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INSECT MALPIGHIAN TUBULES: V-ATPase ACTION IN ION AND FLUID TRANSPORT

BY S. H. P. MADDRELL[1] AND M. J. O'DONNELL[2]

[1]*Department of Zoology, Downing Street, Cambridge CB2 3EJ, UK* and [2]*Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1*

Summary

Insect Malpighian tubules secrete fluid into the lumen as part of their function as excretory organs. The underlying ion transport is, when stimulated, faster than in any other known tissue. It is driven by the activity of an H^+-transporting V-ATPase situated on the luminal cell membranes. This ATPase, together with cation/H^+ antiporter(s), constitutes a common cation pump which can transport sodium ions, potassium ions or both. Treatments that selectively slow cation transport across the epithelium cause the secreted fluid to become alkaline, whereas those that selectively reduce the rate of anion passage lead to secretion of acid fluid.

Introduction

The Malpighian tubules of insects are remarkable transporting tissues. Although insects are very small compared with other terrestrial animals and, so, one would think, very careful to conserve water, some Malpighian tubules, when stimulated, can transport water and ions at rates that, gram for gram, are higher than those of any other known tissues. Each cell in the fluid-secreting portions of the tubules in the blood-sucking insect *Rhodnius prolixus* can transport $3\,pmol\,s^{-1}$ of ions (or $2\times10^{12}\,ions\,s^{-1}$) and their own volume of fluid every 15 s (Maddrell, 1991). They are stimulated to do this by the appearance in the insect's haemolymph of two hormones, a peptide and 5-hydroxytryptamine (5-HT), which act synergistically to accelerate fluid secretion by more than a thousand times (Maddrell *et al.* 1992). Such very fast fluid secretion depends critically on the activity of a V-ATPase located on the luminal cell membrane. There the V-ATPase is easily accessible and the fluid secreted as a consequence of its activity can be collected directly.

Insect Malpighian tubules are epithelia, one cell thick, whose basic function is to carry out ion and fluid transport in the formation of an iso-osmotic primary excretory fluid at the upstream end of the excretory system. As indicated in Fig. 1, the cells also transport into the lumen a variety of organic compounds, such as uric acid (O'Donnell *et al.* 1983), acylamides (Maddrell *et al.* 1974), alkaloids (Maddrell and Gardiner, 1976) and cardiac glycosides (Rafaeli-Bernstein and Mordue, 1978), as well as inorganic ions, such as phosphate, Mg^{2+} and SO_4^{2-} (Maddrell, 1978). The final element in the formation of the presumptive urine is that the cells allow passive diffusive entry of solutes from the

Key words: V-ATPase, insect Malpighian tubules, *Rhodnius prolixus*, ion transport, fluid transport, amiloride, furosemide, cyclic AMP, 5-HT.

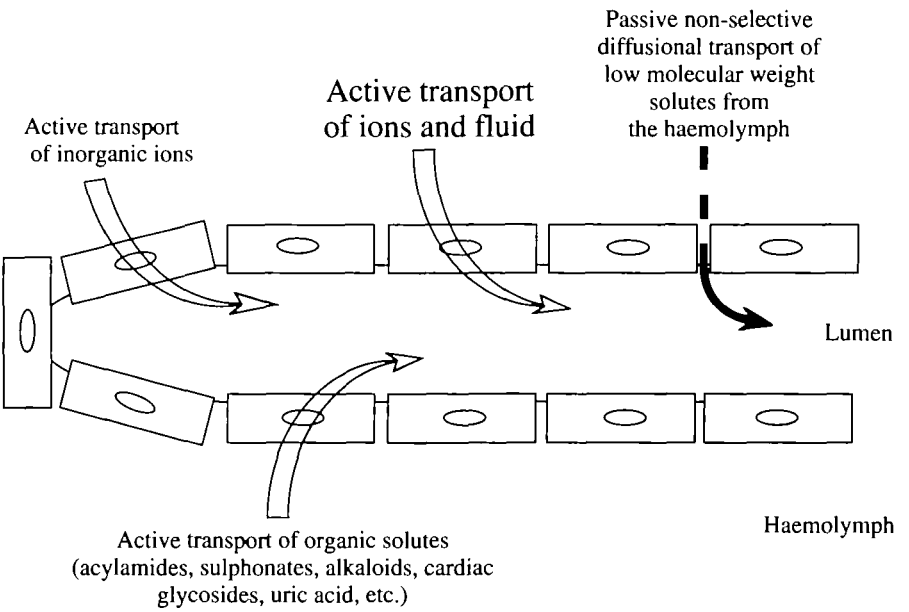

Fig. 1. The various transport processes, active and passive, involved in the operation of insect Malpighian tubules.

surrounding haemolymph into the lumen paracellularly through the cell–cell septate junctions (Fig. 1; Skaer *et al.* 1987). This latter process ensures fail-safe automatic excretion of novel toxins that the insect might encounter in its diet (Ramsay, 1958). Reabsorption of water, ions, sugars, amino acids and other useful substances from the primary excretory fluid occurs lower down the system, to some extent in the anterior hindgut, but more importantly in the rectum. The end result of Malpighian tubule activity is in many ways like that of vertebrate glomeruli, although the mechanism is very different.

The basic functioning of the tubules depends on the transport of fluid into the lumen and this, in turn, requires the active transport of ions, particularly K^+, into the lumen. Our knowledge of how K^+ secretion is achieved has undergone a revolution following the discovery of an H^+-pumping V-ATPase in the luminal cell membranes of Malpighian tubules. The account below first reviews the models used previously to describe the ionic transport processes underlying fluid transport. It goes on to re-interpret them in the light of the new findings. It then describes new experiments which show how the tubules can be induced to secrete fluid of different pH.

Earlier models of ion transport involved in fluid secretion by insect Malpighian tubules

Until recently, ion transport responsible for fluid secretion by insect Malpighian tubules was thought to be rather well understood. Essentially, the significant elements were seen (Fig. 2A) as (1) active transport into the lumen from the cell of sodium ions,

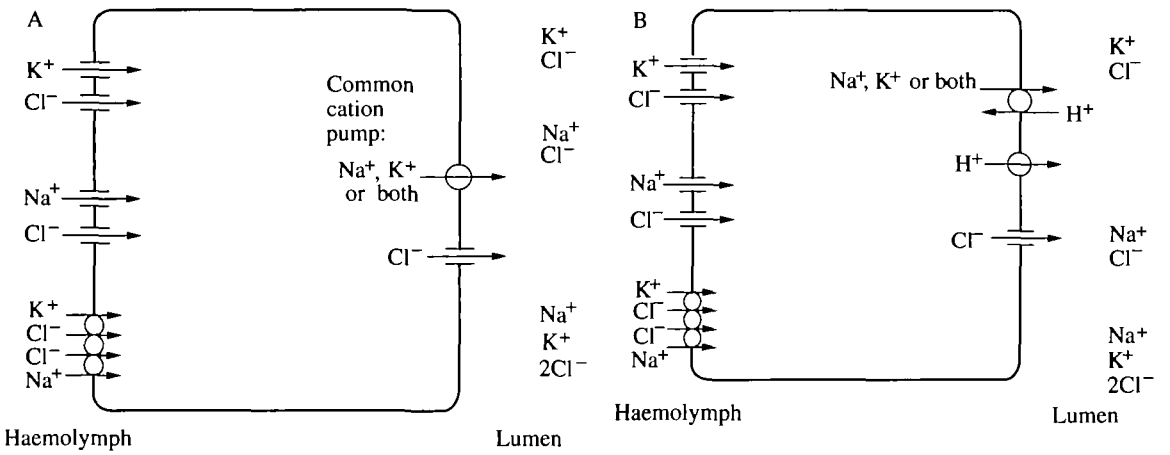

Fig. 2. (A) Model for ion movements underlying fluid secretion by insect Malpighian tubules prior to the discovery that an H^+-pumping V-ATPase was involved. The transport processes shown on the haemolymph-facing membrane (to the left) show successively, from top to bottom, the different ways that ions enter the cell in, respectively, tubules that transport mainly potassium and chloride ions into the lumen, tubules that transport mainly sodium and chloride ions into the lumen and tubules that transport sodium, potassium and chloride ions into the lumen. (B) Model for ion movements underlying fluid secretion by insect Malpighian tubules incorporating an H^+-pumping V-ATPase on the luminal cell membrane. The transport processes shown on the haemolymph-facing membrane are the same as in A.

potassium ions or both by a common cation pump (Maddrell, 1978) sited in the apical microvilli of the plasma membrane; (2) an accompanying movement of chloride ions into the lumen down their electrochemical gradient through chloride-permeable channels; (3) the entry into the cell from the haemolymph of sodium, potassium and chloride ions through channels or transporters selective for these ions at relative rates characteristic of the particular insect and of the state of stimulation of the tubule. The ratio of Na^+ to K^+ in the secreted fluid was thought to be dictated by the entry step 3. Insects vary very much in this respect, some tubules producing almost exclusively a KCl solution and others secreting almost exclusively a NaCl solution. Cases are known in which the tubule carried out relatively fast active transport of anions, such as hippurates or phosphate, to some extent replacing chloride ions in the secreted fluid (Berridge, 1969).

A significant feature of the model shown in Fig. 2A is the omission of the ubiquitous Na^+/K^+-ATPase. In fact, this ATPase does occur in Malpighian tubules, but only in a few cases do its activities affect fluid secretion in any significant way (Anstee and Bell, 1975; Maddrell and Overton, 1988).

Just how the transport of potassium, sodium and chloride ions into the lumen results in accompanying water movements sufficient to produce fluid that is iso-osmotic or slightly hyperosmotic has not been fully worked out, although it is widely assumed that osmotic coupling of ion and water movements is responsible. The extensive membrane elaborations of the basal and apical cell surfaces (O'Donnell *et al.* 1985) are thought to provide the large area required for osmotic water movements. However, there are

objections to this proposal (see, for example, Hill, 1975) that question whether the osmotic permeability of the tubule cell membranes is high enough for sufficiently effective osmosis. Measurements of the osmotic permeability of the cell membranes in the tubules of *Rhodnius prolixus* showed them to have a high osmotic permeability of $4\,cm\,s^{-1}\,(osmol\,l^{-1})^{-1}$ (O'Donnell *et al.* 1982). When this result was incorporated in a mathematial analysis of the system, the results showed that osmosis is sufficient to account for fluid secretion in this particular case (McElwain, 1984). However, other ways of coupling ion and water movements could not be ruled out.

Evidence that V-ATPase energizes the system

Several lines of evidence suggest that V-ATPase is centrally involved in the formation of fluid by Malpighian tubules. In some insects, inhibitors of V-ATPase such as bafilomycin A_1, *N*-ethylmaleimide (NEM) and 4-chloro-7-nitrobenzo-2-oxa-1,3-diazol (NBDCl) also inhibit fluid secretion. Thus, bafilomycin A_1 inhibits fluid secretion by tubules of *Drosophila hydei* (Bertram *et al.* 1991), bafilomycin A_1 and NEM slow urine production by tubules of the ant *Formica polyctena* (Weltens *et al.* 1992), and fluid secretion by tubules of fifth-instar *Rhodnius prolixus* is much slowed by treatment with NBDCl (S. H. P. Maddrell, unpublished observations).

If V-ATPase pumps protons into the lumen, whence they return to the cell cytoplasm *via* cation/H^+ antiporters, then inhibitors of antiporters should interfere with fluid transport. Amiloride inhibits fluid transport by Malpighian tubules of *Drosophila hydei* (Bertram, 1989) and by the tubules of *Rhodnius* (see Fig. 6).

As described above, active cation transport is believed to be sited at the apical microvilli of the plasma membrane. It has now been shown that antibodies to V-ATPase selectively bind to the apical plasma membrane of the Malpighian tubules of *Manduca sexta* (Russell *et al.* 1992).

Reinterpretation of models of action of Malpighian tubules

Replacement of the common cation pump

Perhaps surprisingly, the discovery that the H^+-pumping V-ATPase almost certainly provides the driving force for fluid transport by insect Malpighian tubules has rather few implications for the re-interpretation of tubule action. The revised model is shown in Fig. 2B. Essentially, what was originally termed a common cation pump (Maddrell, 1978) is now seen to be a complex of an H^+-pumping V-ATPase together with a cation/H^+ antiporter(s) that produces effectively the same result, namely the transport of Na^+, K^+ or both into the lumen from the cytoplasm. As before, what is exported into the lumen depends on what comes in across the basal membrane. The major repercussions lie in understanding the results of experiments designed to slow the movements of sodium and/or potassium ions on the one hand and of chloride ions on the other. As can be seen from Fig. 2B, slowing cation transport would be expected to make the secreted fluid acid, as relatively faster movement of chloride ions into the lumen will reduce the potential gradient driving the cation/H^+ antiporter(s); in other words, H^+ and Cl^- will tend to

accumulate in the secreted fluid. In a similar way, slowing chloride ion movement into the lumen produces a large lumen-positive potential (O'Donnell and Maddrell, 1984), which will favour lumen–cell movement of H^+, or cell–lumen movement of OH^-, either of which will result in a more alkaline secreted fluid. Some experiments on these lines are described below (see Figs 5 and 6).

The ability of Malpighian tubules to transport sodium ions or potassium ions or both

Responding to their particular physiological needs, Malpighian tubules of different insects are known to be able to secrete fluid containing, as its major ions, potassium and chloride ions, sodium and chloride ions, or sodium, potassium and chloride ions, In addition, the tubules of some insects can vary the relative proportions of sodium and potassium ions in the fluid they secrete. Most commonly, in herbivorous insects, it is K^+ that is the major transported cation, for example, in *Carausius morosus* (Ramsay, 1953) and in *Calliphora erythrocephala* (Berridge, 1969); many other species are similar in this respect. Bloodsucking insects that have recently fed are faced, initially at least, with surplus sodium and chloride ions from the plasma of the diet. The Malpighian tubules of the tsetse fly *Glossina morsitans* (Gee, 1976) and the mosquito *Aedes egypti* (Williams and Beyenbach, 1983) can both carry out rapid transport of fluid rich in sodium and chloride ions. Unusually, the fluid-secreting parts of the Malpighian tubules of the bloodsucking hemipteran *Rhodnius prolixus*, when stimulated, elaborate fluid rich in both sodium and potassium ions (Maddrell, 1969). The recovery of virtually all potassium ions, together with chloride ions in the lower tubules (Maddrell and Phillips, 1975), allows the insect to eliminate a hypo-osmotic urine, rich in sodium and chloride ions, and containing only very low levels of potassium ions. This selective reabsorption caters exactly for its need to remove surplus sodium and chloride ions together with excess water from its diet, which is hypo-osmotic to the insect's body fluids. When not stimulated to secrete fluid at the very high rates so characteristic of its post-prandial diuresis, *Rhodnius* Malpighian tubules secrete a K^+-rich fluid containing only low levels of sodium ions (Ramsay, 1952; Maddrell, 1980).

It is thus a particular characteristic of insect Malpighian tubules that they can secrete fluid containing sodium and potassium ions in almost any proportion. How is this achieved?

Until recently, this ability was seen as requiring two elements; selective entry of Na^+, K^+ or both across the basolateral cell plasma membranes followed by transfer into the lumen by an apically sited common cation pump. The apical pump was thought to be selective for sodium ions by virtue of higher affinity for them than potassium ions. This selectivity, in turn, automatically provided for the maintenance of the cell interior as a K^+-rich, Na^+-poor environment, catered for in most other cells by the basolateral Na^+/K^+-ATPase. This mechanism explained how it is that the function of most Malpighian tubules is not affected by treatment with ouabain, which so effectively inhibits the Na^+/K^+-ATPase. Only in cases where the apical common cation pump is operating at very slow rates are effects of ouabain seen, most remarkably, for example, in the finding

that ouabain action on unstimulated tubules of *Rhodnius* causes *acceleration* of fluid and Na^+ transport into the lumen (Maddrell and Overton, 1988).

Given that the apical common cation pump would maintain the internal cell milieu at particular activities of potassium and sodium ions, with potassium as the dominant cell cation, any ions entering across the basolateral membrane would be removed apically. So, if, for example, these latter were potassium ions (together with chloride ions) the tubules would secrete essentially an iso-osmotic KCl solution.

We now know that the common cation pump does not exist as a single entity, but consists of an H^+-pumping V-ATPase with cation/H^+ antiporter(s) (Wieczorek, 1992). How might this provide appropriate cation transport into the lumen and still act so as to maintain the intracellular environment? The properties ascribed to the original common cation pump now have to be fulfilled by the antiporter or antiporters. The simplest solution would be a common cation/H^+ antiporter able to handle both sodium and potassium ions but with a higher affinity for sodium than for potassium ions. Alternatively, the apical cell membrane could be equipped with two types of antiporter, one a Na^+/H^+ antiporter, the other a K^+/H^+ exchanger. To maintain the cell interior K^+-rich, the effectiveness of the Na^+/H^+ antiporter would have to be greater than that of the K^+/H^+ one, presumably by virtue of a higher turnover rate, by greater affinity for sodium ions or by its presence at higher density in the luminal cell membrane. We describe below some experiments with amiloride which attempt to distinguish between these two possible ways of achieving Na^+-selectivity in transferring cations to the lumen.

Changes in pH of fluid secreted by *Rhodnius*

It follows from the idea that the motive power for tubule fluid secretion is to be thought of as provided by a V-ATPase pumping protons, that some treatments might well result in changes in pH of the secreted fluid. Unlike other systems, in Malpighian tubules one has easy access to the fluid transported by the activity of the V-ATPase. We now describe our recent experiments with tubules from *Rhodnius*, in which the secreted fluid can be forced by one set of treatments to become alkaline or driven by different treatments to become acid. Loosely speaking, we can make Malpighian tubules act like the lepidopteran midgut or as an acid-secreting system.

As a preliminary, we describe the results of experiments to measure the pH of fluid secreted by Malpighian tubules under control conditions or in bathing fluids of different pH. We used pH-selective microelectrodes based on the H^+ ionophore II Cocktail A (Fluka Chemicals). For measurements of potassium ion activity, the microelectrodes contained K^+ ionophore I Cocktail B (Fluka Chemicals). In each case, a reference microelectrode as well as the ion-selective microelectrode were positioned in a droplet of secreted fluid or of calibration solution under liquid paraffin.

Constancy of pH of fluid secreted by tubules bathed in standard saline at a range of pH values

We measured the pH of fluid secreted by tubules from fifth-instar *Rhodnius* under

conditions of maximum stimulation (10^{-6} mol l^{-1} 5-HT or 10^{-3} mol l^{-1} cyclic AMP; Maddrell *et al.* 1971, 1991) in saline whose ionic composition was that of standard saline but whose pH was adjusted with HCl or NaOH. The results (Fig. 3) showed that the tubules secreted fluid of near neutral pH and this was not affected by the pH of the bathing fluid. This is likely to reflect an ability of Malpighian tubule cells to regulate the intracellular pH in the face of quite large changes in the pH of the extracellular medium.

Treatment that forces *Rhodnius* Malpighian tubules to secrete alkaline fluid

We treated tubules from fifth-instar *Rhodnius* with 10^{-3} mol l^{-1} cyclic AMP as a stimulant, but also with furosemide at 4×10^{-5} mol l^{-1} to slow secretion to a low level (O'Donnell and Maddrell, 1984).

Ten tubules treated in this way secreted fluid at an average rate of 1.47±0.15 nl min^{-1} (in this and subsequent cases the data are recorded as the mean ± S.E.) compared with an expected rate of 60–80 nl min^{-1} for tubules bathed in saline containing stimulant alone. Under these conditions, the secreted fluid was distinctly alkaline, pH 7.69±0.09 (N=10), with an extreme of pH of more than 8.00 in two cases, compared with a pH of 6.93±0.04 (N=10) in 10^{-3} mol l^{-1} cyclic AMP alone. Furosemide slows coupled basal entry of sodium, potassium and chloride ions through the cotransporter. Perhaps because the chloride concentration in stimulated cells is only half that of cations (Gupta *et al.* 1976), the slowing of chloride entry has the greater effect and the luminal potential becomes very large (O'Donnell and Maddrell, 1984) as the action of the common cation pump is not balanced by chloride movements into the lumen. This will tend to make the secreted fluid more alkaline, as discussed above.

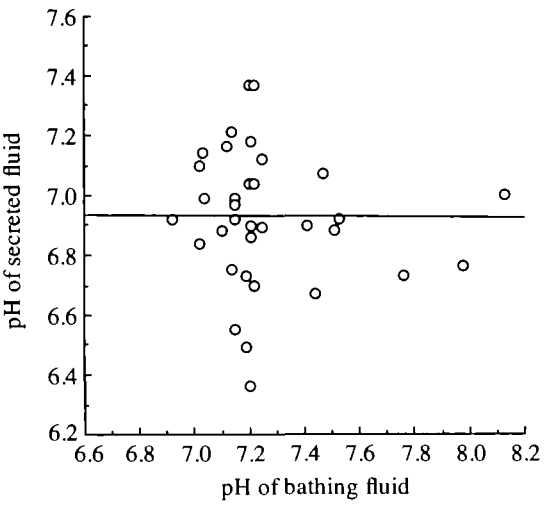

Fig. 3. pH of fluid secreted by *Rhodnius* Malpighian tubules bathed in fluids of different pH. The line through the points is the calculated linear regression line. y=6.96–3.94e^{-3}x, r^2=0.00, N=33.

Treatments that cause *Rhodnius* tubules to secrete fluid of low pH

Amiloride is thought to be an inhibitor of Na^+/H^+ exchangers (Kleyman and Cragoe, 1988). Its use on *Rhodnius* tubules might, on the basis of our working model, be expected to lower the pH of the secreted fluid. We treated isolated tubules with 10^{-6} mol l^{-1} 5-HT and 0.5 or 1.0 mmol l^{-1} amiloride. This treatment depressed the stimulated rate of fluid secretion to low levels (an average of 1.25±0.18 nl min^{-1}; N=11) and caused the pH of the secreted fluid to become acid, by about 1 pH unit (on average to a pH of 5.93±0.09; N=11).

Even lower pH values are seen in the fluid secreted by tubules stimulated to secrete by threshold concentrations of 5-HT (1.25×10^{-8} mol l^{-1}) or of cyclic AMP (5×10^{-5} mol l^{-1}). Such tubules secreted fluid at 1.85±0.21 nl min^{-1}, and the pH of the fluid secreted was 5.84±0.05 (N=27), with three samples having a pH below 5.50. Presumably, under these conditions, chloride passage through the tubule wall (*via* the basal cotransporter and apical anion channel) is more effectively stimulated than is cation transport. As discussed earlier, this will make the secreted fluid acid.

That low pH values are not inevitably found in tubules secreting at a low rate was shown by exposing, in three different experiments, two tubules from an insect to 10^{-3} mol l^{-1} cyclic AMP and 4×10^{-5} mol l^{-1} furosemide and the other two tubules to 5×10^{-5} mol l^{-1} cyclic AMP. All the tubules secreted fluid at about 1–2 nl min^{-1}, but the fluid secreted by the furosemide-treated tubules had a pH close to 2 units higher than fluid from the tubules treated with the low concentration of cyclic AMP (Fig. 4).

Sensitivity of transepithelial transport of fluid and sodium ions to luminal fluid composition

Although covering the surface of relatively long microvilli, the apical membrane that

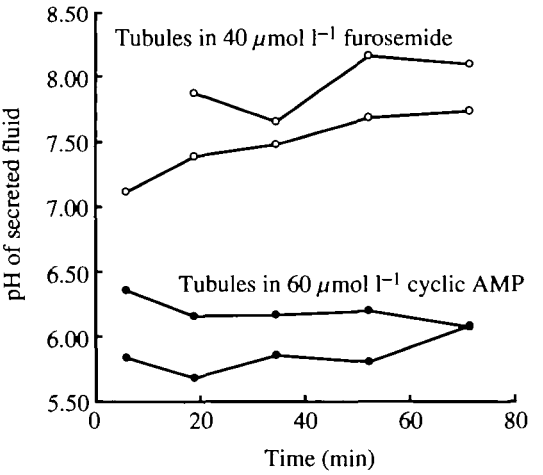

Fig. 4. pH of fluid secreted by Malpighian tubules of *Rhodnius* bathed either in cyclic AMP with furosemide (above) or in a threshold concentration of cyclic AMP alone (below).

carries the V-ATPase is rapidly accessible from the lumen. Mathematical analysis shows that diffusion is so rapid that the conditions in the bulk fluid in the lumen will not be significantly different from those in contact even with those areas of the apical microvillar membrane farthest from the lumen (McElwain, 1984).

We have found that perfusion of fluid along the lumen from a cannula introduced into it can cause the prompt cessation of movements of fluid and ^{22}Na from the bathing fluid into the lumen. The perfused fluid was chosen to be as similar in composition as possible to that naturally occurring in the lumen. It contained, in mmol l^{-1}: NaCl, 94.5; KCl, 83.5; $CaCl_2$, 0.1; $MgCl_2$, 0.2; $NaHCO_3$, 10.2; NaH_2PO_4, 4.3; glucose, 5; and the pH of the solution was 6.7. Four lengths of tubule were cannulated and stimulated with 10^{-6} mol l^{-1} 5-HT. All secreted fluid at normal rates initially but, during subsequent perfusion of fluid through the lumen, the rate of fluid emergence declined, indicating that transepithelial fluid transport was much depressed (Fig. 5A). When perfusion ceased, fluid transport did not recover. ^{22}Na transport into the lumen also rapidly collapsed as perfusion began; it, too, did not recover (Fig. 5B). Similar experiments were performed with the perfusing fluid altered so as to reduce its buffering capacity by reducing the concentrations of $NaHCO_3$ and NaH_2PO_4 to 1.0 and 0.4 mmol l^{-1}, respectively. In four cases, this lowered buffering capacity had the same effect as before, namely that the rates of transepithelial transport of fluid and ^{22}Na were strongly inhibited by perfusion of fluid through the lumen. Increasing the buffering capacity of the perfused fluid by increasing the levels of $NaHCO_3$ and NaH_2PO_4 to 30 and 12 mmol l^{-1}, respectively, still caused failure of transepithelial fluid and ^{22}Na transport (four cases).

These results show that the transport processes on the apical membrane are very sensitive to the conditions in the lumen. What the essential elements in the luminal fluid are will become clearer when we find a luminal perfusing fluid which allows normal

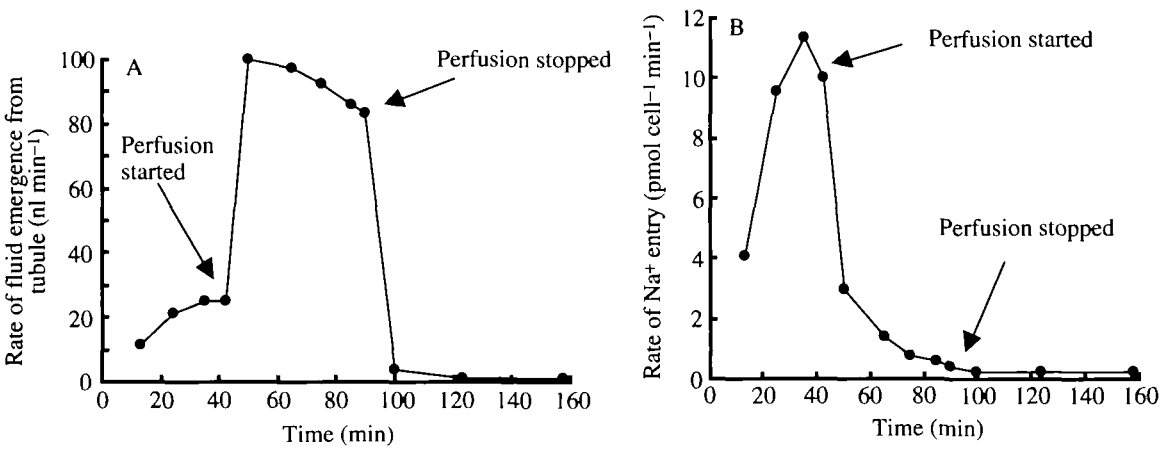

Fig. 5. (A) The rate of fluid emergence from the cut end of a cannulated and perfused Malpighian tubule from *Rhodnius* before, during and after the perfusion of fluid down the lumen from the cannula. (B) The rate of movement of radioactive sodium ions across the wall of a cannulated and perfused Malpighian tubule from *Rhodnius* before, during and after the perfusion of fluid down the lumen from the cannula.

rates of transepithelial ion and fluid transport. For the moment, our findings suggest that the results of experiments involving perfusion of fluid down the lumen of insect Malpighian tubules (Isaacson and Nicolson, 1989; Aneshansley *et al.* 1989) should be interpreted with caution as such perfusion may have profound effects on tubule function.

Effects of amiloride on potassium concentration and rate of fluid secretion

We have argued above that cations reach the lumen in the secreted fluid by involvement either with an antiporter able to exchange both Na^+ and K^+ for H^+, but which has a preference for sodium ions, or with two antiporters, one a Na^+/H^+ antiporter and the other a K^+/H^+ antiporter. If two antiporters are involved, then treatment with amiloride might be revealing, because it might be expected not to affect the two antiporters equally. We measured the potassium concentration in the fluid secreted by 11 tubules exposed to 1 mmol l^{-1} amiloride in standard saline with 10^{-6} mmol l^{-1} 5-HT. Fluid secretion was slowed dramatically from 50±3 nl min^{-1} before addition of amiloride to 1.04± 0.10 nl min^{-1} 30 min after amiloride addition. The potassium concentration in the secreted fluid was 83.0±4.3 mmol l^{-1} before amiloride treatment and 73.2±3.2 mmol l^{-1} 30 min later. In four cases, we followed the potassium concentration in the secreted fluid during the period immediately after exposure to amiloride, when fluid secretion was slowing under its effects; in no case were there significant changes in potassium concentration of the secreted fluid. On the face of it, this result favours the idea that only one antiporter is involved in *Rhodnius* tubules, one that can exchange either sodium or potassium for H^+. However, since sodium and potassium ions enter the cell *via* the $Na^+/K^+/2Cl^-$ cotransporter, they enter at the same rate. So, except in the very short term, they cannot leave at anything other than the same rate. Differential effects of amiloride on two different antiporters could only show up in the secreted fluid to the extent that the levels of ions in the tubule cells would support differential rates of removal from the cells into the lumen. Since the cell volume is not large, changes in the composition of the secreted fluid would be only short-lived. As we have seen, no such changes were observed, so that the evidence, as far as it goes, does favour the idea of a single antiporter.

We have shown that amiloride is a very effective inhibitor of fluid secretion by Malpighian tubules stimulated to secrete by 10^{-6} mol l^{-1} 5-HT. Surprisingly, we have found that amiloride is virtually without effect when the tubules have been stimulated by cyclic AMP.

Fig. 6 shows the results of an experiment to compare the effects of amiloride on tubules stimulated either by cyclic AMP or by 5-HT; 0.2 mmol l^{-1} amiloride reduced fluid secretion by tubules in the presence of 10^{-6} mol l^{-1} 5-HT to less than 1 nl min^{-1} (Fig. 6A), whereas, even at 2.0 mmol l^{-1}, amiloride had almost no effect on the rate of fluid secretion in the presence of 0.4 mmol l^{-1} cyclic AMP (Fig. 6B). Addition of cyclic AMP to tubules inhibited by amiloride in the presence of 5-HT restored high rates of fluid secretion, even when the amiloride concentration was increased at the same time (Fig. 7).

Discussion

The discovery that Malpighian tubules contain, on their luminal cell membranes, an H^+-transporting V-ATPase that powers ion transport has two sorts of consequence. First, it allows one to interpret the changes in pH that one sees following various sorts of interference with a tubule. It explains, for example, why slowing cation passage through

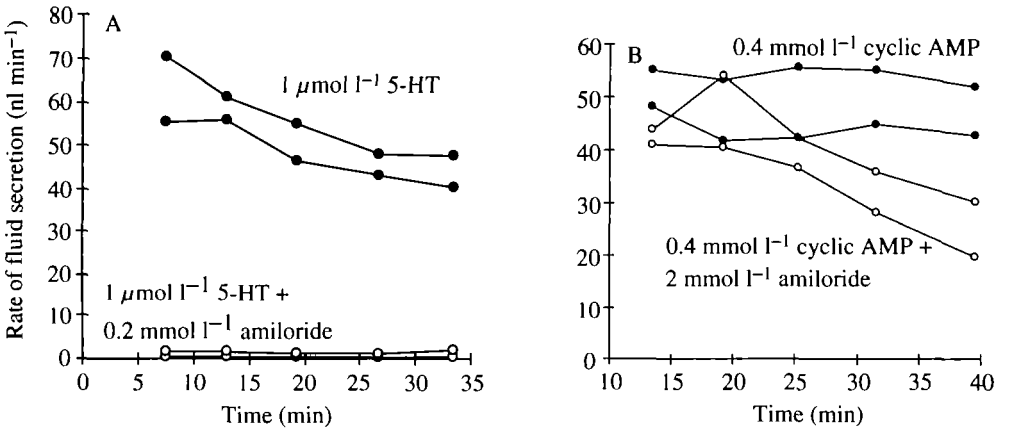

Fig. 6. (A) The rate of fluid secretion by two isolated Malpighian tubules of *Rhodnius* stimulated by 5-HT alone (control tubules, filled circles) or treated in addition with amiloride (open circles). (B) The rate of fluid secretion by isolated Malpighian tubules of *Rhodnius* stimulated by cyclic AMP alone (control tubules, filled circles) or treated in addition with amiloride (open circles).

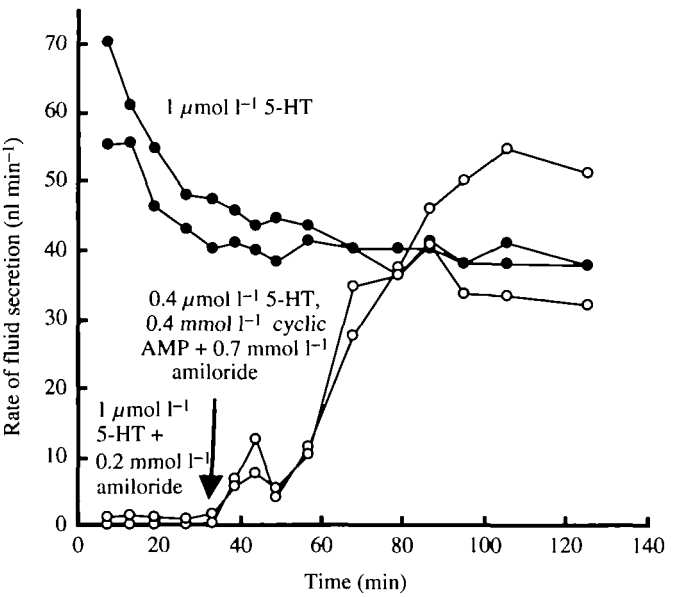

Fig. 7. The effect of adding cyclic AMP on the rates of fluid secretion by two tubules that had been inhibited by treatment with amiloride (open circles). The rates of fluid secretion by two control tubules are shown (filled circles).

the wall of a tubule from *Rhodnius* by treatment with amiloride causes the fluid secreted by the tubule to become acid. It would not have been possible to understand this from the models of tubule action extant before the involvement of an H^+-transporting V-ATPase became clear. Second, it raises new questions about how the complex of V-ATPase and cation/H^+ antiporter(s) can maintain the cell interior as a K^+-rich, Na^+-poor environment. To answer these will require knowledge of the properties of the antiporters found in different Malpighian tubules.

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CHAPTER 9. F-ATPases and genetically related pumps