ELECTROPHYSIOLOGICAL AND CABLE PARAMETERS OF THE MALPIGHIAN TUBULE OF ONYMACRIS PLANA: EFFECTS OF DIURETIC HORMONE, cAMP AND HIGH AMBIENT K

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Summary

Isolated perfused Malpighian tubules of the desert beetle *Onymacris plana* (Coleoptera: Tenebrionidae) have been subjected to cable analysis under the following conditions: control, cAMP, diuretic hormone (DH) and high ambient K (130 mM). In addition, we examined the effects of perfusate composition on transtubular potential (VO), by reducing K, Na or Cl, or adding ouabain, furosemide or dinitrophenol. The effects of cAMP, DH and high K on VO and cable parameters were consistent with increased fluid secretion, i.e. diminished input and core resistances, and increased virtual short-circuit current, length constant and lumen diameter. They differed in that DH had variable effects on VO, and high K did not reduce transepithelial resistance. The effects of cAMP + DH or cAMP + high K were not additive. Alterations in perfusate composition were almost without effect. The stimulatory effects of cAMP, DH and high K appear to be mediated by different mechanisms. Lack of response to changes in the perfusate is attributed to the presence of unstirred layers associated with the apical microvilli.

Isolated perfused Malpighian tubules; desert beetle; cable analysis; mechanisms of stimulation of fluid secretion

INTRODUCTION

Physiological studies on insect Malpighian tubules have depended heavily on the method devised by Ramsay (21), in which unperfused tubules are bathed under liquid paraffin *in vitro*. Although there exists a wealth of information on tubule functioning and control by hormones (for reviews see Refs. 11, 12, and 20), little is known of the cellular mechanisms responsible for movements of ions and water in this epithelium. Only very recently have researchers started to apply electrophysiological techniques to Malpighian tubules (1, 13, 15, 16, 24, 25).

Fluid secretion in the Malpighian tubules of the desert tenebrionid beetle, *Onymacris plana*, is stimulated by a diuretic hormone (DH) and exogenously administered cAMP (14). Although their *in vitro* secretion rates when stimulated are as fast as those of the tubules of blood-sucking insects, *Onymacris* tubules secrete the K-rich fluid which is more typical of insect tubules (20). Stimulation of fluid secretion in *Onymacris* tubules by DH or cAMP is accompanied by pronounced but differing effects on the transepithelial potential (as measured by the 'oil-gap' technique; 15). *Onymacris* tubules thus appear very suitable for detailed study of the electrical phenomena associated with fluid secretion.

Measurement of the transtubular potential (VT) by the 'oil-gap' technique in unperfused tubules is prone to gross errors (7). These errors can be avoided if the tubule

segments are perfused *in vitro*, using the technique developed by Burg and co-workers (3, 4). Another major advantage of the perfusion technique is that cable analysis can be used to assess changes induced by the various diuretic agents in both the physical properties of the tubule (length constant, transtubular resistance, lumen diameter) and in the active transport 'pump(s)' (virtual short-circuit current; see Methods). Furthermore, control of the composition of the perfusate permits exploration of the luminal effects of different ionic concentrations, of specific ion channel blockers, and of metabolic inhibitors. This is of particular interest in insect Malpighian tubules in which the apical membrane is the site of the putative K pump (20). The only previous applications of the perfusion technique to Malpighian tubules have been confined to those of mosquitoes (9, 18, 22, 25).

We report here the results of cable analysis on perfused isolated segments of the Malpighian tubules of *Onymacris plana*, in which fluid secretion was either stimulated (by exposure to cAMP, DH or high ambient K concentrations) or inhibited (by exposure to dinitrophenol (DNP), or to a zero ambient Cl concentration), in an attempt to contribute to the understanding of the mechanisms underlying fluid secretion in this epithelium. The importance of external K and Cl to fluid secretion in Malpighian tubules has been well documented (20). Apical ion channels, and luminal factors possibly contributing to the transtubular potential and so to fluid secretion were also sought; these studies included addition of metabolic inhibitors to the perfusate

and changes in perfusate ionic composition in both control and cAMP- or DH- stimulated tubules.

MATERIALS AND METHODS

Tubules were obtained from adult female beetles (*Onymacris plana*), collected by the Desert Ecological Research Unit at Gobabeb, Namibia, and maintained in the laboratory as described previously (14). A segment of the mid-portion of one of the more accessible dorsal or lateral tubules was dissected out: the tubules of *O. plana* are structurally homogeneous along most of the length of the free segment (5). Usually only one tubule segment was used from any one beetle. The isolated segment was perfused *in vitro* as described by Burg et al. (3). As the transepithelial potential was found to be independent of the rate of perfusion, the height of the reservoir of perfusion fluid was held constant at some 12 cm above the bath, throughout the experiments reported here. The actual rate of perfusion was not measured routinely, but in a few unstimulated tubules was noted to be 40 to 60 nl/min. All experiments were carried out at room temperature (21-23 ^{o}C); most were of 2-5 hr duration.

In those experiments in which the tubule was exposed to cAMP, DH, DNP, high K, or zero Cl concentrations in the bath, and in which cable analysis was to be performed, care was taken to ensure that only short lengths (0.5-1 mm) of tubule were exposed to the bath solution; this minimised errors in measurement of the current-induced voltage jumps at the distal end of the tubule (8). A layer of unpolymerised Sylgard (Dow Corning) within the orifices of both the holding and collecting pipettes ensured electrical

isolation of the tubule lumen from the bath. Changes in stimulant, inhibitor or solute concentrations of the bathing fluid were effected by three-fold washout of the bath (2 ml) with the new solution.

The bath was held at ground potential by a saturated KCl calomel electrode, immersed in bath fluid and joined to the bath by a short wide-bore (5 mm ID) 3% agar saturated-KCl bridge; the dimensions of this bridge were dictated by the need to ensure a non-detectable (<0.5 mV) potential drop at this point during passage of current through the tubule (cf. below). Similar electrodes detected the transtubular electrical potential differences at both the proximal (VO) and distal (VL) ends of the tubule. The electrode at the proximal end was placed within the perfusion reservoir, while that at the distal end, bathed in perfusion fluid, was joined to the luminal fluid within the collecting pipette by a thin 3% agar saturated-KCl bridge. Both VO and VL are expressed relative to the bathing solution. Possible inter-electrode drift was checked by occasionally joining the latter electrodes to the bath by still other 3% agar saturated-KCl bridges; this was found to be a necessary precaution in view of the sometimes small (<5 mV) voltage jumps detected at the far end of the tubule on current injection.

The calomel electrodes sensing VO and VL were joined to high input impedance (>10^{12} ohms) unity-gain voltage followers; that at the proximal end (Model P1, Bioelectric Instruments, U.S.A) incorporated a bridge circuit, permitting

measurement of the jump in proximal transtubular potential on injecting current into the tubule via the perfusion pipette. As the resistance of the perfusion pipette was some 5 to 6 megohms, while the tubule input resistance was only a few hundred kilohms (see Results), it was found advantageous to replace the single-turn bridge-balance potentiometer originally provided in the Model P1 by a 20-turn potentiometer; this permitted precise and reproducible balancing of the bridge. Small changes in bath temperature (1-2 ^{o}C) were found to cause significant bridge imbalance (presumably by altering both the conductivity of the fluids within the bath, and the dimensions of the tip of the perfusing pipette). Accordingly, all solutions - which were stored frozen - were allowed to equilibrate to room temperature before use. The current-induced transtubular voltages, the temporal excursions of which were monitored on an oscilloscope, did not exhibit polarisation potentials (6), i.e. were of constant amplitude in the last two thirds of the 600 msec current pulse; only these latter voltages were recorded. In any given tubule, at any given time, the amplitude of this current-induced voltage was found to be linearly proportional to the amount of current injected, and symmetrical about zero; this relationship was occasionally found useful in checking the bridge balance during prolonged experiments.

VO was displayed on a digital millivoltmeter, of 0.1 mV maximal resolution, and recorded continuously on a millivolt chart recorder. The tubule was subjected to hyperpolarising current pulses, of 600 msec duration and 200 nano-amps

maximum amplitude, at short intervals (usually 5-10 min) throughout each experiment. Where transient changes in VO of only a few min duration were encountered, as for example immediately after addition of DH to the bath, this interval was as short as 1 min. The values of VO and VL, as accessed repeatedly (3x) immediately before and then during the last half of each 600 msec current pulse, were captured on an Apple II micro-computer, equipped with an 8-bit multi-channel A/D converter (Mountain Hardware, U.S.A); maximal resolution was 0.5 mV.

As described previously (15), the Malpighian tubule of *Onymacris plana* frequently displays small and regular oscillations in transtubular potential, each voltage excursion being accompanied by a localised contraction of the muscle associated with the tubule wall. Care was taken to inject intra-luminal current pulses only between contractions, except when the effects of these contractions upon the tubule's cable parameters were being investigated.

In those experiments in which the effects of changes in perfusate composition (altered ionic composition, or addition of inhibitors) on VO were studied, it was found convenient to perfuse longer (2-3 mm) tubule segments, with their distal ends left open to the bath. As shown below, the length constant of *Onymacris* tubules is but a small fraction of a mm. Thus shortening these long tubule segments by 0.5 to 1 mm, or advancing the perfusion pipette 200-300 μm down the length of the tubule, was found to be without effect on VO.

The tubule outer diameter, and the distance between the Sylgard seals at its ends, were measured with an ocular micrometer. Specific resistance of the perfusate, measured with a laboratory conductivity meter, was 61.5 ohm.cm. These values, in conjunction with the changes induced in VO and VL by current injection, were employed to calculate, by cable analysis, the tubule's input resistance, length constant, transepithelial and core resistances, and the lumen diameter. The relevant equations have been described (6), and their use commented upon repeatedly (8, 17), and are accordingly not reproduced here. The short-circuit current, a measure of active transport pump activity, may be calculated either corrected for surface area (SCC; μAmp/cm^2):

SCC = VO/(transepithelial resistance x PI x lumen diameter)

or as the 'virtual' short-circuit current (SCCv; μAmp/cm):

SCCv = VO/transepithelial resistance.

As SCC is a function of both pump activity and of surface area, and as changes in lumen diameter (and so of surface area) featured in most of the experiments reported below (see Results), short-circuit current is reported here as SCCv rather than as SCC (other than in Table 1, where both measures are listed, by way of illustration).

Solutions. The control bath solution, based upon the haemolymph composition of *Onymacris plana* (14), was very similar to that used previously on unperfused tubules (15).It contained (mM): 125 NaCl, 15 KCl, 5 $MgCl_2$, 2 $CaCl_2$,

6 $KHCO_3$, 4 KH_2PO_4, 10 glycine, 10 proline, 10 serine, 10 histidine, 10 glutamine, and 50 glucose. The high-K, low-Na perfusion fluid was intended to approximate the *in vivo* situation and previous experiments on unperfused tubules. The control perfusate contained 20 mM NaCl and 120 mM KCl, but was otherwise identical to the control bath solution; this solution was also used as the bath solution in those experiments in which the tubules were to be exposed to a high ambient K concentration. The basal membrane of *Onymacris* tubules, although highly permeable to K, is apparently impermeable to Na (15), so that the simultaneous change in bath Na concentration is irrelevant.

Bath solutions of zero Cl concentration were made by replacing NaCl by sodium isethionate, KCl by potassium gluconate, $MgCl_2$ and $CaCl_2$ by the respective sulphates, and adding extra glucose to maintain the normal osmolality (385 mOsm/kg water). Perfusates of low Cl (14 mM) concentration were made similarly. Perfusates of low Na (2.5 mM), or K (13 mM) concentration were made by replacing NaCl or KCl with equivalent concentrations of choline chloride. The effects of altering perfusate concentrations of these ions were tested under control conditions, with DH or cAMP in the bath, and also with the Cl-free solution in the bath.

The solution in which the tubules were dissected was identical to that of the control bath, except that additional glucose was substituted for the amino acids. Routine addition of traces of phenol red to all solutions provided a check that the pH was maintained at about 7.0.

Drugs and DH. Where indicated, the following were administered dissolved in either the bath solution or the perfusate: cAMP (1 mM; Na salt, Sigma); ouabain (1 mM; Sigma); 2',4'-dinitrophenol (DNP; 1 mM; British Drug Houses); furosemide (3 mM; prepared from Lasix, National Labs.). Diuretic hormone extract (DH) was prepared by homogenising the corpora cardiaca of the donor beetle in 5 µL of control bath solution.

Statistics. Results are presented as means +/- SD. Paired and independent sample t-tests were used to assess the significance of differences between means. P values of <0.05 were regarded as significant.

RESULTS

Controls. The Malpighian tubule of *Onymacris plana* was usually so heavily pigmented as to render visualisation of the lumen impossible. In a few tubules with relatively little pigment (Fig. 1), the luminal cross section was seen to be markedly irregular, occasional cells protruding prominently into the lumen. Externally, the tubules had a correspondingly beaded appearance. The maximum outer diameter of perfused, non-stimulated and non-contracting tubules was about 138 μm (range 110-184 μm). There was no correlation between the optically measured outer diameter and the calculated lumen diameter; furthermore, as will be shown below, while exposure to cAMP, DH or 120 mM K consistently increased the calculated lumen diameter, the outer diameter remained unchanged (Tables 2 & 4).

The transtubular potentials recorded at the proximal and distal ends of the tubule were almost never identical. At the start of perfusion, VO was always positive. VL was smaller than VO, and sometimes even a few mV negative relative to the bath (when VO was low). During the first half-hour of perfusion, VO usually fell, more or less steeply (Fig. 2), while VL decreased to a lesser extent. Throughout the 2-5 hr period of perfusion, VL was almost always markedly (on average, 10 mV) less than VO. Changes induced in VO by any experimental procedure were accompanied by parallel, and often smaller, changes in VL.

Stability of the bridge balance within the current injection module was checked by monitoring the calculated cable parameters over prolonged periods. Fig. 2 depicts the temporal changes in VO, and in the cable parameters, as seen in four tubules perfused for 120 min; in each tubule, multiple consecutive determinations yielded consistent or smoothly changing values of the various parameters.

Contractions of the tubule muscle often commenced within a few minutes of starting perfusion, were sometimes as frequent as one or two a minute, appeared to be localised constrictions rather than longitudinally peristaltic, and came and went for prolonged periods, for no apparent reason. They were abolished on adding 120 mM K or DNP, and sometimes DH, to the bath. Table 1 lists the values of VO and of the various cable parameters during and between three successive contractions, as found in a single tubule (with 1 mM cAMP in the bath). The contractions occurred at 1 min intervals. During the successive intervening 'relaxed' periods, VO and the various cable parameters were little changed. During contraction, the calculated lumen diameter fell markedly, with corresponding changes in VO, input resistance, length constant, short circuit current (SCC) and core resistance, while the transtubular resistance (Rtrans) and 'virtual' SCC remained essentially constant.

cAMP. In 17 instances, 1 mM cAMP was added to the bath 19 min to 300 min (mean 89 min) after initiating tubule perfusion. In light of the expected increase in VO (15), and in the hope of maximising such concomitant changes in the

cable parameters as might be induced, tubules with lower (<45 mV) rather than higher values of VO were usually selected for exposure to cAMP.

Table 2 lists the values of VO and the various cable parameters immediately before administration of cAMP, with those at the time of maximal cAMP-induced increase in SCCv: this coincided with the peak effect on VO. VO and SCCv increased, while Rtrans fell; the luminal diameter increased, with corresponding falls in the core and input resistances, and an increase in the length constant.

Thus essentially cAMP stimulates both the 'pump' (increases SCCv) and reduces Rtrans.

DH. In 8 tubules, DH was added to the bath solution 16 min to 92 min (mean 54 min) after the start of perfusion. Tubules with higher rather than lower values of VO were usually selected, the rationale being similar to that given for the selection of tubules for exposure to cAMP (see above).

While the response in VO was sometimes (n=3) similar to that found previously in non-perfused tubules (a prompt and sustained fall in VO; 15), here VO usually (n=6) fell promptly, only to return towards (n=2), or even exceed (n=1), its initial value a few mins later; in 2 instances there was no initial fall, but rather a progressive increase in VO after addition of DH to the bath (Fig. 3). As the immediate responses in the various cable parameters were

equally diverse, only the steady-state values of the delayed responses are listed in Table 3. The latter all occurred within a few min of administration of the DH, although not necessarily attaining their maximum values simultaneously. The overall effects of DH were to increase SCCv, reduce Rtrans, and increase the luminal diameter, with a corresponding fall in the input resistance.

The effect of DH on tubules already stimulated by cAMP was examined in another 5 tubules, in order to see whether or not the effects were additive. The response was less complex than that described above, in that an immediate and sustained fall in VO was seen in 4 of the 5 instances, only one tubule displaying a partial return towards the initial value of VO, some min later. Changes in the cable parameters were similarly less diverse. The overall effects of DH in the presence of cAMP were to reduce VO, SCCv and Rtrans (Table 3).

Thus the effect of DH in reducing Rtrans appears to be additive to that of cAMP, whereas its stimulant effect on SCCv appears to compete with that of cAMP.

130 mM K. Table 4 compares the values of VO and the various cable parameters found in 9 tubules, with those found after replacing the control bath with perfusate fluid; the latter values are those pertaining when the increase in lumen diameter was maximal (this being the most consistent effect found), some 6 min after the fluid change. VO and the SCCv increased; Rtrans however did not change significantly. The

luminal diameter increased, with a corresponding fall in the input resistance; the core resistance fell too, but not significantly ($p<.07$), the large drop in the mean value resulting from large falls in only 2 of the 9 tubules, the remainder having had a low level of core resistance initially. The single essential difference between these effects and those seen after exposure to cAMP is that Rtrans was not reduced by increasing the bath K concentration to 130 mM.

Table 4 also lists the response of 5 tubules, exposed to 1 mM cAMP, on replacing the bath fluid by perfusate solution containing a similar concentration of cAMP. The only significant response was an increase in VO.

Thus increasing the K concentration of the bath increased both VO and the SCCv, but had no effect on Rtrans. In the presence of cAMP, the only effect was to increase VO still further.

Zero Cl. Table 5 lists the changes observed on removing Cl from the bath. The only significant change was an increase in Rtrans.

DNP. Table 6 shows that the only effect of the addition of DNP to the bath was to reduce the 'pump' activity (SCCv and VO). Table 7 gives data for two tubules to which DNP was added after previous exposure to cAMP, and in these instances lumen diameter and length constants also decreased, while transtubular, core and input resistances

rose. Similar effects were seen on the application of DNP to tubules previously treated with DH (not shown).

Perfusate changes. As shown in Table 8, reducing the Na (n=13) or Cl (n=8) concentrations of the perfusate, by factors of 8 and 11 respectively, was without effect on VO. However, reducing the perfusate K concentration by a factor of 10 (n=8) caused VO to increase by a few mV.

In the presence of cAMP or DH, changing the perfusate's K, Cl or Na concentration was without effect on VO (Table 8). When the bath contained Cl-free solution, so that fluid secretion was presumably greatly reduced, changes in the K or Cl concentration of the perfusate still had no effect on VO or on the cable parameters (not shown).

Addition of DNP (n=5) or furosemide (n=4) to the perfusate was without effect on VO, as was addition of furosemide to the bath (n=5). VO was also insensitive to perfusion of 1 mM ouabain at either 22°C (n=2) or 37°C (n=2).

DISCUSSION

Although not shown in the Results, VO was consistently several mV larger than VL. This finding is far from new (6). We have shown elsewhere (7) that VL, in turn, is indistinguishable from the transtubular potential as measured in unperfused tubules. In the latter, measurements are based on the 'oil-gap' technique, in which the recording electrode is placed beyond the distal end of the tubule. As the electrode recording VL is similarly placed, distal to the insulated portion of the far end of the perfused tubule, the identity beteen VL and the earlier estimates of transtubular potential is not surprising. Essentially the discrepancy between VO and VL arises in residual electrical activity in the terminal fraction of the tubule within the collecting pipette (7). The phenomenon is relevant here only because it must be borne in mind in comparison of the transtubular voltages (VO) reported here, with those we reported previously in unperfused *Onymacris* tubules (15). It is irrelevant to cable analysis, in which only VO and the current-induced voltage jumps at the proximal and distal ends of the tubule enter into the calculations.

Validation of the cable technique. Direct validation of the application of cable analysis to perfused tubules has been sought in the past by establishing identity between the optically measured and the calculated lumen diameters. However, as pointed out earlier, the lumen of the Malpighian tubule of *Onymacris plana* is not only of highly irregular contour, but very frequently impossible to visualise. Nor was there any consistent relationship between the calculated lumen diameter and the external diameter. Indirect assurance of the validity of cable analysis in perfused *Onymacris* tubules may however be found in the following.

The changes observed in the cable parameters during contraction of the muscular wall of the tubule (Table 1) are just those anticipated for a reduction in lumen diameter, uncomplicated by changes in the intrinsic resistance of the tubule wall or in pump activity. Agents known to stimulate fluid secretion (cAMP, DH, high ambient K concentrations) might be expected to increase the lumen diameter, and so reduce the core resistance and increase the length constant; these were just the effects observed (Tables 2-4); the concomitant increases in 'virtual' short-circuit current are in keeping with their generally accepted effects of stimulating the apical pump. Chloride is thought (2) to diffuse passively through the salivary glands of *Calliphora*, and forms a major fraction of the anionic component of the luminal secretion in this epithelium and in Malpighian tubules (20). Appropriately, as revealed by cable analysis, the only effect of removing chloride from the bath was to increase the transtubular resistance (Table 5). DNP abolishes tubular secretion, while inhibiting the pump; cable analysis revealed no change in transtubular resistance, lumen diameter, core resistance or length constant, while VO plunged and the 'virtual' short-circuit current fell to less than zero (Table 6). When the tubule had previously been exposed to cAMP these changes were accompanied by a fall in lumen diameter and length constant,

and a rise in transtubular, core and input resistances (Table 7), all changes consistent with inhibition of fluid secretion. Some evidence for the validity of the absolute values of the various parameters provided by cable analysis in *Onymacris* Malpighian tubules is given below.

The secretory properties of *Onymacris* tubules have been studied in some detail using Ramsay's method (14, 21). Unperfused, non-stimulated tubules secrete about 3 nl/min/tubule. This increases to 40-60 nl/min/tubule, and occasionally to as much as 100 nl/min/tubule, on exposure to DH. The response to cAMP is similar, if somewhat less marked. The secreted fluid, assumed to be isosmotic with the haemolymph (20), contains about 180 mM K and 20 mM Na, the latter increasing to about 45 mM after exposure to DH. Both K and Na are thought to be translocated by an apical pump (20). On this basis, and assuming an average tubule length of 7 cm within the bathing drop, a secretion rate of 50 nl/min/tubule can be calculated to be equivalent to an open-circuit current of about 2.5 μAmp/cm, or less than a quarter to a third of the maximum SCCv found here (Tables 2-4). This discrepancy is not as large as it may at first appear. Open-circuit current is necessarily less than short-circuit current, the precise relationship being a function of the shunt and series resistances in the equivalent electrical circuit. As these resistances have yet to be determined for *Onymacris* tubules, it is difficult to comment further on the absolute value of the difference between the open- and short-circuit currents. It may be relevant here to point out that at least the paracellular component of Rshunt - that of

the intercellular spaces - can be expected to be large (5, 10). It may also be relevant that the sum of the K and Na concentrations in the fluid secreted by unperfused tubules (above) is about 50 mM greater than that of the perfusate in the experiments reported here; this greater opposing chemical concentration gradient could conceivably have partially reduced the transport capacity of the unperfused tubules.

In short, while we have no evidence for the accuracy of their absolute values, the estimates of the various parameters derived by cable analysis in perfused *Onymacris* tubules appear to be at least relatively valid, and are probably not far removed, if at all, from their actual values. The only previous application of cable analysis to Malpighian tubules is the work of Beyenbach and colleagues on *Aedes*; they measured a similar length constant (300 μm; 19) and also found a drop in Rtrans on stimulation by cAMP or head extract (25). However, mosquito tubules differ greatly from those of *Onymacris* in that the primary effect of cAMP is to stimulate NaCl secretion by increasing the Na conductance of the basolateral membrane (18, 22).

Evidence for different mechanisms of stimulation. The effects of exposure to stimulants - cAMP, DH or a high K concentration - were similar in that in each instance both the SCCv and the lumen diameter increased. This might suggest a common mechanism of fluid secretion secondary only to increased pump activity. Closer examination, however, reveals striking differences in the effects of these various

agents on the transtubular resistance. In the following the intracellular potentials referred to are those found previously (15) in similarly treated but unperfused *Onymacris* tubules. The responses are analysed in terms of the simplest possible equivalent electrical circuit, comprising: a battery (E) representing the electromotive force of the active transport mechanism; a series resistance (Rser) representing the resistance encountered by ions within the active transport pathway; and a shunt resistance (Rshunt), representing the transepithelial leak pathways for all other ions. Thus the transtubular resistance (Rtrans), as measured by cable analysis, represents the parallel combination of Rser and Rshunt. Actively transported Na and K ions are assumed to share a common pump (20).

Following exposure to cAMP (Table 2), Rtrans falls, while VO increases. The potential across the basolateral membrane (Vb) remains unchanged, while that across the apical membrane (Va) increases. Thus the fall in Rtrans reflects a fall in Rser, with little or no change in Rshunt (a conclusion in keeping with our earlier observation that cAMP apparently reduces the Cl permeability of the basolateral membrane). As short-circuit current = E/Rser, and as VO rises proportionately more than the fall in Rtrans, it follows that the cAMP-induced rise in SCCv reflects both a rise in E and a fall in Rser.

Exposure to DH (Table 3) also results in a fall in Rtrans. In unperfused tubules, the transtubular potential falls sharply, while Va approximates to Vb, so suggesting - in

contrast to the response to cAMP - a marked fall in Rshunt (with or without a lesser change in Rser). This could conceivably enhance tubular fluid secretion, by facilitating Cl entry into the lumen. In its electrophysiological responses to stimulants, the Malpighian tubule of *Onymacris* shows some resemblance to the salivary gland of *Calliphora*; in the latter the controlling hormone brings about a cAMP-dependent stimulation of an apical K pump and a Ca-dependent increase in Cl permeability (2).

The response to DH of perfused tubules of *Onymacris* is less consistent than that of unperfused tubules, in that here VO sometimes rises rather than falls; overall, there is no change in VO. We have no explanation for the different responses in transtubular potential, except that they are probably a consequence of using a crude homogenate of the corpora cardiaca: in mosquitoes, for example, head extract contains three fractions with differing effects on transtubular potential and secretion rates of Malpighian tubules (19). SCCv increases when DH is applied to perfused tubules of *Onymacris*. Thus, in addition to reducing Rshunt, the effect of DH, in terms of the equivalent electrical circuit, must be to also induce either a fall in Rser, or a rise in E, or both.

The response of perfused tubules to DH, after previous exposure to cAMP (Table 3), differs significantly from that observed in the absence of cAMP. SCCv falls, suggesting competition between cAMP and DH for the active transport mechanism; in terms of the equivalent electrical circuit,

this reflects either a fall in E, a rise in Rser, or both. As the addition of DH reduces Rtrans still further, as in the absence of cAMP, this presumably reflects the effect of DH on reducing Rshunt, as above. As judged by the absence of change in tubule diameter, core resistance, and length constant, the rate of secretion of fluid into the lumen remains unaltered.

Unlike cAMP or DH, a high K concentration in the bath is without effect on Rtrans (Table 4). Measurement of intracellular potentials reveals that lowering the bath K increases both Vb (in Nernst-like fashion) and Va. Raising the bath K concentration presumably has the opposite effects. Increased entry of K into the cell (in consequence of a more favourable concentration gradient) might be expected to increase the activity of the apical K pump (E), while a reduction in Vb would favour Cl entry into the cell, so permitting increased secretion of KCl into the lumen. In the presence of cAMP, the only effect of increasing the bath K concentration is to increase VO, as expected with reduction of the K-dependent basolateral potential.

Thus the similar effects of cAMP, DH and a raised ambient K concentration on increasing fluid secretion, although all ultimately reflecting increased active transport, might be based upon different mechanisms; that of cAMP in reducing Rser, and increasing E; that of DH in reducing Rshunt, and a cAMP-competitive effect on Rser and/or E; and that of K in increasing E, while enhancing Cl entry into the cell.

Alterations in perfusate composition. The effects of changes in perfusate composition (altered ionic composition, or addition of metabolic inhibitors) on VO were investigated in an attempt to identify luminal factors affecting pump activity, and the presence of specific ion channels. The only effect observed was a 5 mV increase in VO on reducing the lumen K concentration from 130 mM to 13 mM, and even this was absent in the presence of cAMP or DH (Table 8). It is noteworthy here that VO in perfused tubules of the mosquito *Anopheles* is also unaffected by changes in the tubular perfusate (9).

A possible explanation of this paucity of effect might be that continual fluid secretion keeps the perfusate away from the apical membrane. The abolition of the effect of a reduced K concentration following the addition of stimulants - and so presumably enhanced fluid secretion - is in accord with this suggestion. An alternative or additional explanation might be the presence of unstirred layers. As in the Malpighian tubules of other species, the luminal surface of *Onymacris* tubules has a prominent brush-border, with deep channels ('canaliculi') between adjacent cells (5). Unstirred layers here might underlie the similar minimal or absent responses to changes in perfusate K and Cl concentrations obtained with no chloride in the bath, and so presumably in the absence of fluid secretion. On balance, the evidence suggests that both factors - unstirred layers and fluid secretion - are operative. In this respect it is of interest that unstirred layers are thought to play little part in the fluid-absorbing mammalian renal tubule (23).

Although flow rates in perfused preparations of these insect and mammalian epithelia are similar, and both possess apical microvilli, the proximal tubule has nothing analogous to the 'canaliculi' mentioned above and its inner diameter is about a quarter that of the Malpighian tubule.

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TABLE 1. Effects of muscle contractions on VO and cable parameters.

	REL	CON	REL	CON	REL	CON
Time	100	101	101.5	102	102.5	103
VO	41	51	45	52	48	56
Rinput	152	372	165	382	175	356
Lambda	270	105	277	119	269	135
Rtrans	4.01	3.93	4.45	4.55	4.60	4.80
SCCv	10.3	13.0	10.1	11.4	10.5	11.7
SCC	869	2776	874	2328	951	2152
Rcore	5.51	35.40	5.80	32.11	6.37	26.38
ID	38	15	37	16	35	17

VO and cable parameters were recorded over a 3 min period during and between muscle contractions. Tubule had been perfused for 100 min and exposed to cAMP.
Abbreviations and units: REL = muscle relaxed; CON = muscle contracted; Time (min); VO = proximal transtubular potential (mV); Rinput = input resistance (kohm); Lambda = length constant (μm); Rtrans = transepithelial resistance (kohm.cm); SCCv = 'virtual' short-circuit current (μAmp/cm); SCC = short-circuit current (μAmp/cm^2); Rcore = core resistance (megohm/cm); ID = luminal diameter, as calculated by cable analysis (μm).