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STIMULATION OF SODIUM TRANSPORT AND FLUID SECRETION BY OUABAIN IN AN INSECT MALPIGHIAN TUBULE

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Summary

Ouabain, at all concentrations higher than 2×10^{-7} mol l^{-1}, stimulates the rate at which the Malpighian tubules of the insect, *Rhodnius*, transport sodium ions and fluid into the lumen. An effect on paracellular movement of sodium ions is unlikely because ouabain makes the electrical potential of the lumen more positive, which would slow diffusion of sodium into the lumen. Radioactive ouabain binds to the haemolymph-facing sides of the tubule cells but not to the luminal face. This binding is reduced in the presence of elevated levels of potassium or of non-radioactive ouabain. Bound ouabain is only slowly released on washing in ouabain-free saline. The evidence suggests that there is a Na^+/K^+-ATPase on the outer (serosal) membranes of the tubules. Such a pump would transport sodium in a direction opposed to the flow of ions and water involved in fluid transport; poisoning it with ouabain would remove this brake, and fluid flow and sodium transport would increase, as observed.

Introduction

The cardiac glycoside, ouabain, at concentrations higher than about 10^{-6} mol l^{-1}, is a rather specific inhibitor of membrane-bound Na^+/K^+-ATPase (Glynn, 1964). Applied to many salt- and fluid-transporting epithelia of both vertebrates and insects, it causes strong inhibition of sodium transport and of fluid movements (Ussing, 1960; Reuss, Bello-Reuss & Grady, 1979; Farmer, Maddrell & Spring, 1981). The present paper reports an unusual *stimulation* by ouabain of both sodium transport and fluid secretion by an epithelium, the Malpighian tubules of an insect.

Materials and methods

The insects used for our experiments were fifth-stage instars, taken 1–2 weeks after the moult from the fourth-stage instar, of the blood-sucking insect, *Rhodnius prolixus*, from a laboratory culture maintained at 27°C.

In vitro preparations of the upper, fluid-secreting parts of the Malpighian tubules of *Rhodnius* were made (Maddrell, 1969), in which each tubule was

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immersed in a drop of saline (see below) under liquid paraffin (mineral oil), and the cut end of the tubule pulled out and hitched round a glass rod stuck in the wax base of the container. Fluid secreted by the tubule was then collected from a cut made in the wall of that part of the tubule running between the bathing drop and the glass rod. For experiments on sodium transport, the tubules were bathed with saline containing ^{22}Na with test drops containing, in addition, ouabain at concentrations in the range 10^{-8} to 10^{-4} mol l^{-1}. The rates of fluid secretion by the tubules were measured during the first 4 h. Samples of the fluid secreted during the third and fourth hours were assayed for their sodium content. Fluid secreted during the first 2 h was not sampled for radioactive sodium because, at the slow rates of secretion by these unstimulated tubules (Maddrell, Pilcher & Gardiner, 1971), it takes about 100 min to wash out the non-radioactive fluid initially in the lumen.

The saline solution used had the following composition (mmol l^{-1}): NaCl, 142; KCl, 8·6; $CaCl_2$, 2·0; $MgCl_2$, 8·5; Hepes, 8·6; glucose, 34. The pH of the saline was adjusted to 7·0 with 1 mol l^{-1} NaOH (about 2·9 ml l^{-1}).

For ouabain binding studies, we used ^{3}H-labelled ouabain from Amersham International. In these experiments, tubules were exposed for varying times to saline containing radioactive ouabain at a final concentration of 1 μmol l^{-1}. The tubules were then washed in ouabain-free saline to remove surface contamination from radioactive saline. After they had each been immersed in a small drop of distilled water for 1 min to disrupt the tubule by osmotic shock, the tubule and the drop together were assayed for their radioactive content by placing them in scintillation fluid (NE 270; Nuclear Enterprises Limited) and counting them in a Packard Tricarb 3255 liquid scintillation counter.

To perfuse fluid through the lumen of a tubule, the technique used was that of Maddrell & Phillips (1975). In this, a fine cannula (50 μm tip diameter) is pushed through the wall of a tubule held in forceps and fluid driven through it from a motor-driven syringe. The rate of fluid perfusion was 100 nl min^{-1}.

Measurements of potential difference across the wall of Malpighian tubules were made by putting one electrode in the drop of bathing saline and another in a small drop of saline placed where fluid emerges from the cut in the tubule wall. Each electrode was of glass, 0·1 mm in diameter at the tip, filled with 2·5 % agar in saline. Each was connected to a calomel half-cell by an agar–KCl bridge. The potential difference was measured with a Keithley Electrometer model 600B and recorded on a Gould BS-272 chart recorder.

All values are expressed as the mean ± S.E.M. (N = number of observations). All experiments were carried out at room temperature, 20–24 °C.

Results

The effects of ouabain on fluid secretion and sodium transport

The rates of fluid secretion by tubules treated with all concentrations of ouabain above $2·5 \times 10^{-7}$ mol l^{-1} were twice as high as in control tubules. Ouabain-treated

tubules secreted fluid at $1{\cdot}89 \pm 0{\cdot}07\,\text{nl}\,\text{min}^{-1}$ (mean ± S.E.; $N = 36$), whereas control tubules taken from the same insects secreted fluid at $0{\cdot}95 \pm 0{\cdot}05\,\text{nl}\,\text{min}^{-1}$ ($N = 35$). Ouabain treatment also raised the concentration of sodium in the secreted fluid from $56{\cdot}3 \pm 2{\cdot}7\,\text{mmol}\,\text{l}^{-1}$ ($N = 15$) in control tubules to $117{\cdot}9 \pm 3{\cdot}7\,\text{mmol}\,\text{l}^{-1}$ ($N = 15$) in tubules in ouabain-containing saline. The rate of sodium transport was thus about four times higher in ouabain-treated tubules ($264{\cdot}8 \pm 21{\cdot}4\,\text{pmol}\,\text{min}^{-1}$, $N = 15$) than in control tubules ($61{\cdot}8 \pm 4{\cdot}7\,\text{pmol}\,\text{min}^{-1}$, $N = 15$). Significant increases in fluid secretion rate and sodium transport were still seen even at a ouabain concentration of $10^{-7}\,\text{mol}\,\text{l}^{-1}$, but the difference from control tubules was less marked.

To gain a clearer picture of the time course and effectiveness of ouabain treatment on sodium transport, an alternative experimental arrangement was used in which fluid moved rapidly through the lumen of the ouabain-treated tubule. To achieve this, the distal (upstream) part of each tubule was put in a drop of saline containing $10^{-6}\,\text{mol}\,\text{l}^{-1}$ 5-hydroxytryptamine (5-HT) which stimulates very rapid fluid secretion by this part of the tubule (Maddrell *et al.* 1971). The remainder of the fluid-secreting region of the tubule was run through a second drop of saline, free of 5-HT but containing ^{22}Na and, in the experimental drops, ouabain at $10^{-4}\,\text{mol}\,\text{l}^{-1}$. In this arrangement, fluid flows rapidly through the lumen of that part of the tubule bathed in the ^{22}Na-containing drop. Its collection from a cut in the wall of the tubule just downstream from this drop meant that ^{22}Na entry into the lumen could be measured with very little delay. Fig. 1 shows that ouabain

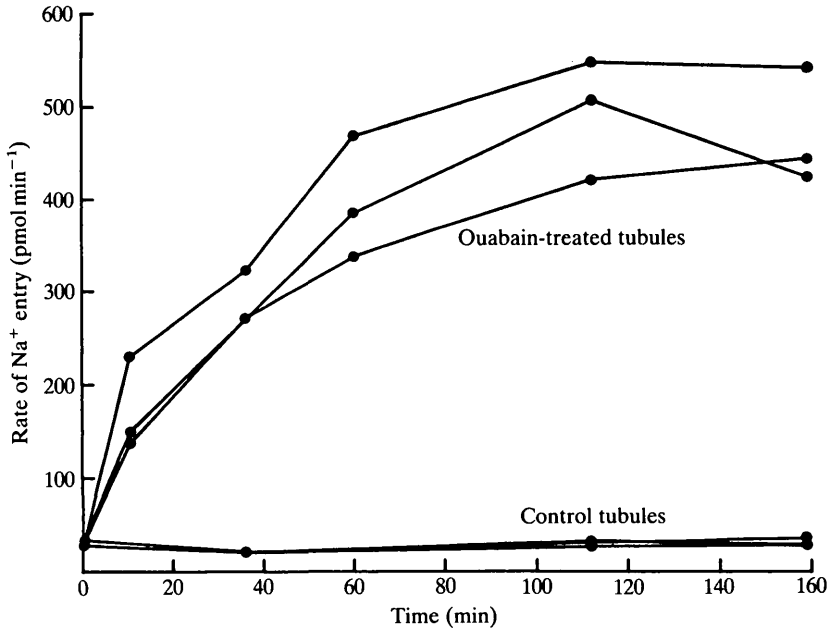

Fig. 1. The effect of treatment with $10^{-4}\,\text{mol}\,\text{l}^{-1}$ ouabain on the rate of movement of Na^+ into the lumen of a Malpighian tubule of *Rhodnius*. The upper traces are for ouabain-treated tubules, the lower ones for untreated control tubules.

affects ^{22}Na transport almost immediately and that its effect steadily increases during the next 2–3 h. Na^+ entry in these relatively short lengths of tubule reached a level of 391 ± 27 pmol min^{-1} ($N = 12$) in ouabain-treated tubules, compared with 32 ± 6 pmol min^{-1} ($N = 7$) in control tubules, a 12-fold difference. The insects used here were younger than in the initial experiments and this may explain the greater effects of ouabain. We have evidence that most aspects of tubule function occur more rapidly in younger insects.

Effects of ouabain on the transepithelial potential difference

The difference in the rate of transport of sodium through the *cells* in ouabain-treated and control tubules may be very much higher than the figures above suggest. This is because Malpighian tubules of *Rhodnius* have permeable intercellular junctions (O'Donnell, Maddrell & Gardiner, 1984). If the junctional permeability to sodium ions were similar to that for sucrose or inulin, it can be calculated that sodium ions could, solely by diffusion into the lumen at the low rates of fluid secretion observed in control tubules, reach a concentration there in the range 40–60 mmol l^{-1}. From the fact that the observed concentration was 56 mmol l^{-1}, it seems likely that the transport of sodium through the cells is very slow in control tubules; most of the sodium ions may cross the wall through the junctions.

Since sodium ions may penetrate through the intercellular clefts by diffusion, it would be expected that such movement would be affected by the transepithelial potential difference (TEP). Conceivably, then, the effect of ouabain in stimulating net Na^+ movement into the lumen could arise from a change in the TEP, if the lumen were to become more negative with respect to the haemolymph (basal) side. We measured the effect of ouabain at 5×10^{-6} mmol l^{-1} on the TEP. In all experiments, the lumen became more positive, on average by $15{\cdot}7 \pm 1{\cdot}7$ mV ($N = 16$). The onset of the effect was rapid; the potential started to rise within 15–30 s of ouabain application. However, a new steady TEP was not reached until 15–20 min later.

These results argue that the effects we observe, a stimulation by ouabain of fluid and sodium transport, are brought about by changes in transcellular movement, rather than by changes in movement through the intercellular clefts. Two types of explanation seem possible.

The first possibility is that the uppermost parts of the tubules might secrete a Na^+-rich primary fluid which is modified by Na^+/K^+ exchange along the length of the tubule, to produce the K^+-rich, Na^+-poor fluid observed. If the tubule were poisoned by ouabain, intracellular [Na^+] would rise, making Na^+/K^+ exchange impossible and giving rise to a high-volume, Na^+-rich secretion. This seems unlikely on two counts. First, the secretory performance of the fluid-secreting part of a tubule does not vary with length along it, at least during hormone stimulation (Maddrell, 1969). Second, even single fluid-secreting cells secrete Na^+ only extremely slowly when not stimulated (Maddrell & Overton, 1985).

Neither of these objections is totally convincing, so we have investigated directly the Na^+ transport capabilities of the different regions of the tubule. To do this we cannulated and perfused tubules with standard saline and bathed, in saline, parts of them 3 mm in length (the fluid-secreting part of the tubule is about 30 mm in length), either as close as possible to the uppermost end or near to the lower end. The bathing saline contained ^{22}Na and we determined the radioactive content of the drops of fluid that had been perfused through the lumen of the tubule. In this way we could determine how fast sodium ions crossed the wall of the tubule in the two different regions. We found that there were no significant differences between the two regions (five experiments). As a further test, we isolated short lengths of the uppermost parts of the tubule into saline containing ^{22}Na and compared the sodium concentration in the secreted fluid with that secreted by whole upper tubules in similar drops. The uppermost lengths of tubule secreted fluid that contained $59{\cdot}1 \pm 4{\cdot}3\,mmol\,l^{-1}$ of sodium ($N = 12$), compared with $56{\cdot}8 \pm 6{\cdot}2\,mmol\,l^{-1}$ of sodium ($N = 5$) in fluid secreted by whole upper tubules.

We believe that these results effectively rule out the possibility that the effects of ouabain treatment in raising the sodium content of the secreted fluid depend on interfering with processes that differ along the length of the tubule. We are left with our alternative explanation that the effects of ouabain are to be understood in terms of uniform action on all the cells of the upper tubule. What this action might be is discussed in detail later.

Ouabain binding studies

The results presented above show that the secretory behaviour of unstimulated *Rhodnius* Malpighian tubules is much affected by treatment with ouabain, even at concentrations as low as $2 \times 10^{-7}\,mol\,l^{-1}$. *Prima facie*, this is powerful evidence for the occurrence in the tubule of a Na^+/K^+-ATPase, the sodium pump. To confirm this, we have exposed the tubules to radioactive ouabain under various conditions to determine whether ouabain binds to the tubules in the manner expected for its attachment to the sodium pump.

In the first experiments, tubules were exposed for 10 min to a low-K^+ saline, modified to reduce its potassium concentration from the usual $8{\cdot}6\,mmol\,l^{-1}$ to $2{\cdot}6\,mmol\,l^{-1}$ (elevated levels of potassium are known to reduce ouabain attachment to its binding site on the sodium pump, Baker & Willis, 1972; Matsui, Homareda & Hayashi, 1985) and containing radioactive ouabain. The tubules were then briefly washed in fresh ouabain-free low-K^+ saline. The results showed that each tubule then had associated with it $46{\cdot}9 \pm 3{\cdot}0$ fmol of ouabain ($N = 14$).

Ouabain is known to bind relatively slowly to its binding sites but also in many cases to wash off slowly, although this is very species-dependent (Baker & Willis, 1972; Bodemann, 1985). Accordingly, we carried out a series of experiments to investigate the rate at which ouabain attached to tubules from ouabain-containing saline and also the rate at which counts subsequently became detached from them in ouabain-free saline.

To be able to determine how rapidly ouabain attaches to the tubules, we needed first to know how rapidly it detaches on washing. So, we bathed tubules in ouabain-containing low-K^+ saline for 5 min and then washed the tubules for periods of between 5 s and 16 min in ouabain-free low-K^+ saline. The quantities of radioactive ouabain still associated with tubules after such treatment are shown in Fig. 2. The results show that about 10 fmol of ouabain is rapidly washed off each tubule but that about 35 fmol of ouabain remains attached to the tubules for at least 16 min during washing.

Therefore, to look at the rate at which ouabain binds to tubules, we soaked tubules in low-K^+ saline containing 1 μmol l^{-1} ouabain for periods of between 10 s and 10 min and then washed each of them for 5 min in low-K^+ saline before disrupting them in distilled water and counting them. The results show that ouabain attaches steadily but progressively more slowly for the first 5 min after which there is no significant increase (Fig. 3). To test the effect of further reducing the potassium concentration of the bathing saline, we carried out parallel experiments with other tubules from the same insects as used with low-K^+ saline but now using K^+-free saline. The results show that a change in potassium concentration from 2·6 mmol l^{-1} to nominally zero does not significantly affect ouabain binding (Fig. 3).

Further to investigate the effects of K^+ levels on binding, we measured the ouabain attaching to tubules after 5 min in salines containing 0, 2·6, 8·6 and 150·6 mmol l^{-1} potassium. In each case, the tubules were washed for 5 min with saline containing 2·6 mmol l^{-1} potassium before counting them. At 0 mmol l^{-1} K^+

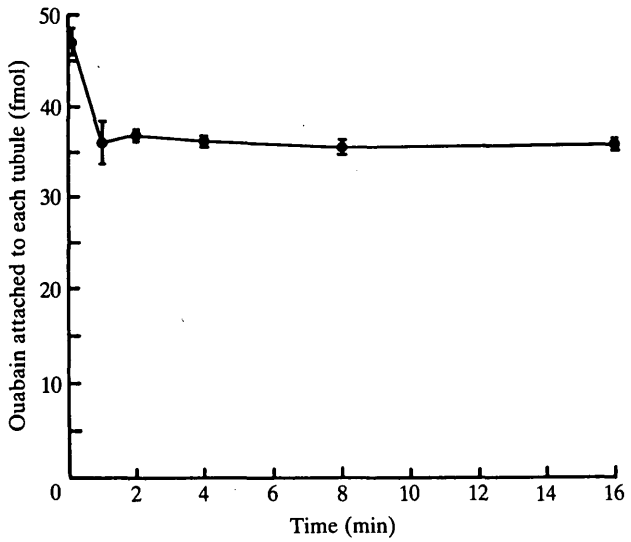

Fig. 2. The effect of different periods of washing in ouabain-free saline on the quantity of radioactive ouabain remaining attached to isolated Malpighian tubules. The points represent the mean values and the vertical lines indicate the extent of the standard error. Each point is the mean of four determinations.

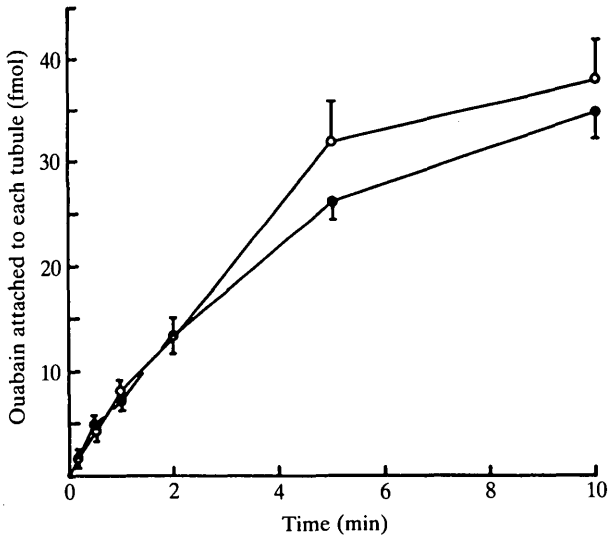

Fig. 3. The rate of attachment of radioactive ouabain to isolated Malpighian tubules, in saline containing no potassium (○, $N = 7$) and in saline containing 2·6 mmol l^{-1} potassium (●, $N = 11$). The points represent the mean values and the vertical lines indicate the extent of the standard error.

the amount of ouabain bound per tubule was 41·9 ± 2·4 fmol ($N = 7$), at 2·6 mmol l^{-1} K^+ the amount of ouabain bound was 36·6 ± 1·3 fmol ($N = 7$), at 8·6 mmol l^{-1} K^+ the amount of ouabain bound was 28·0 ± 2·1 fmol ($N = 7$), and at 150·6 mmol l^{-1} K^+ the amount of ouabain bound was 13·8 ± 0·5 fmol ($N = 7$). Clearly, very much less ouabain binds in the presence of 150·6 mmol l^{-1} potassium than at lower concentrations. In addition, the results show that even increasing the potassium concentration from nominally zero to 8·6 mmol l^{-1} reduces the quantity of oubain bound.

As a further test of the idea that the radioactive counts still bound to tubules after 5 min washing represent ouabain attached to sites on membrane-bound Na^+/K^+-ATPase, we compared the counts attaching to tubules from a low-K^+ saline containing 1 μmol l^{-1} radioactive ouabain with those attaching to tubules from a low-K^+ saline with 1 μmol l^{-1} radioactive ouabain but also 1 mmol l^{-1} non-radioactive ouabain. The results show that the number of counts associated with tubules previously bathed with radioactive ouabain of low specific activity was only 3·4 ± 2·1 % ($N = 16$) of that attaching to tubules bathed in saline containing only radioactive ouabain. This result strongly suggests that the ouabain binding we have studied is specific, as it can be very greatly reduced by dilution with non-radioactive ouabain.

Does ouabain bind to the luminal membranes?

To confirm that the ouabain binding described above is not artefactual – in the sense that it might be non-specific – we have tested the ability of radioactive

ouabain to bind to the membrane on the luminal surface of the cells. Our model, described below, for ion transport by *Rhodnius* Malpighian tubules supposes that a Na^+/K^+ exchange pump exists only on the haemolymph-facing or basal membranes. Accordingly, we perfused the lumen of tubules with the same fluid used to investigate the binding of ouabain to the basal face of the cells, that is a low-K^+ saline containing $1\,\mu mol\,l^{-1}$ radioactive ouabain. The fluid was perfused through the tubule for 5 min while it was immersed in non-radioactive saline and this was followed by 5 min of perfusion with ouabain-free solution. The length of tubule that had been in the bathing drop was then severed from the lengths that had been outside it and, after disruption in distilled water, the tubule together with the distilled water were put into scintillation fluid and counted. As a control, a similar length of tubule from the same insect was bathed as earlier, that is its basal membranes were exposed to ouabain. The quantities of ouabain binding to the two surfaces of the cell were $18{\cdot}52 \pm 3{\cdot}02$ fmol ($N = 6$) bound to the basal side and $0{\cdot}72 \pm 0{\cdot}15$ fmol ($N = 6$) attaching to the luminal surface. The figure for ouabain binding to the basal surface is lower than the experiments described earlier in this paper because shorter lengths of tubule were exposed. This is because, in the perfusion experiments, part of the tubule has the cannula inserted in it, part runs from there to the bathing drop and part runs away from the bathing drop to carry radioactive fluid away from the bathing drop before releasing it through a cut in its wall. What is abundantly clear, however, is that virtually no ouabain binds to the luminal face of the Malpighian tubule cells, providing further evidence that ouabain binding to the basal membranes is specifically attaching to sites on the Na^+/K^+ pumps there.

Discussion

Previous work on transport by the Malpighian tubules of *Rhodnius* (O'Donnell & Maddrell, 1984) has suggested a model in which, during rapid fluid secretion stimulated by 5-HT or the naturally occurring diuretic hormone (Maddrell, 1963), sodium, potassium and chloride ions enter the cells by a $Na^+/K^+/2Cl^-$ cotransport step; sodium and potassium ions are then actively transported out into the lumen with chloride ions following passively down their electrochemical gradient. The secreted fluid thus contains approximately equal concentrations of sodium and potassium ions (Maddrell, 1969). However, as shown above, the fluid secreted by non-stimulated tubules contains only low levels of sodium. A way of reconciling these results and incorporating the findings on ouabain treatment and ouabain binding is shown in Fig. 4; in this model it is suggested that ouabain-sensitive Na^+/K^+ exchange pumps are situated on the basal membranes, but not on the luminal membranes. The model describes only ion movements through the cells although, of course, ion movements may also occur paracellularly through the intercellular clefts. However, we are here attempting to account for an increased transport of sodium ions into the lumen. Since we found that ouabain makes the

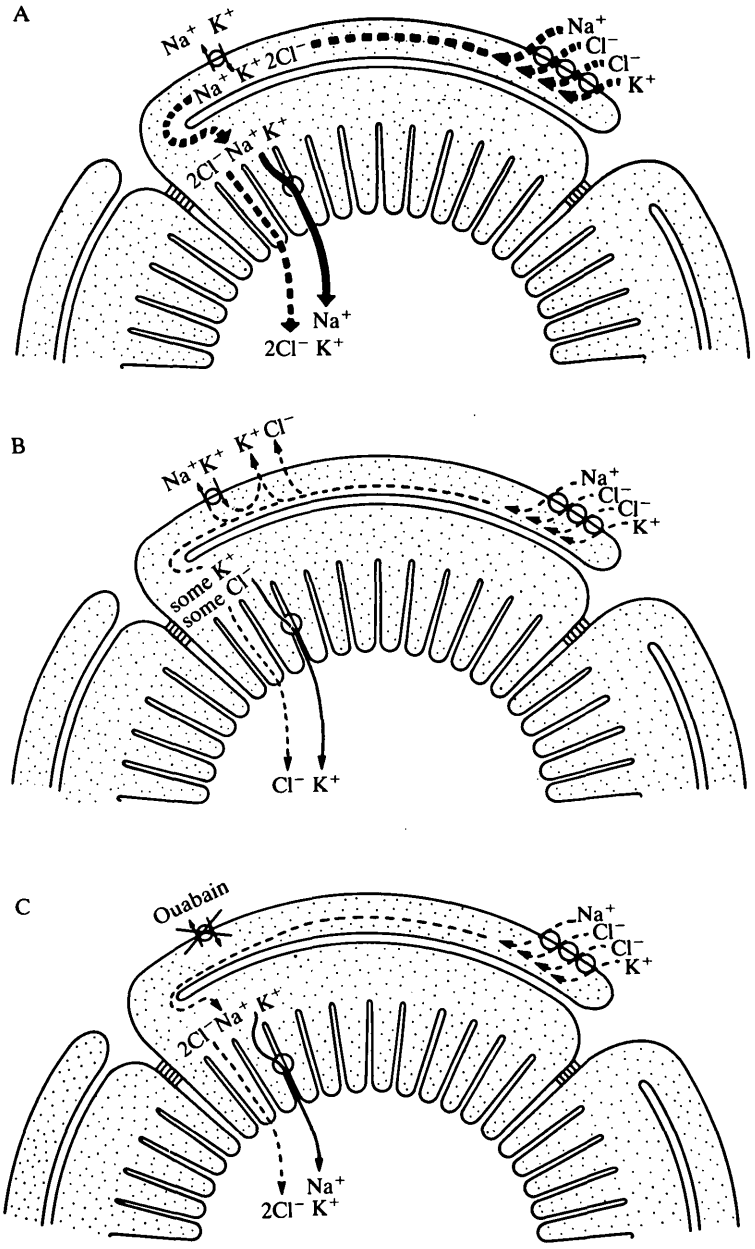

Fig. 4. Proposed mechanisms of ion transport through the cells of Malpighian tubules of *Rhodnius*. (A) During rapid fluid secretion in a 5-HT-stimulated tubule. (B) During slow fluid secretion in a non-stimulated tubule. (C) In a non-stimulated tubule treated with ouabain. The thickness of the lines showing the movements of ions indicates the relative rate of the movement; broken lines indicate passive and continuous lines active movements.

luminal electrical potential more positive, which would slow paracellular diffusion of sodium ions into the lumen rather than speed it up, we believe that the effects of ouabain in speeding transepithelial movements of sodium do not involve changes in paracellular fluxes.

The outer (haemolymph-facing) or basal side of the tubule consists of very many long cytoplasmic finger-like projections which extend circumferentially round the tubule (O'Donnell, Maddrell, Skaer & Harrison, 1985). Because the surface area of the cell membrane on these projections is so large compared with that on the lateral faces of the cell (measurements from electron micrographs show the difference to be about 200 times), most ions are likely to enter the cell *via* these projections. During fast fluid secretion both the apical cation pump and the cotransport entry step operate more than 100 times faster than in an unstimulated tubule (O'Donnell & Maddrell, 1984). If it is assumed that Na^+/K^+ exchange pumps situated on the finger-like projections are not stimulated during fast fluid secretion, they will not be able to affect significantly the very rapid flow of ions entering the cell. However, in an unstimulated tubule, a slow entry of ions could well be much affected by the Na^+/K^+ pumps. Sodium ions would be returned to the bathing fluid and, as the basal membrane is permeable to potassium ions (O'Donnell & Maddrell, 1984), potassium and chloride ions would tend to pass back out of the cell. This would leave a relatively diminished flow of ions, predominantly potassium and chloride, to pass into the cell and in turn be transported into the lumen. This would explain how it is that unstimulated tubules slowly secrete fluid which is largely a solution of potassium chloride.

If now the tubule is exposed to ouabain, ions entering *via* the cotransport step would not be affected by the inhibited Na^+/K^+ pump so that there would be a faster passage of ions into the main body of the cell. This, in turn, would support a faster rate of ion transport into the lumen which, since water movements are coupled to ion transfer, possibly by osmosis (O'Donnell, Aldis & Maddrell, 1982), would lead to the higher rate of fluid secretion that is seen. Since sodium ions after entering the cell would not now be returned to the bathing solution, many more of them would be actively transported into the lumen, making the luminal fluid sodium-rich – as is observed. The rapid change in relative concentrations of Na^+ and K^+ in the basal finger-like projections is shown by the prompt change in transepithelial potential (TEP) after ouabain treatment. The changes in TEP observed of about 16 mV in 20 min suggest that the concentration of K^+ in the projections changes from about 120 mmol l^{-1} to about 65 mmol l^{-1} in this time.

In the period just after a meal, blood-sucking insects have the problem of ridding themselves rapidly of surplus plasma which is a sodium-based fluid. Later, they need a longer-lasting slower excretion of potassium as the blood cells are digested. The model system shown in Fig. 4 provides for an appropriate rapid excretion of sodium (and chloride) ions on hormonal stimulation (the hormone also stimulates the reabsorption of most of the potassium ions from the secreted fluid in a lower, more proximal, part of the tubule; this leaves a fluid to be

eliminated containing sodium and chloride as its major ions). When not hormonally stimulated, the tubule carries out an appropriate slow continuous excretion of potassium (and chloride) ions.

That the Na^+/K^+ pump is confined to the basal cell membranes is confirmed by our finding that virtually no ouabain binds to the luminal membranes. This is in spite of the fact that the area of the luminal cell membrane is at least three times that of the basal membrane (Maddrell, 1980). The density of the ouabain-binding sites on the basal membranes can be calculated, assuming that one ouabain molecule binds to each Na^+/K^+ pump. In K^+-free saline, 42 fmol or 253×10^8 molecules of ouabain bind to each tubule. The apparent surface area of each tubule is $8\,mm^2$ but the basal membrane is amplified 40 times by its surface modifications (Maddrell, 1980), so that its total surface area is $320\,mm^2$ or $320\times10^6\,\mu m^2$. From this it follows that there are close to 80 ouabain binding sites per square micrometre of basal membrane. This compares with values of between 500–1000 sites μm^{-2} of membrane in a variety of cell types but less than 1 site μm^{-2} in erythrocyte membrane (Baker & Willis, 1972). Baker & Willis suggest that the very low figure for erythrocyte membrane may reflect a relatively lower demand on a Na^+ pump in these cells because of the extremely low passive permeability of the cell membrane to cations. What might be the explanation for the low density of ouabain-binding sites in *Rhodnius* Malpighian tubules? One possibility comes from the way that, even in the unstimulated state, cations are pumped from the cells into the lumen of the tubule by a cation pump, thought to be unique to insects, that has a preference for Na^+ over K^+ (Maddrell, 1978). Such a pump will act to keep the cellular concentration of Na^+ at a lower level than K^+. There will therefore be less need of a Na^+/K^+ pump to maintain the intracellular levels of these cations and it may thus be irrelevant for cell volume regulation in Malpighian tubule cells.

The explanation for the apparently paradoxical stimulation of fluid secretion and sodium transport by ouabain in this epithelium, then, is that the Na^+/K^+ pump transports sodium in a direction *opposite* to that of fluid secretion. The normal action of the Na^+/K^+ pump is thus to reduce net Na^+ transfer across the epithelium and so to slow fluid secretion. Ouabain treatment removes this brake and both sodium transfer and fluid secretion are accelerated.

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