Voltages and resistances of the anterior Malpighian tubule of *Drosophila melanogaster*.

Klaus W. Beyenbach

Department of Biology/Chemistry, Division of Animal Physiology, University of Osnabrück, Barbarastrasse 11, Osnabrück 49076, Germany

Send correspondence and reprint requests to:

KWB1@CORNELL.EDU

Phone: 011 49 541 969 12881

FAX: 011 49 541 969 12881

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Summary Statement

Conventional microelectrodes and cable analysis yield first measurements of electrical resistance in *Drosophila* Malpighian tubules.

Abstract

The small size of Malpighian tubules in the fruit fly *Drosophila melanogaster* has discouraged measurements of the transepithelial electrical resistance. The present study introduces two methods for measuring the transepithelial resistance in isolated *Drosophila* Malpighian tubules using conventional microelectrodes and PClamp hardware and software. The first method uses three microelectrodes to measure the specific transepithelial resistance normalized to tubule length or luminal surface area for comparison with resistances of other epithelia. The second method uses only two microelectrodes to measure the relative resistance for comparing before and after effects in a single Malpighian tubule. Knowledge of the specific transepithelial resistance allows the first electrical model of electrolyte secretion by the main segment of the anterior Malpighian tubule of *Drosophila*. The electrical model is remarkably similar to that of the distal Malpighian tubule of *Aedes aegypti* when tubules of *Drosophila* and *Aedes* are studied in vitro under the same experimental conditions. Thus, despite 189 millions of years of evolution separating these two genera, the electrophysiological properties of their Malpighian tubules remains remarkably conserved.

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Introduction

The transepithelial electrical resistance is easily measured in a planar epithelium as the ratio of the transepithelial voltage deflection consequent to a transepithelial current pulse at uniform current density (Ohm's Law). The current density cannot be uniform in a tubular epithelium unless a wire is passed through the tubule lumen, which so far has been accomplished only once in a renal epithelium. Taking advantage of the large size of the renal proximal tubule of the aquatic salamander *Necturus maculosus*, Spring was able to pass a platinized tungsten wire through the tubule lumen to measure 43 Ωcm^2, the specific transepithelial resistance R_t (Spring and Paganelli, 1972). All other measurements of R_t in tubular epithelia are grounded in cable theory (Taylor, 1963) which models the tubule as an electrical cable. The fluid in the tubule lumen is considered the conductive cable core and the surrounding epithelial cells are considered the insulator. Current is passed into the tubule lumen from a point source (microelectrode), and the transepithelial voltage deflections are recorded with microelectrodes at other points in the tubule lumen. The decay of the injected current along the length of the tubule depends on 1) the tubule geometry (length and lumen diameter), 2) the resistivity of the fluid occupying the tubule lumen, and 3) the electrical properties of the epithelial cells, i.e. the transepithelial resistance.

The tubule may be modelled as a cable of finite length or infinite length. In the finite-length model, the two ends of the tubule are electrically isolated from the peritubular medium, defining the tubule length as in isolated perfused renal tubules (Burg et al., 1966; Helman, 1972). When the electrical isolation of the tubule ends is not possible, the tubule is considered a cable of infinite length (Boulpaep and Giebisch, 1978; Froemter, 1986; Hegel et al., 1967). For both models, variations of single- and double-barreled microelectrodes and measuring circuits have been employed to determine the cable parameters. In the present study I introduce the use of three conventional microelectrodes in the tubule lumen to determine the tubule length constant, the transepithelial resistance and the core resistances in the main secretory segment of the anterior Malpighian tubule of *Drosophila*. Intact pairs of anterior Malpighian tubules still attached to a small piece of gut are isolated in Ringer solution and transferred to rest on the bottom of a perfusion bath (see Fig. 1). One microelectrode injects current into the tubule lumen of the main segment and two additional microelectrodes in the tubule lumen measure the transepithelial voltage deflections at different sites downstream. The data are then analyzed on the basis of the infinite-length cable model to yield specific and effective transepithelial and core resistances. The use of only two microelectrodes in the tubule lumen, one to inject current and the other to record voltage, yields relative resistances that are useful in qualitative before and after comparisons in a single tubule.

The usefulness of cable analysis in renal and Malpighian tubules is manifold. Cable analysis yields measures of the transepithelial electrical resistance that spans the range from leaky to tight epithelia. Leaky epithelia usually transport large volumes of fluid in isosmotic proportions, while tight epithelia are capable of separating solute from water thereby forming either dilute or concentrated fluids on one side of the epithelium. Thus, cable analysis gives first clues about the general transport properties of the tubule. Supplemented with ion substitution in the bathing media, cable analysis yields measures of ionic permselectivity, and together with measurements of the intracellular voltages, cable analysis reveals the electromotive forces and resistances of basolateral and apical cell membranes. In brief, cable analysis is the necessary electrical experimental method for unravelling the mechanism and regulation of transepithelial electrolyte transport in tubular epithelia. In the present study, cable analysis has revealed a moderately tight epithelium in the main segment of the anterior Malpighian tubule of *Drosophila melanogaster* and yielded an electrical model of transepithelial electrolyte secretion that is remarkably similar to the model of electrolyte secretion in distal tubules of *Aedes aegypti* when both tubules are studied under similar experimental conditions.

Materials and Methods

Drosophila melanogaster. We considered *white*[1118] (RRID:BDSC 5905) as the wild type. The flies were reared under standard conditions at 22 ºC on cornmeal agar and a 12L:12D light cycle. Malpighian tubules of *Drosophila* are formed during the first 21 hours of embryogenesis at 25 ºC, i.e. before the hatch of the first instar larva. Thereafter, the tubules are not rebuilt during metamorphosis and remain almost unmodified to the adult (Denholm et al., 2003; Singh et al., 2007; Sozen et al., 1997). The development of the tubule stops as the number of cells in the upper tubule (initial, transitional and main segments of the tubule) has reached approximately 150 cells (Singh et al., 2007). Of these, 80% are principal cells with ectodermal origin and 20% are stellate cells with mesodermal origin (Jung et al., 2005; Pugacheva and Mamon, 2003; Singh et al., 2007; Sozen et al., 1997).

Adult female flies at least 5 days old were cold-anesthetized on ice. Pairs of anterior Malpighian tubules were isolated with the cold-anesthetized fly submerged in Ringer solution as shown in the video produced in the laboratory of Rodan (Schellinger and Rodan, 2015). The main segment of the anterior tubule which secretes most of the fluid arriving in the gut (Dow et al., 1994) was the focus of this study.

Ringer Solutions. The basic Ringer solution (BRS) contained in mM: 117.5 NaCl, 20 KCl, 2 $CaCl_2$, 8.5 $MgCl_2$, 10.2 $NaHCO_3$, 15 HEPES, 4.3 NaH_2PO_4, and 20 glucose. For measurements of transepithelial Cl^- diffusion potentials, the Cl^- concentration in BRS was reduced 10-fold and included, in mM: 117.5 Na-gluconate, 20 K-gluconate, 2 $CaCl_2$, 5.95 $MgCl_2$, 3.83 $MgSO_4$, 10.2 $NaHCO_3$, 15 HEPES, 4.3 NaH_2PO_4, and 20 glucose. The pH was adjusted to pH 7.0. The average $[H^+]$ of all solutions was $(9.6 \pm 0.07) \times 10^{-8}$ M (50), pH 7.02. The osmotic pressures of the Ringer solutions containing 100 % Cl^- and 10% Cl^- solution were respectively 335.6 ± 1.2 (43) mOsm/kg H_20 and 332.3 ± 1.3 (6) mOsm/kg H_20. Drosokinin (JPT Peptides, Berlin) was used at a concentration of 1µM. To estimate the epithelial shunt resistance R_{sh}, BRS was initially exchanged with BRS containing 2,4-dinitrophenol (DNP), KCN, and NaN_3 (each 1 mM). However, this inhibitory cocktail of ATP synthesis (and transcellular active transport) proved insufficient for estimates of R_{sh} due to the high K^+ conductance of basolateral membranes. For this reason, a low-K^+ phosphate-free BRS containing 2 mM K^+, 5 mM $BaCl_2$ and the above inhibitors of ATP synthesis was used to raise the transcellular resistance to such a degree that measures of the transepithelial resistance approached the resistance of the transepithelial shunt. The specific resistance of BRS was 59.2 ± 0.2 Ωcm (20) measured with the WTW Multimeter 3430 and the TetraCon 925 conductivity probe (Xylem Analytics, Weilheim, Germany).

Although fluid secretion rates are highest in a 1:1 mixture of BRS and Schneider's medium, *Drosophila* Malpighian tubules are rather insensitive to the choice of saline (Dow et al., 1994). Since the use of Schneider's medium complicated the studies of Cl^- diffusion potentials, it was omitted in the present study.

Isolation of anterior tubules. Stable microelectrode impalements of epithelial cells and the tubule lumen required good immobilization of the Malpighian tubule in the perfusion bath. Glass cover slips were coated in 3 or more drying cycles with 3 short puffs of 0.1 mM poly-lysine (JPT Peptides Technologies GmbH, Berlin) delivered from a vaporizer and stored at 4 ºC. On the day of the experiment 50 µl of BRS was deposited on the poly-lysine coated cover slip to receive a pair of anterior Malpighian tubules still attached to a small piece of gut. Although the tubules stick immediately here and there to the glass they can still be straightened out by handling the piece of gut (tubules and ureter were never handled with forceps). As shown in Fig. 1, slight stretching of the tubules forms a straight line of the main segment which facilitates the impalement with a microelectrode approaching a principal cell or the tubule lumen at an angle of 45º or less in line with the tubule. The bath volume was brought up to 150 µl or 3 ml depending on the need to conserve reagent. Inflow and outflow lines allowed the change of the perfusion bath. The tubules were viewed under a Leica Stereoscope MZ95 at magnification ranging from 16x to 150x. The distances between microelectrode tips in the tubule lumen were measured with an ocular micrometer.

Electrophysiology. Microelectrodes were pulled with a Sutter P-97 Flaming/Brown microelectrode puller (Sutter Instruments, Novato, CA, USA) and filled with 3 M KCl. The electrical resistance for both current-injection and voltage electrodes was on average 48.9 ± 0.9 MΩ (264). After each pull, the microelectrode with the lower resistance was used as the current/voltage electrode. For the measurement of voltage we used the preamplifiers HS-2A Headstage Gain x1LU and HS-2A Headstage Gain x1MGU (Axon Instruments, Molecular Devices, San Jose, CA). The latter preamplifier was also used to pass current for the measurement of resistance. Ag/AgCl bridges in the microelectrodes and a 4% BRS agar bridge in the perfusion bath completed the measuring circuits. When both current- and voltage electrodes were lodged in a principal cell (or in the lumen), we measured respectively the basolateral membrane resistance R_{bl} or the transepithelial resistance R_t in six 200 ms voltage clamp steps of 5 mV bracketing respectively the basolateral membrane voltage V_{bl} or the transepithelial V_t . The current-voltage plots (IV plots) were usually linear in both measurements of R_{bl} and R_t. A GeneClamp 500B along with the Digidata 1322A were used to record electrophysiological data (Molecular Devices, San Jose, CA, USA). To monitor the quality of microelectrode impalements we recorded voltages continuously at a frequency of 10^7 Hz except for the brief periods of IV-plots, during which the voltage clamp was set at a gain of 1K and a stability of 200 µs.

The Malpighian tubule modelled as an infinite electrical cable. The measurement of the transepithelial resistance in a tubular epithelium requires cable analysis. The specific cable equations depend on the experimental condition. When the two ends of the tubule can be electrically isolated from the bathing medium as in isolated tubules perfused by the method of Burg (Burg et al., 1966), the cable length is defined and cable analysis yields direct measurements of the length constant and the transepithelial and core resistances in renal tubules

(Helman, 1972) and Malpighian tubules (Pannabecker et al., 1993; Williams and Beyenbach, 1984). When the tubule ends are not electrically isolated as in studies of renal tubules in vivo or in isolated Malpighian tubules in vitro as in the present study, the tubule is considered a cable of infinite length (Boulpaep and Giebisch, 1978; Froemter, 1986). The infinite cable model requires that three microelectrodes are placed in the tubule lumen for the measurement of the tubule length constant and the transepithelial and core resistances (Fig. 2).

Measurement of specific resistances. Fig. 2 illustrates the Malpighian tubule modelled as an electrical cable consisting of a series of 1) core resistances r_c, 2) transepithelial resistances r_t in parallel to transepithelial capacitances c_t, and 3) resistances of the peritubular medium r_p (bath). Since the core resistance (fluid in the tubule lumen) is much greater than the bath resistance, the latter can be neglected in the analysis of the cable. For the measurement of the specific transepithelial resistance (sR_t) current is injected into the tubule lumen via the current/voltage electrode E_0 to yield the voltage deflections ΔV_{t1} and ΔV_{t2}. Since the distance d_2 between electrodes E_1 and E_2 can be measured through the microscope, Eqn 1 yields the length constant λ of the tubule, namely the distance in cm from the current injection site at which ΔV_t has decayed to 37%.

$$\frac{\Delta V_{t2}}{\Delta V_{t1}} = e^{-d_2/\lambda} \qquad (1)$$

Knowledge of the length constant and the distance d_1 allows determination of ΔV_0 at the site of current injection (Eqn 2).

$$\Delta V_{t0} = \frac{\Delta V_{t1}}{e^{-d_1/\lambda}} \qquad (2)$$

Since the injected current (ΔI_0) is known and controlled by the GeneClamp, the ratio of ΔVt_0 and ΔI_0 yields the input resistance, R_{input} (Eqn 3).

$$R_{input} = \frac{\Delta V_0}{\Delta I_0} \qquad (3)$$

The specific transepithelial resistance sR_t normalized to luminal surface area (Ωcm^2) depends on four variables: the length constant λ, R_{input}, the radius of the tubule lumen (r_e), and the resistivity of the luminal fluid (ρ). However, any combination of only three variables yields sRt (eq. 4) (Boulpaep and Giebisch, 1978). Accordingly, there are 4 expressions of the equation for sRt

$$sR_t = \frac{2\rho\lambda^2}{r_e} = \sqrt{8\pi\rho\lambda^3 R_{input}} = 4\pi r_e \lambda R_{input} = \frac{8\pi^2 r_e^3 R_{input}^2}{\rho} \qquad (4)$$

where r_e is the electrical radius of the tubule lumen. Since the optical radius of the tubule lumen measured through the microscope varies considerably with length along the tubule (see Fig. 1), sR_t was determined in the present study with known values λ, R_{input}, and ρ, the resistivity of secreted fluid in the tubule lumen; i.e. the 2^{nd} equivalence of eq. 4 does not require knowledge of the lumen radius. The resistivity of secreted fluid in the tubule lumen was assumed to be similar to that of the peritubular Ringer solution in view of 1) the secretion of fluid isosmotic to the peritubular Ringer bath, and 2) the largely electrolyte composition of both peritubular and luminal fluids.

Rearranging Eqn 4 yields the electrical radius of the tubule lumen r_e (Eqn 5).

$$r_e = \sqrt{\frac{\rho\lambda}{2\pi R_{input}}} \tag{5}$$

Equation 6 relates the length constant to the specific transepithelial resistance sR_t and the specific core resistance sR_c, the resistance of the tubule lumen.

$$\lambda = \sqrt{\frac{r_e sR_t}{2sR_c}} \tag{6}$$

Thus, the greater the lumen radius r_e and the specific transepithelial resistance, the greater the length constant and the smaller the loss of current injected into the tubule lumen. Rearrangement of Eqn 6 yields the specific core resistance sR_c

$$sR_c = \frac{r_e\, sR_t}{2\lambda^2} \tag{7}$$

Measurement of effective resistances. Specific resistances (Eqn 4) are normalized to the area of luminal surface. Effective resistances are normalized to tubule length. Eqn 8 and 9 show respectively the conversions of specific resistances sR to effective resistances eR.

$$eR_t = \frac{sR_t}{2\pi r_e} \tag{8}$$

$$eR_c = \frac{sR_c}{\pi (r_{e)}^2} \tag{9}$$

For effective resistances the length constant of the tubule is

$$\lambda = \sqrt{\frac{eR_t}{eR_c}} \tag{10}$$

and the electrical radius of the tubule lumen is

$$r_e = \sqrt{\frac{\rho}{\pi(eR_c)}} \tag{11}$$

Relative resistance. The use of only two microelectrodes in the tubule lumen yields the relative transepithelial conductance rg_t, as the ratio of I_o and V_{t2}, or its inverse, the relative transepithelial resistance rR_t (Fig. 2). The relative transepithelial resistance decreases with increasing distance between current and voltage electrodes as epithelial mass between the two electrodes increases.

For this reason, measures of rR_t are relative and useful only in before/after comparisons in the same tubule (paired comparisons using each tubule as its own control).

Non-linear or distorted I-V plots resulted when the tip of one electrode is not free in the tubule lumen or in the cell as in touching a membrane. Currents as high as 300 nA were injected into the tubule lumen without ill effects on the tubule.

<u>Measurement of the fractional resistance of the basolateral membrane of principal cells.</u> The fractional resistance of the basolateral membrane fR_{bl} is defined as the ratio $\Delta V_{bl} / \Delta V_t$ consequent to the passage of transepithelial current (Eqn 12).

$$fR_{bl} = \frac{\Delta V_{bl}}{\Delta V_t} = \frac{\Delta V_{bl}}{\Delta V_{bl} + \Delta V_a} = \frac{R_{bl}}{R_{bl} + R_a} \qquad (12)$$

where V is voltage, R is resistance and bl and a denote respectively the basolateral and apical membrane. In the typical measurement of fR_{bl}, the current electrode E_0 and the voltage electrode E_2 were lodged in the tubule lumen, and the voltage electrode E_1 was in the cytoplasm of a principal cell (Fig. 2). The I/V plot yielded the voltage deflection across the basolateral membrane of the impaled principal cell ($\Delta E_1 = \Delta V_{bl}$). Electrode E_1 was subsequently advanced across apical membrane into the tubule lumen. With all three microelectrodes located in the tubule lumen, the transepithelial I/V plot yielded λ and consequently ΔV_t at the site of the previously impaled principal cell for the determination fR_{bl} (Eqn 12).

<u>Statistical analysis</u>. Significant difference was evaluated using the Student's t-test for either unpaired or paired samples.

Results

STUDIES WITH THREE MICROELECTRODES: CABLE ANALYSIS.

Studies with three microelectrodes yield resistances normalized to luminal surface area or tubule length for comparisons with Malpighian tubules from other species and other epithelia. The tubule lumen of Malpighian tubules is easily reached by passing the microelectrode through thin stellate cells (< 5 µm). Passing a microelectrode through principal cells (> 15 µm) usually yields the basolateral membrane voltage before reaching the tubule lumen (as in Fig. 5). Under control conditions when the tubules were bathed in BRS, the transepithelial voltage was 31.6 ± 4.0 mV, the basolateral membrane voltage of principal cells was -39.3 ± 3.8 mV, and the apical membrane voltage was 70.9 ± 4.0 mV in eleven main segments of the anterior Malpighian tubule studied in Table 1.

Estimate of the shunt resistance. According to the Ussing/Windhager conception of transepithelial transport, active and passive transport pathways are parallel across the epithelium (see Fig. 4A). The passive transport pathway is considered the shunt. In initial estimates of the shunt resistance, the cocktail of DNP, NaN_3 and KCN was added to the peritubular bath to inhibit ATP synthesis and hence active transcellular transport. The inhibition was expected to increase the transcellular resistance to such a degree that measures of the transepithelial resistance approach the resistance of the shunt as in in *Aedes* Malpighian tubules (Pannabecker et al., 1992). Not so in *Drosophila* Malpighian tubules: the inhibitor cocktail failed to significantly increase the effective transepithelial resistance (control, 14.2 ± 2.0 KΩcm; cocktail 16.1 ± 3.0 KΩcm, $p < 0.34$, 13 anterior tubules). However, the inhibitory cocktail of ATP synthesis significantly brought all voltages towards zero with two kinetics of voltage depolarization (Fig. 3A). The first fast depolarization (phase 1, Fig. 3A) took off as soon as the cocktail was added to the peritubular medium and lasted about the time it took to change the bath, ~25s. In phase 1, V_a depolarized from 80 mV to 24 mV (lumen-positive), V_t depolarized from 33 mV and reversed polarity to -12 mV, and V_{bl} depolarized from -47 mV to -38 mV. Thereafter, in phase 2 of slow depolarization, voltages gradually depolarized and converged at values near 0 mV in the time of 196 s (Fig. 3A).

To elicit a sharp drop of all voltages together with a sharp increase in the transepithelial resistance, it was necessary to reduce the K^+ conductance of basolateral membranes in addition to the inhibition of ATP synthesis. As shown in Fig. 3B, the inhibitory cocktail together with 5 mM Ba^{2+} in low K^+ (2 mM) BRS promptly depolarized all voltages in the time it took to replace the peritubular bath. Moreover, the K^+ channel blocker Ba^{2+} in low K^+ peritubular Ringer solution obliterated phase 2 of depolarization as well as the reversal of the transepithelial voltage to lumen-negative values. In subsequent studies of 11 main segment of anterior Malpighian tubules, the combined effects of metabolic inhibition and reduced K^+ conductance significantly increased the tubule length constant from 540.0 µm to 772.4 µm and significantly increased the effective transepithelial resistance eR_t from 17.1 KΩcm to 26.9 KΩcm (Table 1). No significant effect on the effective core resistance eR_c and the electrical radius of the tubule lumen point to the successful inhibition of specifically the transcellular active transport pathway (Table 1).

Electrical equivalent model of transepithelial electrolyte secretion in the main segment of the anterior Malpighian tubule of *Drosophila melanogaster*. The transepithelial secretion (or absorption) of electrolytes such as Na^+, K^+, and Cl^- carries current and generates voltages. Accordingly, the transepithelial secretion of electrolytes in the main segment of the anterior Malpighian tubule can be modeled with an electrical circuit consisting of two transepithelial routes for current flow: an active transport pathway leading through cells where active transport finds ATP, parallel to a passive transport pathway, the shunt that does not require ATP (Fig. 4A). In general, Na^+ and K^+ are actively transported through principal cells, and Cl^- is passively transported through stellate cells and/or the paracellular pathway (Beyenbach and Piermarini, 2011; O'Donnell et al., 1996; Pannabecker et al., 1993). The analysis of electrical equivalent circuits is useful in that it reveals 1) the driving forces for electrolyte secretion across the basolateral and apical membranes, and 2) the electrical resistances of all three barriers: basolateral membrane, apical membrane, and shunt. Knowledge of these variables is useful for further dissecting transport pathways. For example, the analysis of an electrical equivalent circuit of Malpighian tubules in *Aedes aegypti* has shown inter alia that 1) the conductance of the basolateral membrane of principal cells is dominated by K^+, 2) the conductance of the apical membrane is dominated by the V-type ATPase, and 3) the diuretic hormone leucokinin increases the Cl^- conductance of the shunt pathway (Beyenbach, 2012; Beyenbach and Masia, 2002; Beyenbach et al., 2000b; Beyenbach and Wieczorek, 2006; Pannabecker et al., 1993).

In the case of the electrical equivalent circuit of transepithelial electrolyte transport in anterior Malpighian tubules of *Drosophila melanogaster*, the estimate of the shunt resistance allows estimates of the electromotive forces and resistances of the transcellular pathway under control conditions (Fig. 4). Effective rather than specific resistances are used from here on for comparison with Malpighian tubules of *Aedes aegypti* (Pannabecker et al., 1992). However, the purpose of this paper is not this comparison but to introduce a new experimental method, the measurement of resistance in tubular epithelia as small as Malpighian tubules of *Drosophila melanogaster* using cable analysis and conventional microelectrodes rather than the method of in vitro microperfusion of tubules.

According to the minimal electrical model of transepithelial electrolyte secretion (Fig. 4A), the open-circuit current I_{oc} is

$$I_{oc} = \frac{E_{cell}}{eR_{cell}+eR_{sh}} = \frac{V_t}{eR_{sh}} \quad (13)$$

where E_{cell} and eR_{cell} are respectively the electromotive force and the effective resistance of the transcellular transport pathway, and eR_{sh} is the effective resistance of the shunt (Fig. 4A,B). Accordingly,

$$V_t = \frac{E_{cell} eR_{sh}}{eR_{cell}+eR_{sh}} \quad (14)$$

The effective transepithelial resistance eR_t is

$$eR_t = \frac{(eR_{cell})(eR_{sh})}{eR_{cell}+eR_{sh}} \qquad (15)$$

Since eR_t is 17.1 KΩcm and eR_{sh} is 26.9 KΩcm (Table 1), the effective transcellular resistance eR_{cell} is 46.9 KΩcm (Fig. 4A).

The ratio of the transepithelial voltage and transepithelial resistance is the short-circuit current I_{sc}, i.e. the maximum current under any condition when transcellular current is not slowed down by the resistance of the epithelial shunt.

$$I_{sc} = \frac{V_t}{eR_t} = \frac{E_{cell}}{eR_c} \qquad (16)$$

Since V_t is 31.6 mV and eR_t is 17.1 ± 2.4 KΩcm (Table 1), I_{sc} is 1.85 μAcm^{-1} and E_{cell} is 86.7 mV for a transcellular resistance of 46.9 KΩcm (Fig. 4A). The fractional resistance of the basolateral membrane of principal cells was 0.33 ± 0.05 in 9 main segments of the anterior tubule (Eqn 12). Accordingly, the effective resistance of the basolateral membrane eR_{bl} is 15.5 KΩcm, and the effective apical membrane resistance eR_a is 31.4 KΩcm (Fig. 4B). Knowledge of these resistances yields the voltage drops across eR_{bl} and eR_a under open-circuit conditions as well as the electromotive force E of the basolateral and apical membrane when the tubule secretes electrolytes and fluid.

According to Eqn 13 the open-circuit current I_{oc} is 1.17 μAcm^{-1} (Fig. 4A). Hence the voltage drop across eR_{bl} is 18.2 mV (cell negative) and E of the basolateral membrane (E_{bl}) is cell-negative 21.1 mV (Fig. 3B). The voltage drop across eR_a is 36.9 mV and E of the apical membrane (E_a) is also cell-negative 107.8 mV (Fig. 4B).

STUDIES WITH TWO MICROELECTRODES.

Studies with two microelectrodes, one to inject current and the other to record voltage, yield relative resistances that are useful for comparing before/after effects using each tubule as its own control. Since relative resistances are not normalized to tubule length or area, they should not be used for comparison with other tubules or epithelia.

<u>The transepithelial voltage profile.</u> Fig. 5 illustrates a representative experiment using two microelectrodes. The two microelectrodes are located within 600 µm of each other in the main segment of an anterior tubule; on average the distance between the two electrodes was 357.4 ± 30.1 µm in 25 tubules. The tip of the current/voltage microelectrode has entered the tubule lumen at point A to record the lumen-positive transepithelial voltage (V_t). V_t is usually constant with time, but oscillating voltages were observed occasionally. The tip of the other microelectrode has penetrated the basolateral membrane of a principal cell at point B to record

the cell-negative basolateral membrane voltage (V_{bl}). Significantly, V_{bl} does not oscillate but remains constant near -50 mV. It follows that oscillations of V_t parallel oscillations of the apical membrane voltage (V_a). V_a was not measured directly but determined as the difference between V_t and V_{bl} in accordance with Kirchhoff's Law. At point C, the voltage microelectrode has been advanced across the apical membrane of the impaled principal cell into the tubule lumen. Both microelectrodes now measure V_t. With both microelectrode tips lodged in the tubule lumen, the relative transepithelial resistance (rR_t) is measured with a current-voltage plot. When V_t had hyperpolarized to 25 mV, rR_t was 122 KΩ; when V_t had depolarized to 10 mV rR_t was 91 KΩ (Fig. 5).

The effect of drosokinin on transepithelial voltage and resistance. In the experiment shown in Fig. 6 the tips of both microelectrodes were lodged in the tubule lumen of the main segment of an anterior Malpighian tubule and recorded similar values of the transepithelial voltage. Shortly before adding drosokinin (1 μM) to the peritubular bath, V_t was 26 mV and rR_t was 109 KΩ. Upon the addition of drosokinin, V_t promptly dropped towards 0 mV; in parallel, rR_t dropped to 87 KΩ.

In paired t-tests, using each tubule as its own control, the addition of 1μM drosokinin to the peritubular bath significantly ($p < 0.04$) hyperpolarized V_{bl} to -53.5 ± 2.5 mV (4), significantly ($p < 10^{-6}$) depolarized V_t to 4.7 ± 2.1 mV (11), but had no significant effect on V_a 64.0 ± 2.5 mV (3). The relative transepithelial resistance significantly ($p < 0.02$) decreased from 111.5 KΩ to 55.0 ± 11.8 KΩ (25).

The Cl^- dependence of the effect of drosokinin. Since the kinins, leucokinin in *Aedes* Malpighian tubules and drosokinin in *Drosophila* Malpighian tubules, are known to increase the permeability of Malpighian tubules to Cl^-, the effect of drosokinin on transepithelial Cl^- diffusion potentials was of interest (Cabrero et al., 2014; Halberg et al., 2015; Lu et al., 2011; Pannabecker et al., 1993; Yu and Beyenbach, 2001; Yu and Beyenbach, 2002). A representative experiment is shown in Fig. 7. In BRS containing 158.5 mM Cl^- V_t was 25 mV and the rR_t 149 KΩ (Fig. 7, 9min). The basolateral membrane voltage of a principal cell was -40 mV and the apical membrane voltage was 65 mV (data not shown). The tenfold reduction of the peritubular Cl^- concentration had negligible effects on V_t and rR_t in the absence of drosokinin (Fig. 7, 9-31 min). The addition of drosokinin (1 μM) to the peritubular bath had major effects on the transepithelial voltage and resistance. Drosokinin depolarized V_t from 25 mV to 4 mV and reduced rR_t from 161 KΩ to 49 KΩ (Fig. 7, 43 min). In the presence of drosokinin, the tenfold reduction of the peritubular Cl^- concentration now induced a large transepithelial Cl^- diffusion potential of 40 mV (Fig. 7, 67 min) and rR_t increased from 49 KΩ to 128 KΩ. After 44 minutes in the presence of 10 % peritubular Cl^- concentration, the return to 100 % Cl^- concentration in the peritubular bath returned V_t to 2 mV, consistent with the transepithelial electrical short circuit induced by drosokinin as rR_t decreased to 92 KΩ (Fig. 7, 97 min).

Discussion

Measurements of the transepithelial electrical resistance in Malpighian tubules of *Drosophila* have not been thought feasible because of their small size (Blumenthal, 2001). Since the time of this assessment *Drosophila* Malpighian tubules have been successfully perfused in vitro, which renders them suitable for measurements of the transepithelial resistance (Wu et al., 2015). The present paper presents less demanding methods using conventional microelectrodes and commercially available electronic hardware. Resistance measurements normalized to luminal surface area or tubule length require the use of 3 microelectrodes. The use of only two microelectrodes yields relative resistances for before/after comparisons in a single tubule.

The specific transepithelial resistance of the main segment of the anterior tubule of *Drosophila melanogaster* is 229.3 Ωcm^2 (Table 1). By comparison, sR_t of the renal proximal tubule, a leaky epithelium, is only 6 and 7 Ωcm^2 in the rat and dog respectively, 30 Ωcm^2 in the rabbit gall bladder, 75 Ωcm^2 in frog choroid plexus, 100 Ωcm^2 in the rabbit ileum, between 150 and 300 Ωcm^2 in the rat distal tubule, 867 Ωcm2 in rabbit cortical collecting tubules, 1500 Ωcm^2 in the toad urinary bladder, and 3600 Ωcm^2 in the frog skin (Boulpaep and Seely, 1971; Dor'o, 1968 ; Erlij, 1976; Frizzell and Schultz, 1972; Hegel et al., 1967; Helman et al., 1971; Malnic and Giebisch, 1972; Reuss and Finn, 1974; Ussing and Windhager, 1964; Wright, 1972). Accordingly, the specific transepithelial resistance of the main segment of the anterior Malpighian tubule of *Drosophila melanogaster* falls in the range of renal distal tubules, namely moderately tight epithelia, reflecting selective barrier and transport properties consistent with a wide quantitative and qualitative range of transepithelial transport (Beyenbach, 1993a; Beyenbach, 2016). Resistances can also be expressed in terms to tubule length (Eqn 8,9). Accordingly, the specific transepithelial resistance of 229.3 Ωcm^2 is equivalent to the effective transepithelial resistance of 17.1 K Ωcm (Tables 1,2, Fig. 4).

<u>Validation of the cable analysis</u>. Table 2 lists the voltages and effective resistances of the main segment of the anterior Malpighian tubule of *Drosophila melanogaster* measured in the present study. The transepithelial voltage, 31.6 mV, measured with microelectrodes is similar to that measured in the lab of Rodan using the method in vitro microperfusion of Malpighian tubules (Wu et al., 2015), and the basolateral membrane voltage of principal cells, -45.3 mV, is similar to that measured in the lab of O'Donnell using double-barreled microelectrodes (Ianowski and O'Donnell, 2004). The ratio of the transepithelial voltage and the effective shunt resistance yields the open-circuit current I_{oc} (Eqn 13), namely the intraepithelial current when tubules are secreting solutes and water into the lumen. The I_{oc} of 1.17 $\mu A cm^{-1}$ is equivalent to a transepithelial flux of 12.1 pmol/s-cm monovalent ions. Accordingly, 12.1 pmols of cations pass per second through principal cells from bath to tubule lumen in a tubule segment 1 cm long, and 12.1 pmols of anions per second pass via the transepithelial shunt (paracellular pathway and/or stellate cells). Rates of the secretory cation flux measured in the laboratories of Dow, O'Donnell and Rodan are on average 89 pmol/min that stem largely from the main segment that comprises approximately 60% of the tubule length (Dow et al., 1998; Linton and O'Donnell, 1999;

O'Donnell and Maddrell, 1995; Rodan et al., 2012; Wu et al., 2015). Since the average *Drosophila* Malpighian tubules is 2.2 mm long (Rheault and O'Donnell, 2004), the flux of 89 pmol/min for the whole tubule is equivalent to 11.2 pmol/s-cm tubule length. The latter is in good agreement with 12.1 pmol/s-cm measured by electrophysiological methods in the present study, and confirms 1) the cable analysis and the model of the tubule as a cable of infinite length, and 2) the largely electrogenic nature of transepithelial cation secretion.

Estimate of the epithelial shunt resistance. According to the Ussing/Windhager conception of transepithelial NaCl transport by the frog skin, Na^+ takes the active transport pathway in parallel with Cl^- through the shunt (Ussing and Windhager, 1964). The separation of transepithelial cation and anion transport applies widely to absorptive and secretory epithelia. In the case of insect Malpighian tubules in general and *Drosophila* Malpighian tubules in particular, the cations Na^+ and K^+ are secreted through principal cells, and Cl^-, the counter ion of Na^+ and K^+, is secreted through the shunt (Beyenbach and Piermarini, 2011; O'Donnell et al., 1996). Thus, the transepithelial secretion of Na^+, K^+, and Cl^- in *Drosophila* Malpighian tubules can be modelled with an electrical circuit consisting of the electromotive force E_{cell} for the transcellular secretion Na^+ and K^+ in series with the transcellular resistance eR_{cell}, both parallel to the transepithelial shunt R_{sh} for Cl^- (Fig. 4A). An E_{cell} of 86.7 mV polarized to move positive charge (Na^+, K^+) into the tubule lumen is the sum of the electromotive forces of the basolateral and apical membranes for transcellular Na^+ and K^+ secretion (Fig. 4A). The distributed circuit reveals an electromotive force of 21.1 mV across the basolateral membrane of principal cells that reflects in part the K^+ equilibrium potential across that membrane (Fig. 4B). The E_a of 107.8 mV across the apical membrane likely reflects the electromotive force of the V-type H^+ ATPase located at that membrane.

In *Aedes* Malpighian tubules, the inhibition of ATP synthesis brings transepithelial electrolyte and fluid secretion to a halt and all voltages to zero in a matter of seconds (Beyenbach et al., 2000b; Pannabecker et al., 1992; Wu and Beyenbach, 2003). With transcellular cation secretion inhibited and eR_{cell} at a maximum, the transepithelial resistance approaches the resistance of the shunt 16.8 KΩcm (Table 2). This approach for estimating the shunt resistance is successful in epithelia powered largely if not exclusively by active transport pumps. The approach does not work in epithelia powered in addition by secondary active transport mechanisms and diffusion potentials. For example, the inhibition of ATP synthesis in the gall bladder has no effect on the transepithelial resistance, and it hyperpolarizes both basolateral and apical membrane voltages on account of ATP-sensitive K^+ channels (Bello-Reuss et al., 1981). In the present study of *Drosophila* Malpighian tubules, the inhibition of ATP synthesis failed also to significantly increase the transepithelial resistance during the initial phase 1 of voltage depolarization because of the large K^+ conductance of the basolateral membrane (Fig. 3A). The large K^+ conductance accounts for nearly 75% of the basolateral membrane voltage and the lumen-negative transepithelial voltage during phase 1 (Fig. 3A). Subsequently in phase 2 of slow depolarization, all voltages decay gradually to zero as K^+ leaks to the peritubular bath in the absence of ATP

(Fig. 3A). In order reduce the K^+ conductance of the basolateral membrane, the inhibitory cocktail was fortified with the K^+ channel blocker barium and the peritubular K^+ concentration was reduced to 2 mM. As shown in Fig. 3B, this maneuver brought all voltages to zero in the time it took to replace the peritubular medium. At the same time, the transcellular resistance increased to values that allowed the estimate of the effective shunt resistance, 26.9 KΩcm (Fig. 4, Tables 1, 2).

Transepithelial voltage and resistance oscillations and the effect of kinins. Oscillations of the transepithelial voltage (V_t) are widely observed in Malpighian tubules of insects, among them *Carausius* (Pilcher, 1970), *Locusta* (Morgan and Mordue, 1981), *Aedes* (Williams and Beyenbach, 1984) and *Drosophila* (Davies et al., 1995). V_t oscillations are also observed in the present study which paralleled oscillations of the transepithelial resistance as in *Aedes* Malpighian tubules (R_t) (Fig. 5). As V_t depolarized towards zero, R_t decreased, and as V_t hyperpolarized, R_t increased, as in *Aedes* Malpighian tubules where the oscillations are dependent on the peritubular Cl^- concentration (Beyenbach et al., 2000a). In particular, as R_t decreases V_t goes to the transepithelial Cl^- equilibrium potential in *Aedes* Malpighian tubules, revealing cyclical changes in transepithelial Cl^- conductance.

While oscillations of V_t and R_t reflect transient changes in the shunt Cl^- conductance, drosokinin brings both voltage and resistance to lower constant values in *Drosophila* Malpighian tubules as in *Aedes* Malpighian tubules in the presence of leucokinin (Fig. 6) (Miyauchi et al., 2011; Pannabecker et al., 1993; Schepel et al., 2010; Yu and Beyenbach, 2001). Drosokinin is the leucokinin of *Drosophila*. It has a single gene that encodes the longest known leucokinin with 15 amino acid residues (Radford et al., 2002). So far, only one drosokinin G protein-coupled receptor (GPCR, CG10626) has been identified in specifically stellate cells of Malpighian tubules of *Drosophila, Anopheles, and Aedes* (Lu et al., 2011; Radford et al., 2002; Radford et al., 2004). Binding to its GPCR at the basolateral membrane of stellate cells, drosokinin increases the intracellular [Ca^{2+}] in selectively stellate cells in *Drosophila* Malpighian tubules (Cabrero et al., 2013; Cabrero et al., 2014; O'Donnell et al., 1998; Terhzaz et al., 1999). In a comprehensive study of the mechanism of action of drosokinin, Cabrero et al. offer strong evidence for localizing the kinin-activated transepithelial Cl^- shunt to stellate cells of *Drosophila* Malpighian tubules (Cabrero et al., 2014). Ca^{2+} imaging, Ramsay fluid secretion assays, measurements of transepithelial voltage, and transgenic chloride reporter technology, are consistent with the chloride channel CLC-a at the apical membrane of stellate cells as part of the kinin-activated transepithelial shunt (Cabrero et al., 2014).

Effect of the peritubular K^+ concentration in Malpighian tubules of *Drosophila* and *Aedes*. Table 2 compares the electrophysiological variables of the main secretory segments of Malpighian tubules in two dipterans, *Drosophila* and *Aedes*, separated by 189 Mya of evolution (Chen et al., 2015). Every variable is significantly different. Though time for evolution could account for the differences, they can largely be attributed to the use of different peritubular K^+

concentrations. *Drosophila* Malpighian tubules were bathed in 20 mM K^+ and *Aedes* Malpighian tubules in 3.4 mM K^+ Ringer solution (Table 2). The physiological differences between *Drosophila* and *Aedes* Malpighian tubules disappear when both tubules are bathed in either low K^+ or high K^+ Ringer solutions.

In the presence of the usual 20 mM K^+ in the Ringer solution, Malpighian tubules of *Drosophila* secrete KCl-rich fluid with a K^+ concentration (180 mM) 9-fold higher than the concentrations of Na^+ (Dow et al., 1994; Dow et al., 1998; Linton and O'Donnell, 1999; O'Donnell and Maddrell, 1995; Rheault and O'Donnell, 2001; Rodan et al., 2012). However, in K^+-free Ringer solution, the tubules secrete a NaCl-rich fluid containing 150 mM Na^+, like *Aedes* Malpighian tubules (Linton and O'Donnell, 1999). What is more, in K^+-free Ringer solution, cAMP stimulates fluid secretion by 48%, presumably via the stimulation of transepithelial Na^+ secretion as in *Aedes* Malpighian tubules (Beyenbach, 1993b; Beyenbach, 2003; Beyenbach and Petzel, 1987; Linton and O'Donnell, 1999; Williams and Beyenbach, 1983). The corollary is observed in *Aedes* Malpighian tubules.

As shown in Fig. 8A, Malpighian tubules of *Aedes* secrete NaCl-rich fluid (151 mM) when bathed in Ringer solution containing 3.4 mM K^+ (Hine et al., 2014). However, in the presence of 34 mM K^+ (Fig. 8B) the tubules secrete a KCl-rich fluid like *Drosophila* Malpighian tubules (Hine et al., 2014). In parallel, the basolateral membrane voltage of 12 principal cells significantly ($p < 10^{-6}$) depolarizes from -76.5 ± 4.4 mV to -41.2 ± 1.7 mV, similar to the depolarization of V_{bl} in *Drosophila* Malpighian (Table 2), and the basolateral membrane resistance of principal cells significantly ($p < 10^{-5}$) decreases from 258.6 ± 13.6 KΩ to 165.2 ± 9.2 KΩ in same cells. Thus, Malpighian tubules of both *Aedes* and *Drosophila* respond to peritubular Na^+ by increasing transepithelial Na^+ secretion at the expense of K^+ secretion, and both tubules respond to peritubular K^+ by increasing transepithelial K^+ secretion at the expense of Na^+ secretion (Fig. 8). The tubules do so spontaneously in vitro without signaling by extracellular natriuretic or kaliuretic agents. *Aedes* evolved 189 million years after *Drosophila*. Thus it appears that the capacity for secreting K^+ and Na^+ by *Drosophila* Malpighian tubules has allowed females of *Aedes* to adopt hematophagous habits and to excrete the Na^+ load of blood meals because their Malpighian tubules had retained ancestral mechanisms.

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Figures

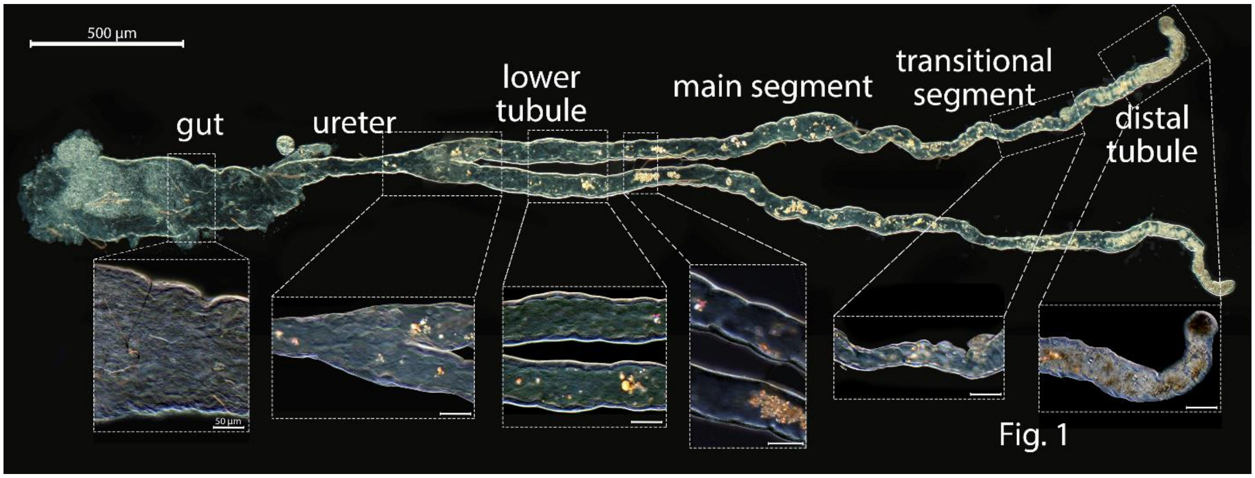

Fig. 1. Pair of anterior Malpighian tubules of *Drosophila melanogaster* secreting fluid in vitro. The distal tubule secretes xanthines, uric acid and Ca oxalates which precipitate (flocculent material) in the tubule lumen. The transport function of the transitional segment is unknown. The main segment secretes an isosmotic fluid, however, the concentrations of secreted K^+ and Na^+ can vary widely depending on the peritubular K^+ and Na^+ concentrations; Cl^- is the preferred counter-ion. The lower tubule and the ureter may reabsorb solute and water. Peristaltic contractions of the ureter propel secreted fluid into the gut; the contractions can also be observed to move secreted fluid back and forth in the upper tubule. The upper tubule (distal, transitional and main segments) consists of ~80 % principal cells and 20% stellate cells, while the lower tubule and the ureter do not have stellate cells (Denholm et al., 2003). Note the differences in lumen diameter along the length of the tubule. The bar diagram in the cut-outs indicate 50 µm. Contractions of the ureter and the flow of tubular fluid were indicated by the movement of flocculent material in the tubule lumen.

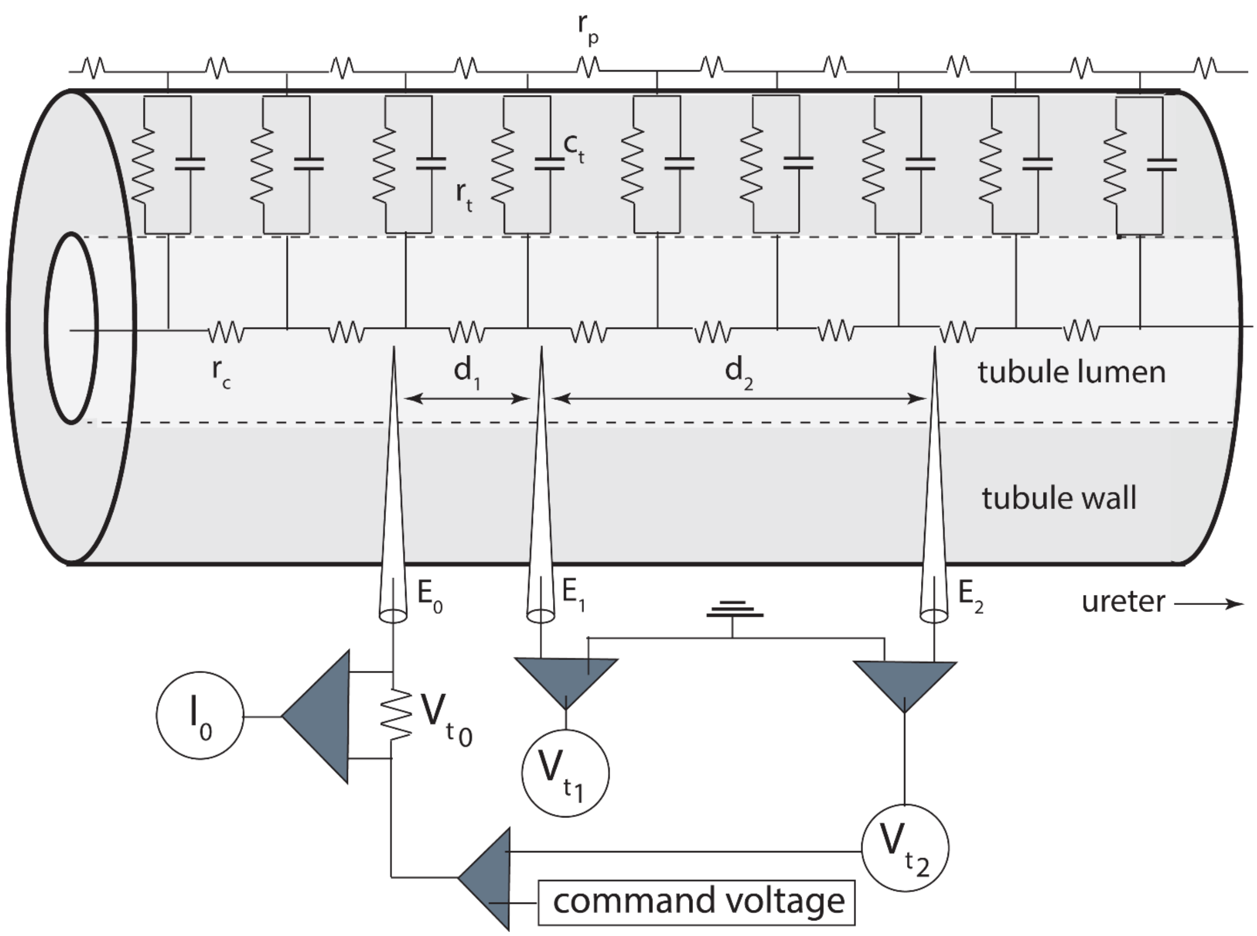

Fig. 2. The Malpighian tubule of *Drosophila melanogaster* modelled as an infinite electrical cable. The cable consists of a conductive, central luminal core (secreted fluid) and a less conductive, "insulating" single layer of epithelial cells (tubule wall). The isolated Malpighian tubule attached to the ureter and a small piece of the intestine is placed in a perfusion bath containing basic Ringer solution, as in Fig. 1. Current I_o is injected into the tubule lumen of the main segment via microelectrode E_0 with respect to ground in the bath, and the transepithelial voltage deflections are recorded downstream at d_1 and d_2 for the determination of the cable parameters. V, voltage; I, current; E, microelectrode; r_t, series transepithelial resistance; c_t, series transepithelial capacitance; r_p, series peritubular bath resistance; r_c, series resistance of tubule lumen (core); d, distance.

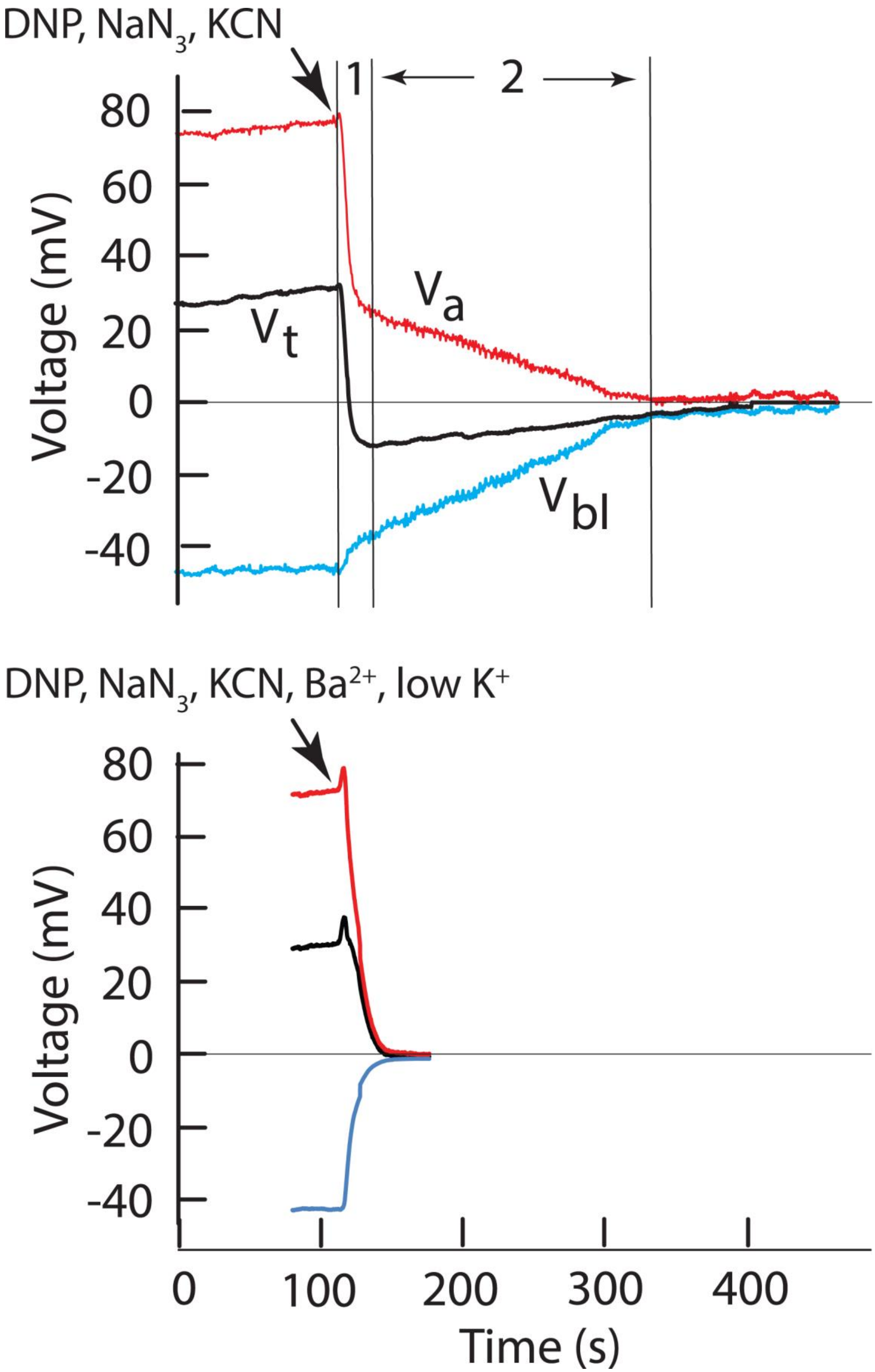
DNP, NaN3, KCN
1
2
80
60
40
20
0
-20
-40
Voltage (mV)
Va
Vt
Vbl
DNP, NaN3, KCN, Ba2+, low K+
0
100
200
300
400
Time (s)

Fig. 3. Inhibition of voltage generation in the main segment of the anterior Malpighian tubule of *Drosophila melanogaster*. A, fast (1) and slow (2) kinetics of voltage depolarization upon adding DNP, NaN_3, KCN (1 mM each) to the peritubular bath (arrow). B. Loss of the slow phase of depolarization when the peritubular medium was replaced with BRS containing 2 mM K^+ and 5 mM Ba^{2+} as well as the inhibitory cocktail of ATP synthesis. V_a, apical membrane voltage of a principal cell; V_{bl}, basolateral membrane voltage; V_t, transepithelial voltage.

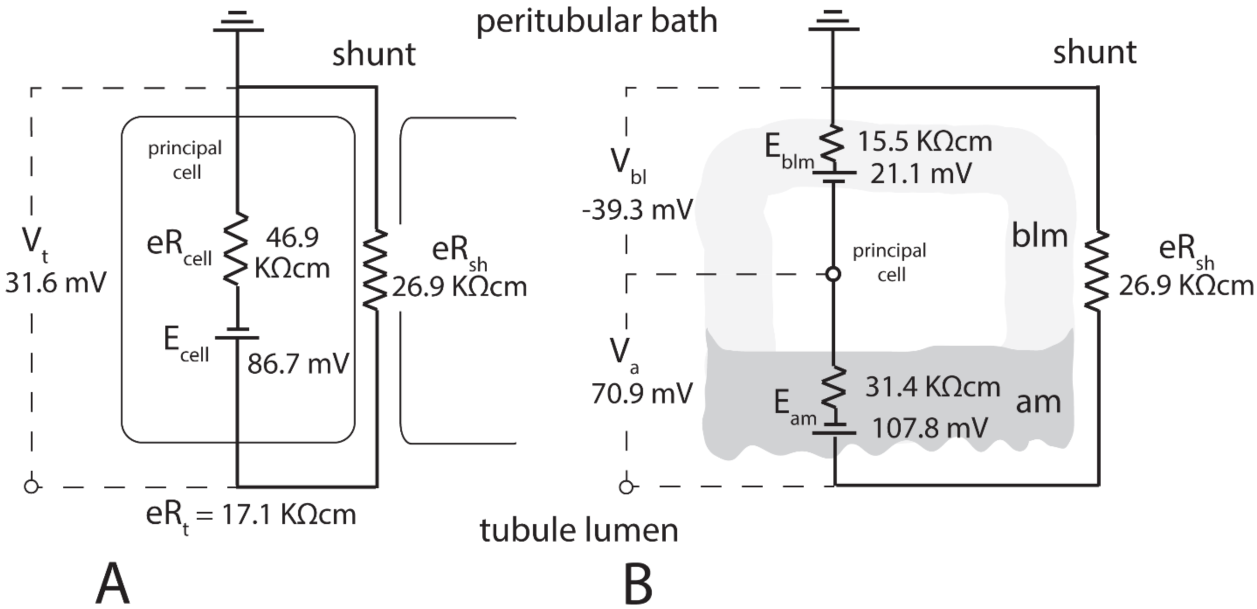

Fig. 4. The Ussing/Windhager model of transepithelial electrolyte transport (A) and the distributed circuit model (B) of transepithelial electrolyte secretion in the main segment of the anterior Malpighian tubule of *Drosophila melanogaster*. A. Parallel transepithelial active and passive (shunt) transport pathways (Ussing and Windhager, 1964). The active transport pathway leads through cells where the electromotive force (E) for transport is generated; the passive transport pathway, i.e. the shunt, may pass through cells or between cells. Thus, positive Na^+ and K^+ current passes through principal cells into the tubule lumen; positive current returns to the peritubular bath via Cl^- secretion through the shunt. B. Electromotive forces and resistances of the basolateral and apical membrane of principal cells of the main segment. Electrical resistances are expressed in terms of tubule length (eR). V, voltage; am, apical membrane; blm, basolateral membrane; sh, shunt.

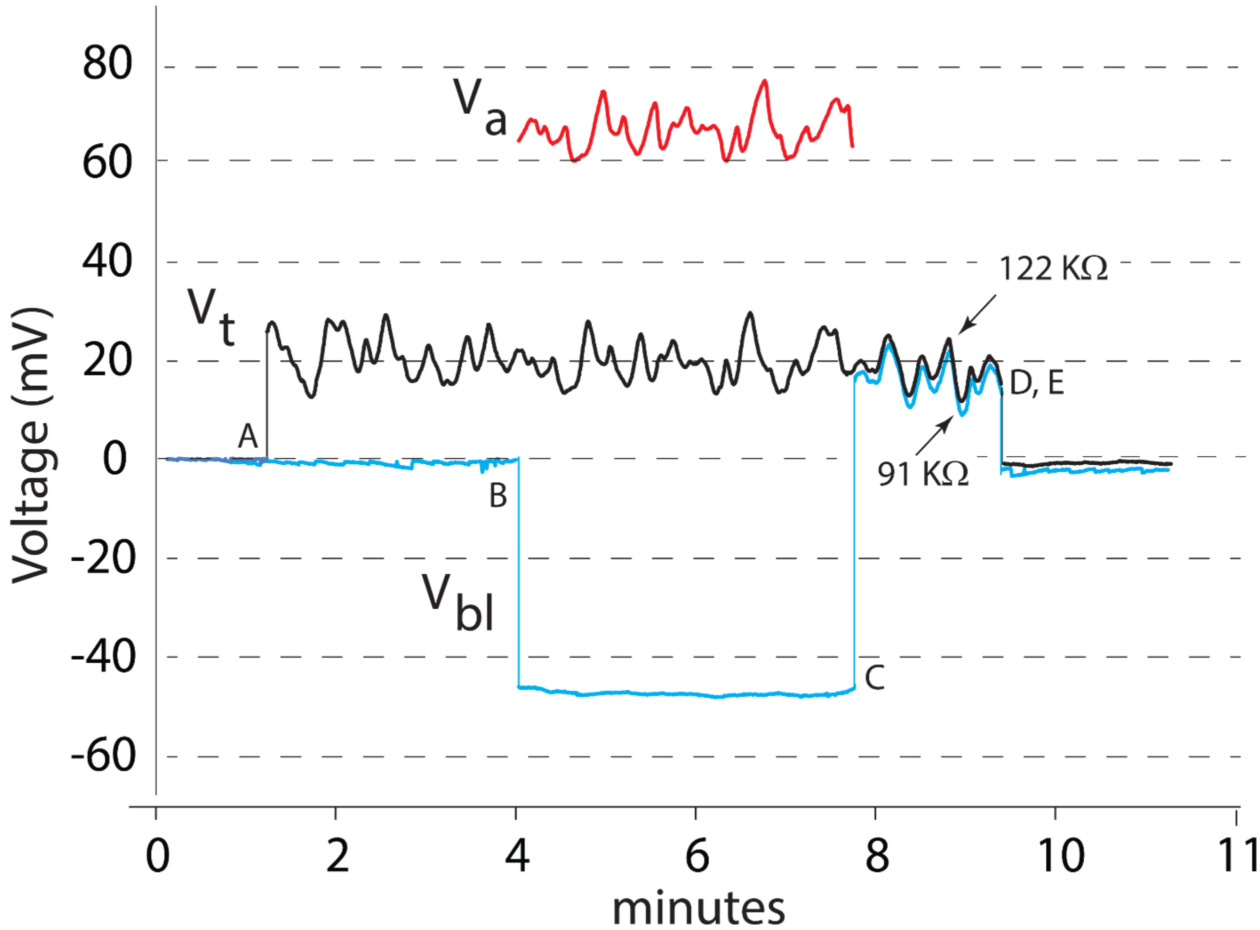

Fig. 5. Spontaneous oscillations of the transepithelial voltage and resistance in the main segment of an anterior Malpighian tubule of *Drosophila melanogaster*. A, impalement of the tubule lumen with the current/voltage microelectrode to measure the transepithelial voltage V_t. B, impalement of a principal cell with the voltage microelectrode to measure the basolateral membrane voltage V_{bl}. C, passing the voltage microelectrode through the apical membrane into the tubule lumen to record V_t. D,E, withdrawal of both microelectrodes to the peritubular bath (ground). The apical membrane voltage V_a is the difference between V_t and V_{bl}. Relative resistance (not normalized to tubule length) was measured in this study with two microelectrodes. Note that the effective transepithelial resistance (rR_t) and V_t cycle in parallel: rR_t increases as V_t increases.

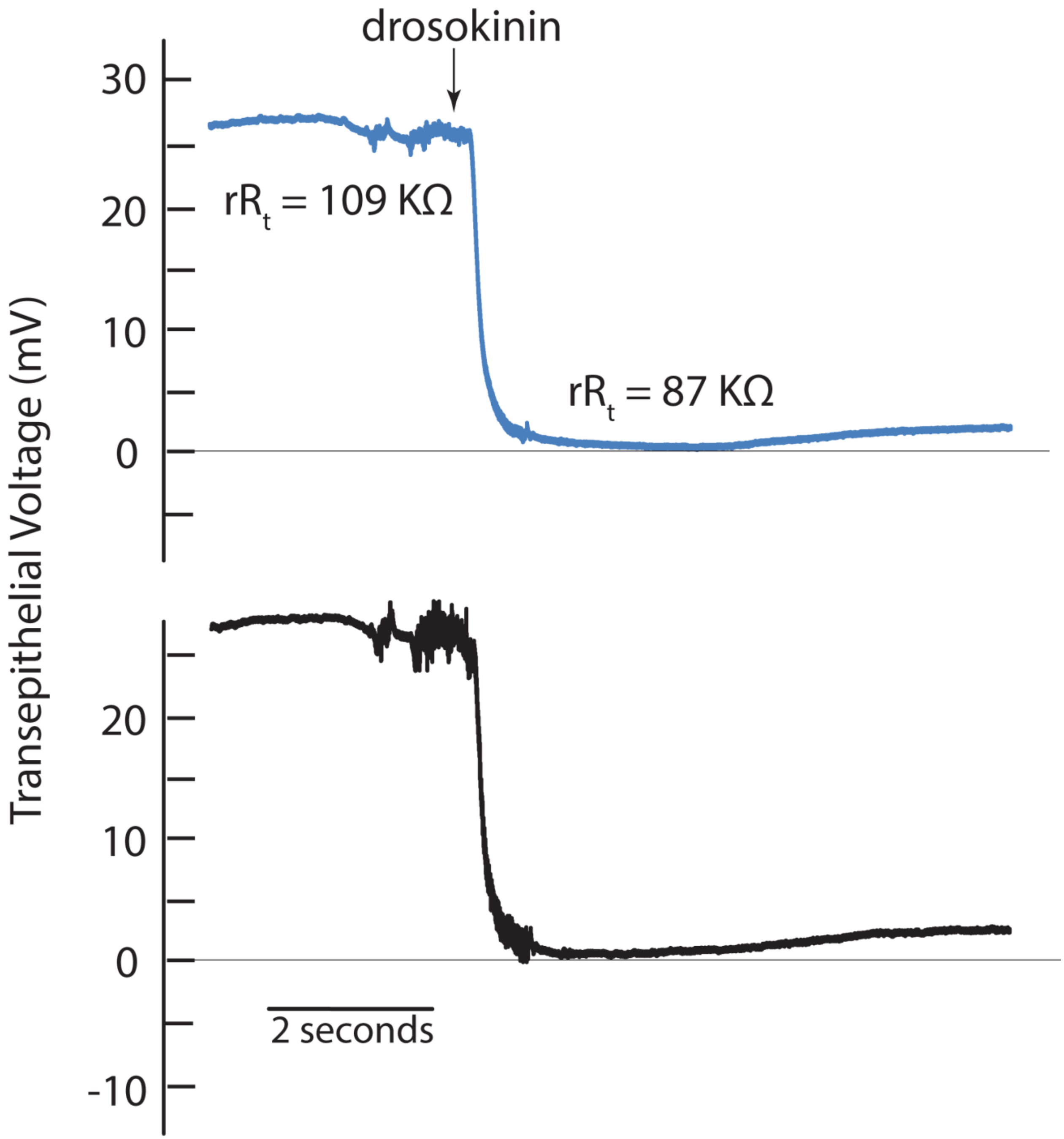

Fig. 6. The effect of drosokinin (1 µM) on the transepithelial voltage and the relative resistance (rR_t) in the middle segment of an anterior Malpighian tubule of *Drosophila melanogaster*. The tips of both microelectrodes are located in the tubule lumen and record the

transepithelial voltage. One electrode is used to pass current for measurement of the relative transepithelial resistance. Drosokinin effectively short-circuits the transepithelial voltage (V_t = 0 mV) and significantly lowers the relative transepithelial resistance consistent with the decrease in the transepithelial shunt resistance. Two electrode studies are suitable for before/after comparisons of the transepithelial resistance in single tubules.

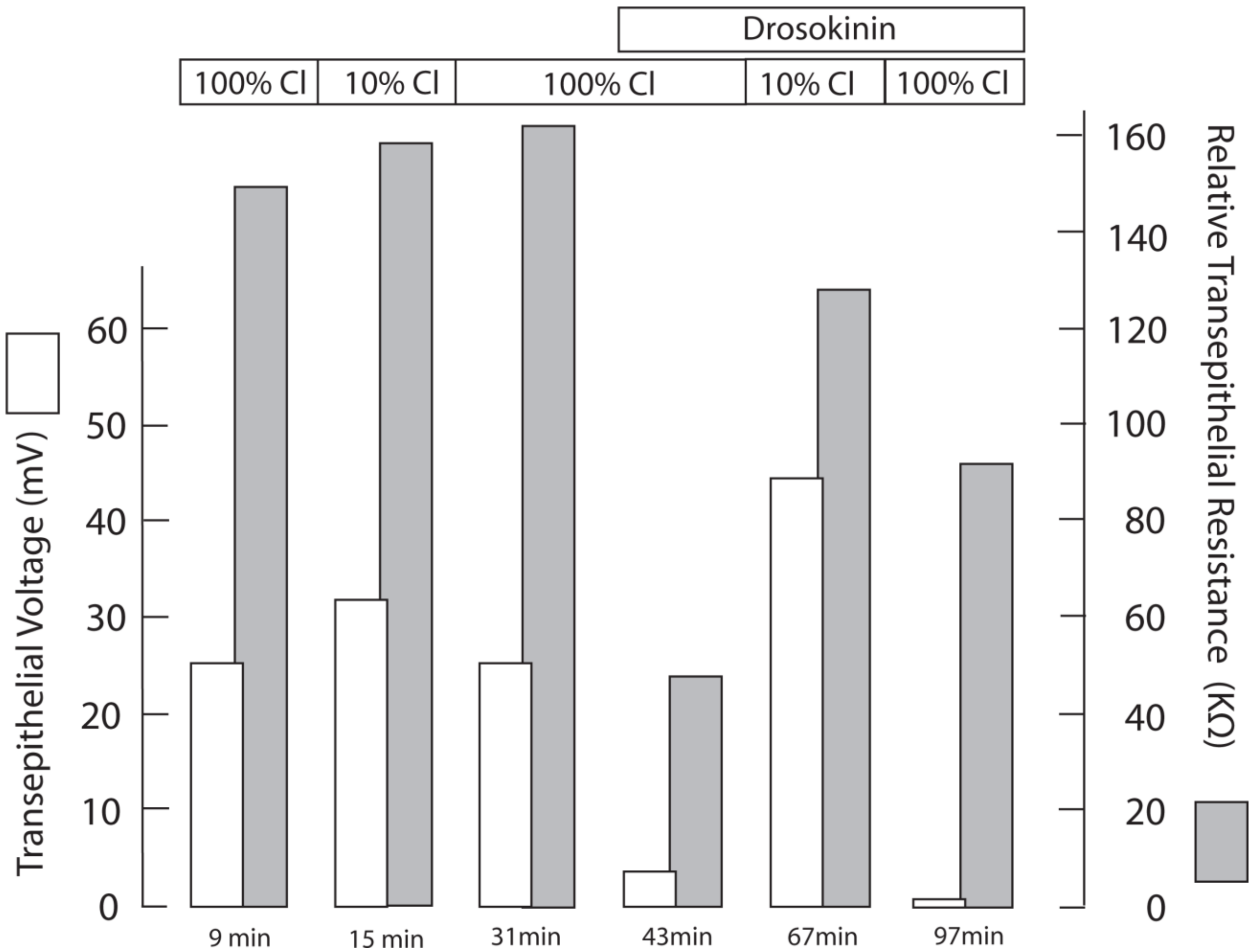

Fig. 7. The effect of drosokinin (1 µM) on the transepithelial voltage and the relative transepithelial resistance of the main segment of an anterior Malpighian tubule of *Drosophila melanogaster*. The Cl^- dependence of the drosokinin effect is demonstrated in tenfold reductions of the peritubular Cl^- concentration which hyperpolarizes the transepithelial voltage (V_t) by 40 mV while increasing the relative transepithelial resistance (rR_t) from 49 KΩ to 128 KΩ. Note that V_t and R_t change together as in spontaneous oscillations (Fig. 6), consistent with the shunt as the route for Cl^- secretion. Relative resistance was measured with two microelectrodes for before/after comparisons in a single tubule.

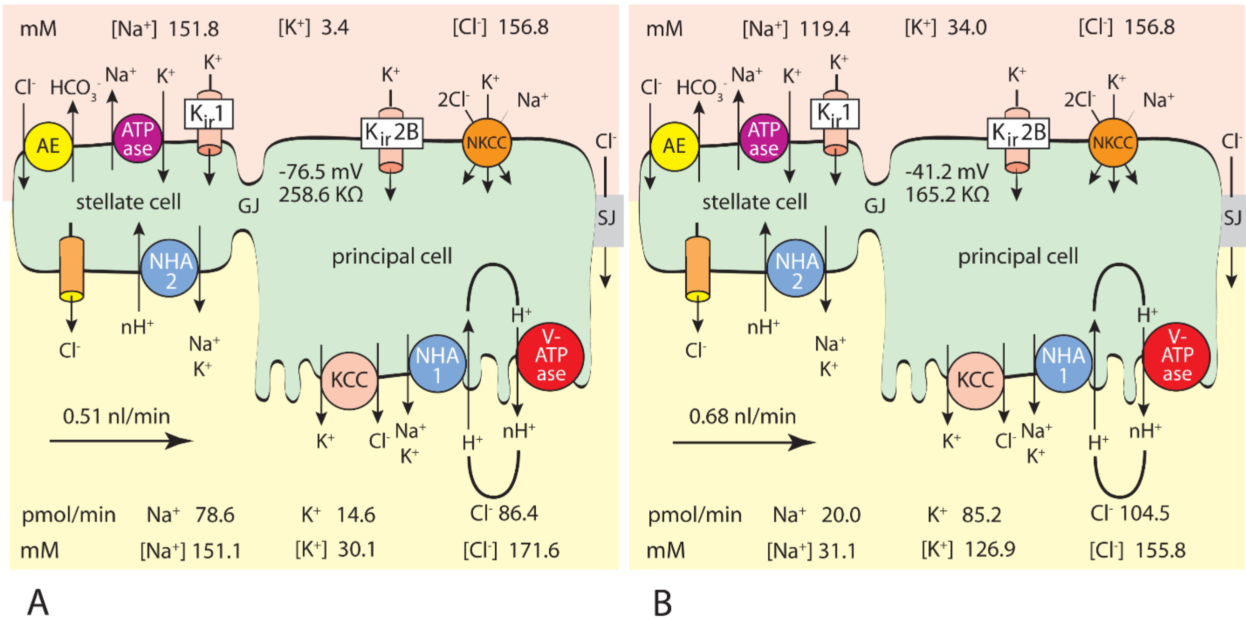

Fig. 8. The effect of peritubular K^+ concentration on transepithelial electrolyte and fluid secretion in *Aedes* Malpighian tubules. Distal segments, i.e. the blind ends of tubules were studied in presence of 3.4 mM K^+ (A) and 34 mM K^+ (B) in the peritubular medium. The voltage and resistance of the basolateral membrane of principal cells was measured with both current and voltage electrodes located in principal cells. GJ, gap junction; SJ, septate junction; AE, anion exchanger; ATPase, Na/K pump; K_{ir}, inward rectifier K^+ channel; NKCC, Na/K/2Cl cotransporter; NHA, Na/H exchanger; KCC, K/Cl cotransporter; V-ATPase, vacuolar-type proton pump. Data are in part from (Beyenbach and Masia, 2002; Hegarty et al., 1991; Hine et al., 2014; Ianowski et al., 2004; O'Connor and Beyenbach, 2001; Petzel, 2000; Piermarini et al., 2015; Piermarini et al., 2010; Piermarini et al., 2011; Piermarini et al., 2013; Rheault et al., 2007; Rodan et al., 2012; Tiburcy et al., 2013; Weng et al., 2003; Weng et al., 2008; Xiang et al., 2012).

Tables

<u>Table 1</u>. Effect of inhibiting transcellular cation transport through principal cells on the cable parameters of the main segment of the anterior tubule of *Drosophila melanogaster*.

		Control	**Metabolic Inhibition,** low K^+ BRS, Ba^{2+}	**p**
	tubule length constant λ	540.0 ± 87.8 µm	772.4 ± 102.1 µm	<0.0491
	electrical radius of the tubule lumen r_e	21.0 ± 2.6 µm	21.3 ± 2.6 µm	NS
Normalization to luminal surface area	specific transepithelial resistance sR_t	229.3 ± 4.4 Ωcm^2	357.3 ± 64.7 Ωcm^2	<0.0022
	specific core resistance sR_c	55.8 ± 5.0 Ωcm	63.5 ± 3.4 Ωcm	NS
Normalization to tubule length	effective transepithelial resistance eR_t	17.1 ± 2.4 KΩcm	26.9 ± 4.0 KΩcm	<0.0004
	effective core resistance eR_c	5.5 ± 1.3 $M\Omega cm^{-1}$	6.7 ± 1.6 $M\Omega cm^{-1}$	NS

The data are from 11 Malpighian tubules; each tubule is used as its own control. Control, in the presence of BRS in the bath; metabolic inhibition, in the presence of low K^+ BRS (2 mM K^+), 5 mM Ba^{2+} plus DNP, NaN3, and KCN (1 mM each). The transepithelial resistance approximates the shunt resistance after metabolic inhibition in low K^+ BRS containing Ba^{2+}.

Table 2. Electrical variables of the main (secretory) segment of anterior tubule of *Drosophila melanogaster* and the main secretory distal (blind end) Malpighian tubule of the yellow fever mosquito *Aedes aegypti*. Note the significant difference of every variable when *Drosophila* and *Aedes* Malpighian tubules are bathed respectively in 20 mM and 3.4 mM K^+ Ringer solution.

	Transepithelial		Shunt	Basolateral Membrane		Apical Membrane	
	Voltage (mV)	Effective Resistance (KΩcm)	Effective Resistance (KΩcm)	Voltage (mV)	fR_{bl}	Voltage (mV)	fR_a
Drosophila melanogaster	31.6 ± 4.0 (11)	17.1 ± 2.4 (11)	26.9 ± 4.0 (11)	-45.3 ± 1.0 (54)	0.33 ± 0.05 (9)	69.4 ± 2.0 (52)	0.67 ± 0.05 (9)
Aedes aegypti	52.6 ± 10.4 (7)	11.4 ± 1.6 (7)	16.8 ± 2.2 (7)	-58.0 ± 5.2 (7)	0.68 ± 0.04 (7)	110.6 ± 6.3 (7)	0.32 ± 0.04 (7)
Significance	$p < 0.025$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.005$	$p < 0.0005$	$p < 0.005$

V and R are respectively voltage and resistance, and a, bl, and sh are respectively apical membrane, basolateral membrane, and transepithelial shunt. The data for *Aedes aegypti* are from (Pannabecker et al., 1992). Data are mean ± SE (number of tubules). V_t measured in *Drosophila* Malpighian tubules (31.6 mV) is substantially less than 50 to 60 mV measured by Blumenthal (Blumenthal, 2003). However, Blumenthal studied tubules bathed in a 1:1 mixture of BRS:Schneider's which is known to increase V_t.