a matrix would be patent to K^+ but not 'open' to free diffusion of TMA^+. The chemistry responsible for this selectivity would have to be quite different from that of the synthetic ion-selective resins now available.

The authors' work, cited herein, was supported by USPHS GM 26287–01 and NSF DCB 8315739 and 8811354.

References

ANDERSON, E. AND HARVEY, W.R. (1966). Active transport in the *Cecropia* midgut. II. Fine structure of the midgut epithelium. *J. Cell Biol.* **31**,107–134.

BERRIDGE, M. J. (1977). Cyclic AMP, calcium and fluid secretion In *Transport of Ions and Water in Animals* (ed. B. L. Gupta, R. B. Moreton, J. L. Oschman, and B. J. Wall), pp. 225–238. New York, London: Academic Press.

CHAO, A. C., KOCH, A. R. AND MOFFETT, D. F. (1989). Active chloride transport in isolated posterior midgut of tobacco hornworm (*Manduca sexta*). *Am. J. Physiol.* **257**, R752–R761.

CHAO, A. C., KOCH, A. R. AND MOFFETT, D. F. (1990). Basal membrane uptake in potassium-secreting cells of midgut of tobacco hornworm (*Manduca sexta*). *Am. J. Physiol.* **258**, R112–R119.

CHAO, A. C., MOFFETT, D. F. AND KOCH, A. R. (1991). Cytoplasmic pH and goblet cavity pH in the posterior midgut of the tobacco hornworm (*Manduca sexta*). *J. exp. Biol.* **155**, 503–414.

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

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

DOW, J. A. T. (1984). Extremely high pH in biological systems: a model for carbonate transport. *Am. J. Physiol.* **246**, R633–R635.

DOW, J. A. T. (1986). Insect midgut function. *Adv. Insect Physiology* **19**, 187–328.

DOW, J. A. T., GUPTA, B. L., HALL, T. A. AND HARVEY, W. R. (1984). X-ray microanalysis of elements in frozen-hydrated sections of an electrogenic K^+ transport system: the posterior midgut of tobacco hornworm (*Manduca sexta*) *in vivo* and *in vitro*. *J. Membr. Biol.* **77**, 223–241.

DOW, J. A. T. AND PEACOCK, J. M. (1989). Microelectrode evidence for the electrical isolation of goblet cell cavities in *Manduca sexta* middle midgut. *J. exp. Biol.* **143**, 101–114.

FLOWER, N. E. AND FILSHIE, B. K. (1976). Goblet cell membrane differentiation in the midgut of a lepidopteran larva. *J. Cell Sci.* **20**, 357–375.

GUPTA, B. L., DOW, J. A. T., HALL, T. A. AND HARVEY, W. R. (1985). Electron microprobe analysis of the effects of *Bacillus thuringiensis* var *Kurstaki* delta-endotoxin on an electrogenic K^+ transport system, the midgut of the larval lepidopteran, *Manduca sexta*. *J. Cell Sci.* **74**, 137–152.

HARVEY, W. R. AND NEDERGAARD, S. (1964). Sodium-independent active transport of potassium in the isolated midgut of the *Cecropia* silkworm. *Proc. natn. Acad. Sci.* **51**, 757–765.

HARVEY, W. R. AND ZERAHN, K. (1972). Active transport of potassium and other alkali metals by isolated midgut of the silkworm. *Curr. Topics Membranes Transport* **3**, 367–410.

JUNGREIS, A. M. AND HARVEY, W. R. (1975). Role of active potassium transport by integumentary epithelium in secretion of larval–pupal molting fluid during silkworm development. *J. exp. Biol.* **62**, 357–366.

JUNGREIS, A. M. AND VAUGHN, G. L. (1977). Insensitivity of lepidopteran tissues to ouabain: absence of ouabain binding and Na^+-K^+ATPase in larval and adult midgut. *J. Insect Physiol.* **23**, 503–509.

KEYNES, R. D. (1973). Comparative aspects of transport through epithelia. In *Transport Mechanisms in Epithelia* (ed. H. H. Ussing and N. A. Thorn), pp. 505–511. New York, London: Academic Press.

KLEIN, U., LOEFFELMANN, G. AND WIECZOREK, H. (1991). The midgut as a model system for insect K^+-transporting epithelia: immunocytochemical localization of a vacuolar-type H^+ pump. *J. exp. Biol.* **161**, 61–75.

MADDRELL, S. H. P. (1977). Insect Malpighian tubules. In *Transport of Ions and Water in Animals* (ed. B. L. Gupta, R. B. Moreton, J. L. Oschman and B. J. Wall), pp. 541–569. New York, London: Academic Press.

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., HUDSON, R. L., MOFFETT, S. B. AND RIDGWAY, R. L. (1982). Intracellular K^+ activities and cell membrane potentials in a K^+-transporing epithelium, the midgut of tobacco hornworm (*Manduca sexta*). *J. Membr. Biol.* **70**, 59–68.

MOFFETT, D. F. AND KOCH, A. R. (1988*a*). Electrophysiology of K^+ transport by midgut epithelium of lepidopteran insect larvae. I. The transbasal electrochemical gradient. *J. exp. Biol.* **135**, 25–38.

MOFFETT, D. F. AND KOCH, A. R. (1988*b*). Electrophysiology of K^+ transport by midgut epithelium of lepidopteran insect larvae. II. The transapical electrochemical gradients. *J. exp. Biol.* **135**, 39–49.

MOFFETT, D. F. AND KOCH, A. (1991). Lidocaine and barium distinguish separate routes of transbasal K^+ uptake in the posterior midgut of the tobacco hornworm (*Manduca sexta*). *J. exp. Biol.* **157**, 243–256.

MOFFETT, D. F. AND LEWIS, S. A. (1990). Cation channels of insect midgut goblet cells: conductance diversity and Ba^{++} activation. *Biophys. J.* **57**, 85a.

REUSS, L. (1985). Changes in cell volume measured with an electrophysiologic technique. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6014–6018.

RHEUBEN, M. B. (1972). The resting potential of moth muscle fibre. *J. Physiol., Lond.* **225**, 529–554.

SCHULTZ, T.W., LOZANO, G. AND CAJINA-QUEZADA, M. (1981). Histochemical analysis of the goblet cell matrix in the larval midgut of *Manduca sexta*. *Trans. Am. microsc. Soc.* **100**, 204–209.

SMITH, D. S., COMPHER, K., JANNERS, M., LIPTON, C. AND WITTLE, L. W. (1969). Cellular organization and ferritin uptake in the mid-gut epithelium of a moth, *Ephestia kuhniella*. *J. Morph.* **127**, 41–72.

SOLEIMAN, M., LESOINE, G. A., BERGMAN, J. A. AND ARONSON, P. S. (1991). Cation specificity and modes of the Na^+:CO_3^{2-}:HCO_3^- cotransporter in renal basolateral membrane vesicles. *J. biol. Chem.* **266**, 8706–8710.

THOMSON, R. B., THOMSON, J. M. AND PHILLIPS, J. E. (1988). NH_4^+ transport in acid-secreting insect epithelium. *Am. J. Physiol.* **254**, R348–R356.

THURM, U. AND KÜPPERS, J. (1980). Epithelial physiology of insect sensilla. In *Insect Biology in the Future* (ed. M. Locke and D. S. Smith), pp. 734–764. New York: Academic Press

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.* A **148**, 303–311.

WIECZOREK, H., PUTZENLECHNER, M., ZEISKE, W. AND KLEIN, U. (1991). A vacuolar-type proton pump energizes K^+/nH^+ antiport in an animal plasma membrane. *J. biol. Chem.* **266**, 15340–15347.

WOOD, J. L. AND MORETON, R. B. (1978). Refinements in the short-circuiting technique and its application to active potasium transport across the cecropia midgut. *J. exp. Biol.* **77**, 123–140.

ZEISKE, W., VAN DRIESSCHE, W. AND ZIEGLER, R. (1986). Current–noise analysis of the basolateral route for K^+ ions across a K^+-secreting insect midgut epithelium (*Manduca sexta*). *Pflügers Arch.* **407**, 657–663.

ZERAHN, K. (1971). Active transport of the alkali metals by the isolated mid-gut of *Hyalophora cecropia*. *Phil. Trans. R. Soc. Lond. B* **262**, 315–321.

ZERAHN, K. (1977). Potassium transport in insect midgut. In *Transport of Ions and Water in Animals* (ed. B. L. Gupta, R. B. Moreton, J. L. Oschman and B. J. Wall), pp. 381–401. New York, London: Academic Press.