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FLUID REABSORPTION AND ION TRANSPORT BY THE LOWER MALPIGHIAN TUBULES OF ADULT FEMALE *DROSOPHILA*

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Summary

The properties of the Malpighian tubules of *Drosophila melanogaster* change along their length. The upstream main segments secrete K^+-rich fluid at a high rate. From this, the lower tubules reabsorb significant amounts of water and K^+. Under stimulation, K^+ reabsorption is accelerated. In addition, the lower tubules acidify the fluid passed to them by the main segments and secrete Ca^{2+} into it, adding to that transported there by the upstream epithelium. In contrast to the lumen-positive transepithelial potential difference (TEP) of the main segments, the TEP in the lower tubules is much lower and becomes lumen-negative close to their downstream junction with the common ureter. We suggest that the role of the lower tubule is to reduce the flow of K^+-rich fluid that passes to the hindgut; this allows the hindgut to process the flow of excretory fluid more thoroughly.

Key words: reabsorption, Malpighian tubules, potassium ions, fluid transport, calcium transport, excretion, pH, *Drosophila melanogaster*.

Introduction

The isolated Malpighian tubules of adult female *Drosophila melanogaster* secrete fluid at high rates (Dow *et al.* 1994*b*). It is noticeable, even under a dissecting microscope, that the morphological appearance of the tubules changes somewhat along their length. At the ultrastructural level, there are similar observable changes (Wessing and Eichelberg, 1975). These latter authors concluded that the anteriorly directed tubules were divided into regions (Fig. 1) which they termed, in order from the distal tip of the tubule, the distal segment, the middle segment, the proximal segment and the ureter. The posteriorly directed tubules they described as consisting of a distal segment, a proximal segment and the ureter. We have previously shown that the distal white regions of the anterior tubules do not secrete fluid at measurable rates in isolated preparations (Dow *et al.* 1994*b*), and that the middle segments of anterior tubules and distal segments of posterior tubules secrete fluid at similar rates. We propose, therefore, that the ultrastructurally similar, fluid-secreting portions of both anterior and posterior tubules might properly be referred to by the same term, the main segment (Fig. 1). We also suggest that the ultrastructurally similar lengths of tubule that are interposed between the main segments and the ureters be called the lower tubules (Fig. 1); this avoids the potential confusion of calling them proximal, a term that in kidney tubules refers to the most upstream epithelia. Here we show that fluid secretion is confined to the main segments of the Malpighian tubules and that the lower lengths of both anterior and posterior tubules reabsorb significant amounts of fluid and K^+. They acidify and increase the Ca^{2+} content of the fluid that passes to them in the lumen from the upstream main segments, and their transepithelial potential difference differs markedly from that in the main segment.

Materials and methods

Adult female *Drosophila melanogaster* (Oregon R strain) flies were taken from a laboratory culture maintained at the Department of Zoology, University of Cambridge, UK. Except where noted, Malpighian tubules were isolated into 6 μl drops of medium held under liquid paraffin (mineral oil) as described elsewhere (Dow *et al.* 1994*b*). The medium consisted of equal parts of a standard *Drosophila* saline (in mmol l^{-1}; NaCl, 135; KCl, 20; $CaCl_2$, 2; $MgCl_2$, 8.5; $NaHCO_3$, 10.2; NaH_2PO_4, 4.3; Hepes, 15; pH 6.75) and Schneider's *Drosophila* medium. Cyclic AMP and leukokinin-1 were from Sigma.

The pH or concentration of Na^+, K^+ or Ca^{2+} in drops of haemolymph or tubule fluid was measured using ion-selective microelectrodes, as described previously (Maddrell and O'Donnell, 1992; Maddrell *et al.* 1993). Ionophore cocktails used were as follows: H^+ ionophore II Cocktail A; K^+ ionophore I Cocktail B; Ca^{2+} ionophore I Cocktail A; Na^+ ionophore II Cocktail A (Fluka Chemical, Ronkonkoma, New York, USA) and Na^+ ion-exchanger IE-110 (World Precision Instruments, Sarasota, Florida, USA). Na^+ ionophore II is appropriate for measurements of $[Na^+]$ in extracellular fluids (i.e. high $[Na^+]$, low $[K^+]$, high $[Ca^{2+}]$), whereas IE-110 is used

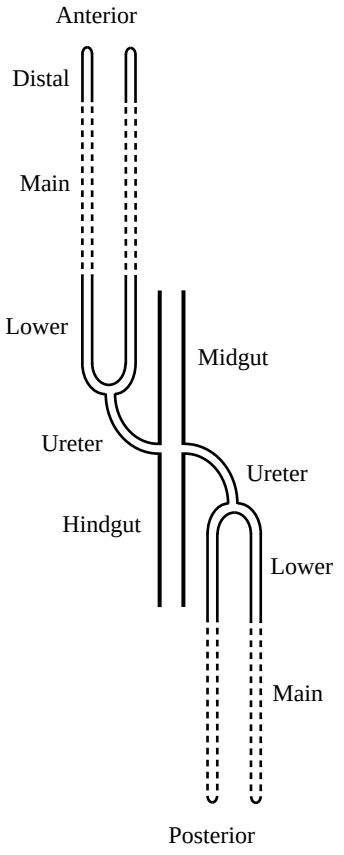

Fig. 1. Schematic diagram of the arrangement of the regions of the Malpighian tubules in *Drosophila melanogaster*. The distal, main and lower segments, the ureters and a short length of the gut are shown.

for measurement of $[Na^+]$ in fluids resembling the intracellular milieu (i.e. low $[Na^+]$, high $[K^+]$, low $[Ca^{2+}]$). Ion concentrations or pH in drops of haemolymph or secreted fluid were measured under paraffin oil by positioning ion-selective and reference microelectrodes in the drop and measuring the change in electrical potential relative to that in drops of calibration solutions. Although ion-selective electrodes measure ion *activity* and not *concentration*, Na^+, K^+ and Ca^{2+} were expressed in terms of concentrations by assuming that the activity coefficients in the calibration and experimental solutions were the same (see Maddrell *et al.* 1993). Expressing the data as concentrations simplifies comparisons with studies involving elemental analysis by techniques such as flame photometry.

Transepithelial potential differences (TEPs) were measured by inserting microelectrodes filled with $3\,mol\,l^{-1}$ KCl into the lumen of Malpighian tubules. The procedures for inserting micropipettes were adapted from those used to cannulate and perfuse the tubule lumen (Maddrell and Phillips, 1975). A length of tubule was pulled out of the bathing saline and held by microforceps under paraffin oil. The microelectrode was inserted into the lumen and advanced axially several hundred micrometers until its tip was inside a segment of the tubule within the bathing saline. The TEP was then measured with respect to a reference microelectrode positioned in the bathing drop.

Where appropriate, experiments were done first with a whole tubule (i.e. main segment plus lower tubule – plus distal segment in the case of anterior tubules) in the bathing drop, and then by pulling the lower tubule out, leaving the main segment alone in the bathing drop. This allowed a direct comparison, in the same tubule, of the composition of the fluid leaving the main segment with the fluid after passage through the lower tubule.

All experiments were carried out at room temperature, 23–30 °C. Values are given as means ± 1 S.E.M.; significance of differences between means was evaluated using the appropriate Student's *t*-test.

Results

Fluid reabsorption by the lower Malpighian tubule: changes in rates of fluid secretion with length of tubule in the bathing drop

During our earlier studies on fluid secretion and its control in isolated Malpighian tubules of *Drosophila* (Dow *et al.* 1994*a*,*b*), it became increasingly evident that the rate at which fluid was secreted was surprisingly little affected by changes in the length of a tubule that was in the drop of bathing medium. Neither adding drops of fluid to the bathing drop nor pulling a longer length of a tubule out from the bathing drop caused any noticeable changes in the rate of fluid secretion. It seemed possible that the downstream regions of the tubule did not secrete fluid at the same rate per unit length as the more distal, upstream regions. So we tested the effects of systematically varying the length of a tubule in a drop of bathing fluid. In each case, we started with as much of the tubule in the bathing drop as possible and then pulled the tubule out, stage by stage, by repositioning the metal pin to which the proximal end of the tubule was attached. After each change, we measured the length of tubule pulled out and the new rate of fluid secretion, and expressed the latter as a fraction of that measured initially with the maximal length bathed. Tubules removed entirely from the bathing drop immediately ceased secretion. The results are summarised in Fig. 2, from which it is clear that, far from secreting fluid, the downstream regions of the tubule reabsorb significant amounts of fluid. The key point is that removing the first short length of the tubule from the bathing drop produces an *increase* in the rate of fluid secretion. In one experiment specifically to test this, the length of tubule in the bathing drop was reduced from 89 to 76 % of the entire length from the upstream end of the main segment to the ureter; the rate of fluid secretion then increased from 0.97±0.07 to $1.10\pm0.09\,nl\,min^{-1}$ ($P<0.001$, paired *t*-test; $N=16$).

It is clear from Fig. 2 that fluid secretion into the lumen occurs in approximately the first 65 % of the tubule, measuring from the upstream end of the main segment. Under phase-contrast microscopy, this is where there is a clear difference in the appearance of the cells at the junction of the main segment

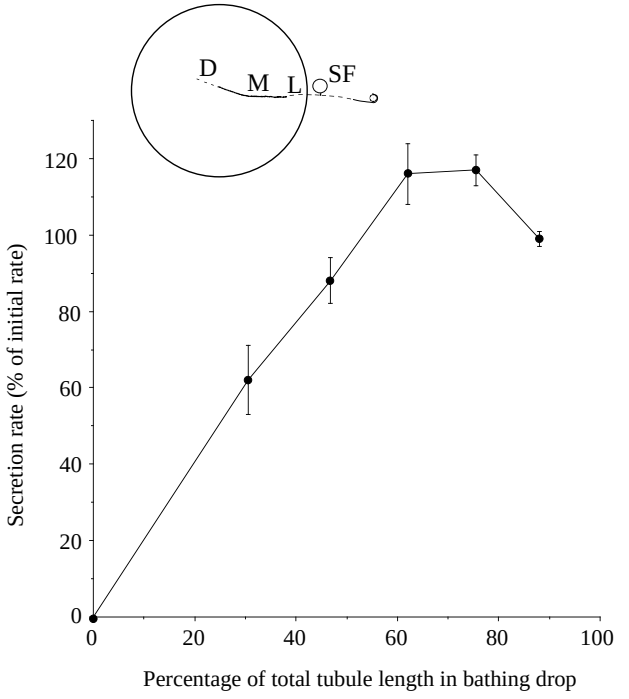

Fig. 2. Changes in relative rates of fluid secretion by Malpighian tubules when successive lengths were pulled out from the bathing drop. Each point is the mean value determined from eight tubules. The vertical lines attached to the mean values indicate ±1 S.E.M. The inset shows the disposition of a tubule at the start of the experiment, with as much of it as possible in the bathing drop. A droplet of secreted fluid (SF) is indicated by the open circle. D, M, L, distal, main and lower parts, respectively, of the tubule.

with the lower tubule. In other words, physiological function correlates with morphology.

K^+ reabsorption by the lower Malpighian tubule

The K^+ concentrations in *Drosophila* haemolymph and in the 1:1 mixture of Schneider's medium and *Drosophila* saline were 34.7±2.4 mmol l^{-1} (*N*=14 flies) and 22.7±0.1 mmol l^{-1} (*N*=4), respectively. The K^+ concentration in fluid secreted by whole unstimulated tubules was 105.0±4.2 mmol l^{-1} (*N*=13 tubules); this value is significantly lower ($P<0.05$) than the concentration of 118.5±5.4 mmol l^{-1} in fluid secreted by the main segment of the same tubules. The combined effect of reabsorption of fluid and K^+ resulted in a large reduction in K^+ loss in tubule fluid. K^+ flux from the bathing saline to the secreted droplet was calculated as the product of fluid secretion rate (nl min^{-1}) and K^+ concentration (mmol l^{-1}). K^+ flux into the main segment was 67±14 pmol min^{-1}, significantly greater ($P<0.025$) than the value for fluid leaving the whole tubule, 40.5±11 pmol min^{-1}, a reduction of 39 %. Potassium ions are thus recovered at a rate of about 26 pmol min^{-1}.

When fluid secretion was stimulated by addition of 1 mmol l^{-1} cyclic AMP to the bathing saline, the K^+ concentration in fluid secreted by the whole tubule was 117.7±1.9 mmol l^{-1} (*N*=20), also significantly lower ($P<0.001$) than the value of 129.3±1.3 mmol l^{-1} in fluid secreted by the main segment of the same tubules.

Fluid secretion can also be stimulated by application of an aqueous extract of the larval central nervous system (CNS; Dow *et al.* 1994*b*). The difference in K^+ concentrations in drops secreted by CNS-stimulated whole tubules (105.0±6.6 mmol l^{-1}; *N*=13) compared with main segments (125.3±2.2 mmol l^{-1}) was larger than when tubules were stimulated with cyclic AMP; this increase may indicate more effective stimulation of reabsorptive processes by larval CNS extracts than by cyclic AMP; we suppose that the CNS contains more than one natural stimulant. The data also show that the K^+ concentrations varied, from 105.0 to 117.7 mmol l^{-1}, for example (see above), in fluid secreted by whole unstimulated tubules from different groups of insects. For this reason, all comparisons between secreted fluid composition were based on paired experiments using whole tubules compared with main segments of the same tubules.

For the experiments described above, fluid was collected first from the whole tubule, then the lower segment was pulled out of the bathing saline and subsequent drops were collected after passage through the main segment. However, to prevent the droplet secreted by the whole tubule from merging with the bathing saline drop, a short length of the lower segment was necessarily pulled out into the paraffin oil (see Fig. 2 inset for the experimental arrangement). As a result, the data for whole tubules may underestimate the extent of K^+ reabsorption by the lower segment, as only a part of it was bathed in our preparations.

To overcome this limitation, an alternative preparation was developed. The four Malpighian tubules in *Drosophila melanogaster* consist of an anterior and posterior pair, and each pair is connected to the hindgut through a short ureter. All four tubules, still connected to a very short length of the gut, were dissected from the insect. One pair was placed in the bathing droplet. One tubule of the other pair was cut away and discarded, and the remaining tubule was pulled out into the paraffin oil and used to anchor the preparation. Fluid was thus collected after it had passed through the entire length of two tubules upstream of their common ureter (see Fig. 3A inset). The lower segments were subsequently pulled out into the paraffin oil, so that only the main segments (and distal segments where anterior tubules were used) remained in the bathing saline.

Experiments with this potentially improved preparation indicated effective K^+ reabsorption by the lower segment (Fig. 3), even when fluid secretion rates increased from 0.6±0.05 nl min^{-1} to 1.2±0.09 nl min^{-1} in response to 1 mmol l^{-1} cyclic AMP. This means that reabsorption by the lower segment reduced K^+ flux by 58 %, from approximately 160 pmol min^{-1} in the main segment to approximately 65 pmol min^{-1} for the whole tubule. By comparison with the corresponding data for unstimulated tubules, this suggests that reabsorption is more effective in stimulated tubules.

Na^+ concentrations in secreted fluid

Na^+ concentrations in fluid secreted by whole tubules

compared with the main segments of unstimulated tubules did not differ significantly. Using Na^+ microelectrodes based on the Na^+ ion exchanger IE-110, Na^+ concentrations were 34.8±5.7 mmol l^{-1} and 31.2±4.3 mmol l^{-1} (N=9) for fluid secreted by the whole tubule and main segment respectively. High Ca^{2+} concentrations in secreted fluid would lead to an overestimate of Na^+ concentrations determined using microelectrodes based on IE-110. For this reason, the measurements were repeated using Na^+ microelectrodes based on Na^+ ionophore II. In this experiment, Na^+ concentrations were 31.2±4.4 mmol l^{-1} and 34.8±5.9 mmol l^{-1} (N=12) for fluid secreted by the whole tubule and main segment respectively.

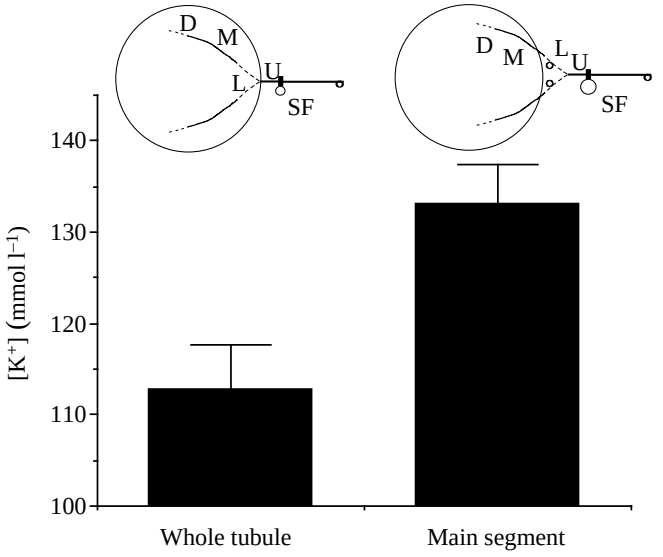

Fig. 3. Comparison of K^+ concentrations (mean + S.E.M.; N=10 tubules) in fluid secreted by whole tubules *versus* main segments. K^+ concentrations were reduced significantly (P<0.002) by passage through the lower tubule. The insets above each column show the corresponding arrangements of the tubules and bathing drops. Glass pins for securing the tubules are indicated by filled circles. U, ureter; other labels as in Fig. 2. No differences between anterior and posterior tubules were noted, and results were pooled. Further details are given in the text.

Ca^{2+} transport by the lower tubule and the main segments

As a preliminary to investigating possible Ca^{2+} transport by the tubules, we measured the Ca^{2+} concentrations in the haemolymph and in a 1:1 mixture of Schneider's *Drosophila* medium and *Drosophila* saline. These were 0.49±0.08 mmol l^{-1} (N=11) and 4.15±0.02 mmol l^{-1} (N=4) respectively.

Whole tubules bathed in the 1:1 mixture secreted fluid containing 0.19±0.04 mmol l^{-1} Ca^{2+} (N=10). When stimulated with 1 mmol l^{-1} cyclic AMP, this concentration rose to 0.38±0.05 mmol l^{-1}. The total Ca^{2+} flux, the product of the rate of fluid secretion and the Ca^{2+} concentration in it, rose from 0.12 pmol min^{-1} to 0.46 pmol min^{-1} (Fig. 4A). When the lower segment was then pulled out of the bathing saline, the Ca^{2+} concentration in the secreted fluid dropped to 0.21 mmol l^{-1} and the flux to 0.26 pmol min^{-1} (Fig. 4B). These data show that the lower segment is involved in Ca^{2+} transport, since both [Ca^{2+}] and flux rates decline when the lower tubule is removed from the bathing saline. Indeed, since the length of lower tubule pulled out of the bath was only about one-quarter of the whole bathed length of tubule and yet the Ca^{2+} flux fell by 43 %, it follows that the Ca^{2+} flux per unit length is higher in the lower tubule.

The finding that the tubules transport Ca^{2+} was unexpected, so, to characterize it further, we determined the rates at which Ca^{2+} was transported into the lumen (from knowledge of the rate of fluid secretion and the concentration of Ca^{2+} in it) at three differing bathing Ca^{2+} concentrations, 0.2, 1.8 and 4.15 mmol l^{-1}. Fig. 4B shows that, even at 0.2 mmol l^{-1}, stimulated main segments transport Ca^{2+} at high rates and these rates are not significantly lower than at higher bath Ca^{2+} concentrations. At the lowest bath concentration, 0.2 mmol l^{-1}, the Ca^{2+} concentration of the secreted fluid (0.37±0.09 mmol l^{-1}, N=12) exceeds that in the bathing

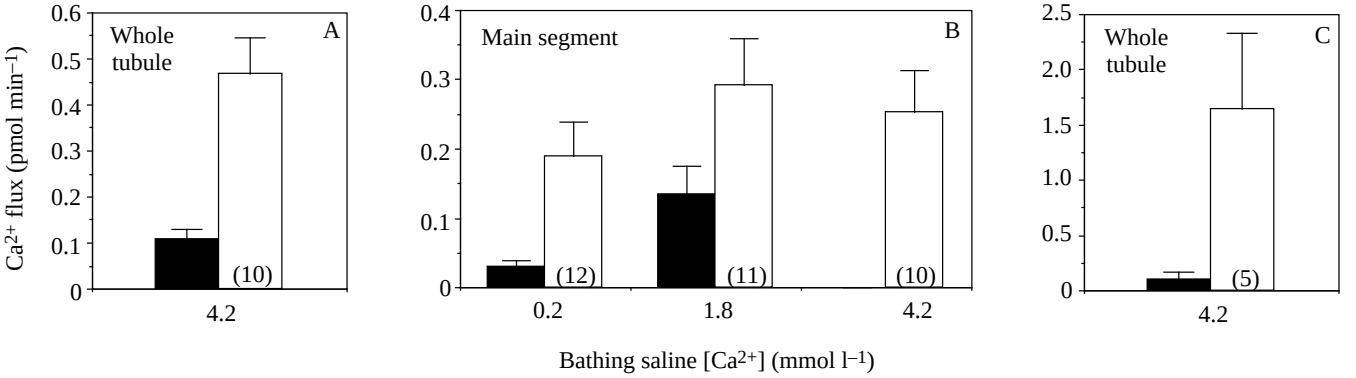

Fig. 4. Ca^{2+} flux in *Drosophila* Malpighian tubules. Tubules were bathed in a 1:1 mixture of Schneider's *Drosophila* medium and *Drosophila* saline ([Ca^{2+}]=4.2 mmol l^{-1}) or in *Drosophila* saline containing 0.2 or 1.8 mmol l^{-1} Ca^{2+}. Mean values for Ca^{2+} flux for unstimulated and stimulated tubules are indicated by filled and open columns, respectively. Tubules in A and B were stimulated with 1 mmol l^{-1} cyclic AMP; those in C were stimulated with 1 mmol l^{-1} cyclic AMP and 100 μmol l^{-1} leukokinin-1. The vertical lines attached to the columns indicate +S.E.M. for the number of tubules indicated in parentheses.

medium. Since the transepithelial potential difference is about 50 mV, lumen-positive, increasing to about 60–70 mV on stimulation with cyclic AMP (M. J. O'Donnell and S. H. P. Maddrell, in preparation), this is *prima facie* evidence for active transport of Ca^{2+} into the lumen. Stimulation with leucokinin-1 causes an opposite change in TEP, that is a depolarization to about 20–25 mV; the electrochemical gradient against which any Ca^{2+} transport occurs is thus greatly reduced. We measured Ca^{2+} transport by whole tubules before and after stimulation with a mixture of 1 mmol l^{-1} cyclic AMP and 100 μmol l^{-1} leucokinin-1 [this gives very high rates of fluid secretion (Dow *et al.* 1994*b*) while still depolarizing the TEP (M. J. O'Donnell and S. H. P. Maddrell, in preparation)]. Under these conditions, Ca^{2+} transport increased by more than twentyfold (Fig. 4C).

Acidification of primary urine by the lower Malpighian tubule

Measurements of the pH of drops of secreted fluid indicated that the primary urine becomes acidified as it passes through the lower segment. When unstimulated tubules were bathed in a 1:1 mixture of Schneider's *Drosophila* medium and *Drosophila* saline at pH 7.1, the pH of fluid secreted by the main segment was 7.74±0.07 (N=9), whereas fluid secreted by the same whole tubules had a significantly (P<0.05) more acidic pH, 7.56±0.05. The difference in pH between fluid secreted by whole tubules and the main segments exceeded 0.4 units when fluid secretion was stimulated by addition of cyclic AMP (Fig. 5).

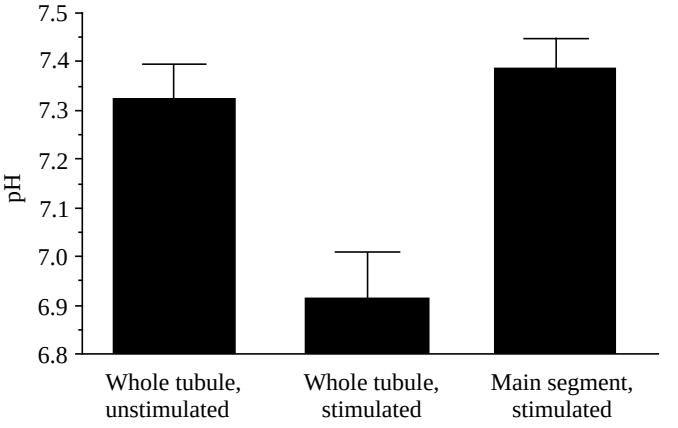

Fig. 5. pH of fluid secreted by whole, unstimulated tubules, whole tubules stimulated with 1 mmol l^{-1} cyclic AMP and main segments stimulated with 1 mmol l^{-1} cyclic AMP. Data are presented as means + S.E.M. for the same 24 Malpighian tubules. Student's t-tests indicated significant differences between unstimulated and stimulated whole tubules (P<0.001) and between whole tubules and main segments of stimulated tubules (P<0.001).

Transepithelial potential in main versus *lower segments of Malpighian tubules*

The transepithelial potential difference of the lower segment was substantially less positive than that of the main segment (Fig. 6). Moreover, when TEP was measured in the region of the lower segment within 200 μm of the ureter, the TEP reversed sign and was as much as 35 mV negative to the bathing saline.

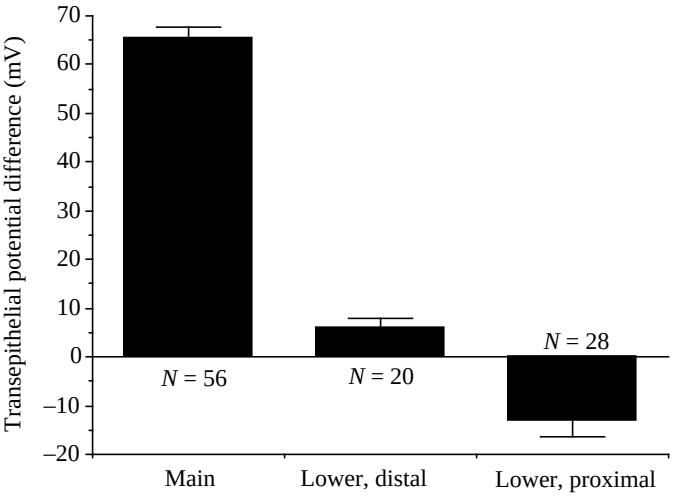

Fig. 6. Transepithelial potential difference (mV) in main *versus* lower Malpighian tubule segments. Luminal microelectrodes were positioned randomly along the length of the main segment. For the lower tubule, the microelectrode tip was positioned either within 200 μm of the ureter (lower, proximal) or at a distance of more than 400 μm from the ureter (lower, distal). Each histogram shows mean potential + S.E.M. for the indicated number of tubules.

Discussion

Possible functional significance of fluid modification by the lower tubule

Our results show that the lower Malpighian tubule of adult female *Drosophila* modifies the fluid passed to it from the upstream fluid-secreting regions by reabsorbing water and K^+. Strictly speaking, we have not shown that K^+ is reabsorbed into the haemolymph, as we measured only a fall in the concentration of K^+ in the secreted fluid passing through the lower tubule. The rate of removal of K^+, however, is so high (26 pmol min^{-1}, see above) that it is impossible that K^+ could be retained in the cells of the lower tubule, whose volume is only about 250 pl.

In addition to reabsorption of water and K^+, the lower tubule can acidify the fluid by more than 0.4 pH units and actively transports Ca^{2+} into the lumen.

The main segments of the Malpighian tubules of *Drosophila* can secrete K^+-rich fluids at very high rates when stimulated (Dow *et al.* 1994*b*). Among other second-messenger pathways involving intracellular cyclic AMP, cyclic GMP and Ca^{2+}, stimulation may also involve nitric oxide (Dow *et al.* 1994*a*), the first such demonstration in insect epithelia. Secretion rates *in vitro* can approach 6 nl min^{-1} $tubule^{-1}$ (Dow *et al.* 1994*b*), which means that maximal *in vivo* fluid output by all four main segments is likely to be at least 25 nl min^{-1}. We weighed adult female *Drosophila* from our stocks. Typical females not

carrying mature eggs weighed between 0.8 and 1.1 mg each, while females with large mature eggs weighed as much as 1.5 mg. Adult flying insects, such as honeybees, contain haemolymph equivalent to between 16 and 23 % of their mass (Dr T. Wolf, personal communication). Adult female *Drosophila* might, therefore, contain as much as 250 nl of haemolymph. It follows that fluid secretion by the main segments of the Malpighian tubules could potentially remove all fluid from the haemolymph in 10 min.

These very high rates of fluid secretion from stimulated main segments may have three types of explanation. The first is that *Drosophila* is much smaller than most insects whose Malpighian tubules have been studied. Since specific metabolic rates inexorably increase as size decreases (see, for example, Schmidt-Nielsen, 1990), we should expect to find that small Malpighian tubules transport fluid at higher rates than larger ones.

The second point is that adult *Drosophila* feed on fluids of various sorts – the very name *Drosophila* derives from the Greek meaning, roughly, 'fond of dew'. The ingested fluid may be much more dilute than the haemolymph, and small size again dictates that it will equilibrate rapidly with the haemolymph. If nectar is used as fuel for flight and flight consumes energy more rapidly in smaller insects, then the production of surplus water (Bertsch, 1984; Maddrell, 1986, 1987) will also be rapid. It may well be that adult *Drosophila* need on occasion to eliminate fluid at rates that, at first sight, might seem unnecessarily high.

Lastly, rapid secretion of fluid by the main segment, provided that it is followed by appropriate reabsorption in the lower tubule and hindgut, will permit prompt clearance of metabolic wastes from the haemolymph without concomitant water loss. Observation of hormonally mediated acceleration of fluid secretion by the Malpighian tubules of the desert beetle *Onymacris* led Nicolson (1991) to suggest that the active factors involved should be referred to as clearance hormones rather than diuretic hormones. The term 'clearance' is used in discussions of vertebrate kidneys in which filtration is fast but most of the fluid is recycled, leading to the elimination of unwanted substances but recovery of useful ones. Similarly, the high rates of fluid secretion by unstimulated *Drosophila* tubules, and further augmentation of these rates through intracellular second messengers produced in response to hormones in circulation, may derive from the need for rapid haemolymph filtration, during and after flight for example, as opposed to simple elimination of water.

Particularly during such clearance activities, the speed of fluid production by the main segments of the Malpighian tubules poses serious problems for the reabsorptive parts of the excretory system. Our results show that the lower tubules can carry out significant reabsorption of fluid and, in particular, can reabsorb K^+ at high rates. One residual difficulty is that the K^+ level in fluid secreted by whole tubules is still some two- to threefold greater than that in the haemolymph so, to maintain K^+ homeostasis, further reabsorption of these ions is required, probably in the hindgut. Similarly, the hindgut must reabsorb fluid at a high rate if the insect is to avoid rapid water loss. It is worth noting in this context that the K^+ concentration in the haemolymph of *Drosophila*, 35 mmol l^{-1}, is considerably higher than that found in Orthoptera and other Diptera, but similar to that of Hymenoptera and Lepidoptera (Altman and Dittmer, 1971).

We propose that the activity of the lower tubule is preparatory, reducing the amount of water and ions which the hindgut must reabsorb. In this regard, the lower tubule in *Drosophila* may be analogous to the ileum of the locust hindgut, where water and ions are recovered, allowing the rectum, downstream of the ileum, to process more thoroughly the reduced amount of fluid delivered to it (Phillips *et al.* 1986).

Previous studies have provided evidence for ion reabsorption by the lower Malpighian tubule in *Rhodnius prolixus* (Maddrell and Phillips, 1975; Maddrell *et al.* 1993) and in the crickets *Acheta domesticus* (Spring and Hazelton, 1987) and *Teleogryllus oceanicus* (Marshall *et al.* 1993). In *Rhodnius prolixus*, reabsorption of KCl reduces tubule fluid osmolarity by as much as 100 mosmol l^{-1} (Maddrell and Phillips, 1975). Although the ultrastructure of the lower tubule is homogeneous along its length, reabsorption is confined to the lowermost one-third of the lower tubule (Maddrell, 1978). The blood meal of *Rhodnius* is Na^+-rich and hypo-osmotic to the insect's haemolymph, whereas the primary urine elaborated by the upper tubule is iso-osmotic and rich in both NaCl and KCl. The lower tubule maintains osmotic and ionic homeostasis by reabsorbing KCl but only very small amounts of water, resulting in elimination of Na^+-rich urine, hypo-osmotic to the haemolymph. In contrast, the lower tubule of *Drosophila* is characterized by reabsorption of K^+, albeit less dramatically than is the case in *Rhodnius*, and by much more extensive fluid reabsorption. This suggests that the activity of the lower tubule of *Drosophila* can act as part of a clearance mechanism in which fluid is rapidly secreted into the tubule lumen by the main segments and is partly reabsorbed by the lower tubule and, presumably, also by the hindgut. The situation in *Rhodnius* is different as the lower tubule of this species is designed to permit reabsorption of KCl but not water, so that excess fluid from the blood meal is eliminated.

In *Acheta domesticus*, solute reabsorption is thought to occur in the lower Malpighian tubule, the ampulla, or both. When tubules are maintained *in vitro*, the secreted fluid is hyperosmotic to the medium by 5–10 mosmol l^{-1} under control conditions, but becomes 10–12 mosmol l^{-1} hypo-osmotic to the bathing saline when the tubules are stimulated with a homogenate of the corpora cardiaca (Spring and Hazelton, 1987). Both lower tubule and ampulla consist of columnar cells whose ultrastructure is typical of insect reabsorptive epithelia (Hazelton *et al.* 1988). In *Teleogryllus oceanicus*, fluid from the main segment of both stimulated and unstimulated tubules is hypo-osmotic to the bath (Marshall *et al.* 1993). The possibility that a downstream region of the main segment is specialized for ion reabsorption can be ruled out, since hypo-osmotic fluid can be collected from different lengths of the main segment. Reabsorptive cells may be dispersed throughout the epithelium.

Ca^{2+} elimination

We have found that both the main segments and lower segments of *Drosophila* Malpighian tubules rapidly transport Ca^{2+} into the lumen. Transport is much affected by the sign and size of the transepithelial potential. Thus, although the main segment transports Ca^{2+} at a high rate even when the TEP is made markedly lumen-positive (as, for example, when fluid secretion is stimulated by cyclic AMP), Ca^{2+} transport is greatly accelerated when the TEP is depolarized (for example, in the presence of leukokinin-1). In addition, as noted above, the lower tubules transport Ca^{2+} at a higher rate per unit length than does the main segment, and this can be correlated with the fact that the TEP in the lower tubule is much more favourable for Ca^{2+} movement into the lumen. Active transport of Ca^{2+} into the lumen of *Drosophila* tubules is a surprising finding, as isolated tubules from *Rhodnius*, for example, restrict transepithelial Ca^{2+} movements (Maddrell *et al.* 1991). Most of the calcium from the diet in *Rhodnius* is not eliminated but is deposited at very high concentration in concretion bodies in the cells of the upper Malpighian tubules (equivalent to the main segment of *Drosophila* Malpighian tubules) and any Ca^{2+} escaping from the concretions passes ten times faster to the haemolymph than to the lumen. Even in another dipteran, *Musca domestica*, Ca^{2+} is sequestered in concretions in the Malpighian tubules (Sohal, 1974), and so, presumably, is not transported into the lumen. In *Drosophila*, given that the haemolymph Ca^{2+} activity is about $1\,mmol\,l^{-1}$, the Malpighian tubules could remove when stimulated the entire Ca^{2+} content from the haemolymph within 10–20 min. The question therefore arises as to the Ca^{2+} content of the diet. We ashed a known mass of the medium by heating in a glass tube over a Bunsen flame on which the flies are raised and dissolved the resulting mineral ash in very dilute HCl. Even though not all the ash dissolved, the Ca^{2+} content of the fluid showed that the diet must have contained in excess of $4\,mmol\,l^{-1}$ Ca^{2+}. It may well be, therefore, that flies need to excrete excess Ca^{2+} from their food. Malpighian tubules of larvae of the alkali fly *Ephydra hians*, which live in Ca^{2+}-rich hypersaline waters, can also transport Ca^{2+} at high rates, in this case high enough to lead to deposits of calcium carbonate in the lumina of the tubules (Herbst and Bradley, 1989).

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