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# ELECTRICAL TRANSIENTS IN THE CELL-VOLUME RESPONSE TO CYCLIC AMP OF THE TSETSE FLY MALPIGHIAN TUBULE

LEON C. ISAACSON[1] AND SUSAN W. NICOLSON[2,*]

[1]*Department of Physiology, University of Cape Town Medical School, Observatory 7925* and [2]*Department of Zoology, University of Cape Town, Rondebosch 7700, South Africa*

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## Summary

**1. Using cyclic AMP to stimulate perfused tsetse fly Malpighian tubules bathed in $SO_4^{2-}$ Ringer frequently causes an immediate but transient peak in transtubular potential ($V_t$), before stabilisation of $V_t$ at an increased value.**

**2. These transients were investigated by monitoring the associated changes in cable properties and current–voltage (*I*/*V*) relationships. Tubules were perfused and bathed in either $Cl^-$ Ringer or $SO_4^{2-}$ Ringer (containing 8 mmol $l^{-1}$ $Cl^-$).**

**3. Tubules bathed in $Cl^-$ Ringer showed a transient swelling of the cells on exposure to cyclic AMP. Cable analysis confirmed the visually observed narrowing of the tubular lumen and revealed transient increases in core resistance ($R_c$) and transtubular resistance ($R_t$). As the cells returned to their initial volume, the lumen became distended, and $R_c$ and $R_t$ fell below their initial levels. These changes were accompanied by an increase, and a subsequent decrease, in the slope of the *I*/*V* plot.**

**4. None of the above changes was apparent in $SO_4^{2-}$ Ringer, other than a fall in $R_t$ and in the slope of the *I*/*V* plot.**

**5. The results suggest that, in $Cl^-$ Ringer, cyclic AMP induces swelling of the tubular cells by promoting increased basolateral solute (and water) entry and that the subsequent rapid return to normal cell volume, with a concomitant progressive increase in the rate of tubular secretion, reflects the operation of a specific cell-volume regulatory mechanism of transepithelial transport.**

**6. The cyclic-AMP-induced peak that occurs in $V_t$ in $SO_4^{2-}$ Ringer appears to be primarily due to a transient overshoot in the fall in series resistance (i.e. an increase in basolateral $Na^+$ conductance), accompanied by a proportionately lesser increase in shunt resistance.**

Key words: tsetse fly, Malpighian tubules, cyclic AMP, transients, cable analysis, *I*/*V* plots, cell volume, *Glossina morsitans morsitans*.

## Introduction

In earlier studies of the secretory effect of cyclic AMP on the isolated and perfused Malpighian tubules of the tsetse fly *Glossina morsitans morsitans* (Isaacson and Nicolson, 1994; Nicolson and Isaacson, 1996), the addition of cyclic AMP to the $Cl^-$-rich bathing fluid was observed to cause immediate and marked tubular swelling, which persisted for just a few minutes. Replacing the bathing fluid with one in which $Cl^-$ was largely substituted by $SO_4^{2-}$ abolished this transient, but frequently introduced another: the abrupt onset of a rapid rise in transtubular potential to a shortlived peak, subsiding a few minutes later to a lower and relatively stable, but still elevated, potential (Fig. 1).

We have explored the mechanisms of these cyclic-AMP-induced transients by monitoring the associated changes in tubular cable properties and current–voltage (*I*/*V*) relationships.

## Materials and methods

### *Insects*

Tsetse flies were obtained as pupae from the International Centre for Insect Physiology and Ecology, Nairobi, Kenya, and from Insect Investigations Ltd, Cardiff, UK. After emergence, flies were maintained at 13 °C and were not fed. Flies of both sexes, between 1 and 4 days old, were used.

### *Solutions*

The Ringer's solutions contained either chloride or sulphate as the predominant anion (Isaacson and Nicolson, 1994). The $Cl^-$ Ringer (138 mmol $l^{-1}$ $Cl^-$) was identical to the recipe of Gee (1976*b*), with the addition of 5 mmol $l^{-1}$ proline and 5 mmol $l^{-1}$ alanine. The $SO_4^{2-}$ Ringer had a $Cl^-$ concentration of 8 mmol $l^{-1}$, the remaining $Cl^-$ being replaced by $SO_4^{2-}$. The cyclic AMP was a solution (5 mmol $l^{-1}$) of dibutyryl cyclic AMP (sodium salt, Sigma).

*Author for correspondence.

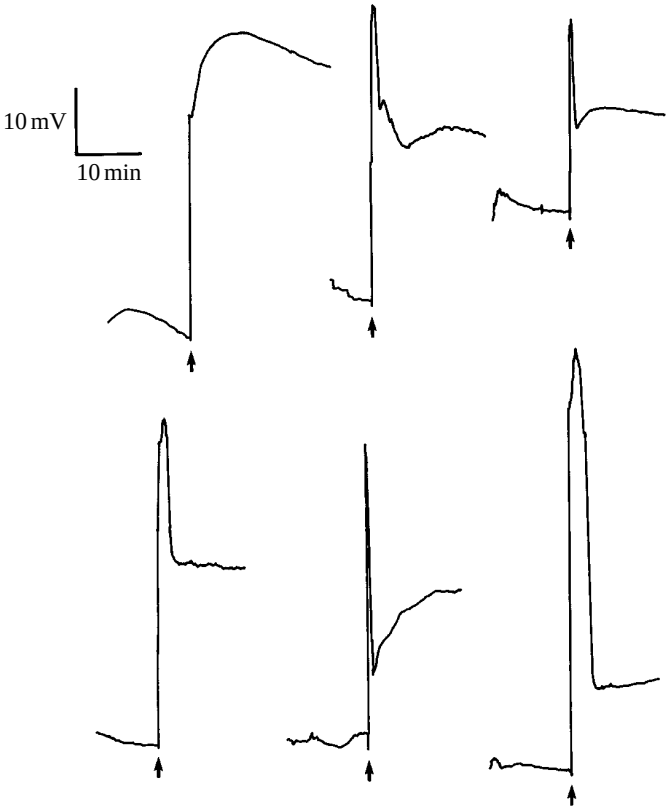


Fig. 1. Examples of transients in transtubular potential ($V_t$) occurring immediately after exposure of tsetse fly Malpighian tubules to cyclic AMP. Tubules bathed in $SO_4^{2-}$ Ringer. Control values of $V_t$ at the time of cyclic AMP addition to the bath (arrows) ranged from –1 to +22 mV. Also shown is a trace for a tubule (top left) in which no $V_t$ transient occurred.

### *Tubule perfusion*

Short segments of Malpighian tubule, 0.3–0.7 mm in length, were perfused and bathed with either $Cl^-$ or $SO_4^{2-}$ Ringer, as described previously (Isaacson and Nicolson, 1994). The perfusion pipette was constructed of double-barrelled glass (tip diameter 20–25 μm), one barrel being used to perfuse the tubule and to measure the spontaneous transtubular potential ($V_t$), and the other being used to inject current. The current source, the output of which was computer-controlled, was electrically isolated from the voltage-recording circuit.

The bath fluid (3 ml) was stirred continuously by aspiration and reinjection (about 20 times a minute) of about a quarter of its volume, using a peristaltic pump. When required, 0.06 ml of a 5 mmol $l^{-1}$ solution of cyclic AMP was added to the bath, at a site close to the inlet of the tubing to the peristaltic pump (and remote from the tubule); the stirring rate ensured mixing within a few seconds of this solution with that of the bath, giving the desired final concentration of 0.1 mmol $l^{-1}$ cyclic AMP.

### *Cable analysis and current–voltage relationships*

Cable analysis and determination of current–voltage relationships (*I*/*V* plots) were effected by injection of current into the second barrel of the perfusion pipette; detailed descriptions of our use of these procedures have been given previously (Isaacson *et al.* 1989; Isaacson and Nicolson, 1994; Nicolson and Isaacson, 1996) and are not repeated here.

In essence, cable analysis was initiated by measurement of the increments in transtubular potential at the proximal and distal ends of the tubule, following the passage of a single 200 nA hyperpolarising current pulse into the tubular lumen. These voltage increments, in a tubule of known length, permit the immediate computer calculation of the transtubular resistance ($R_t$), the core resistance ($R_c$), the lumen diameter ($D$), the length constant ($L$), and the virtual short-circuit current ($I_{sc,v}$; calculated as $V_t/R_t$). The entire procedure takes less than 1 s.

*I*/*V* plots were initiated by injection of a series of current pulses, incrementing in 20 nA steps from –200 nA to 200 nA, into the tubular lumen. The *I*/*V* plot, embodying the resultant changes in transtubular potential ($V_t$), was then generated by microcomputer, the entire procedure taking about 20 s. Analysis of the *I*/*V* plot (Helman and Fisher, 1977) depends on the assumption that the tubular electrical response is equivalent to that of a simple electrical circuit, consisting only of shunt and series resistances ($R_{sh}$ and $R_{ser}$) and a battery ($E1$), and is conditional on the plot being curvilinear. The values of $E1$ and $R_{sh}$ can then be derived from the slope resistances ($R1$ and $R2$, respectively) of the upper and lower linear regions of the curvilinear *I*/*V* plot. $R_{ser}$ can then be calculated as:

$$R_{ser} = R_{sh}(E1/V_t - 1) .$$

Thus, the tubular response to cyclic AMP can be described in terms of changes in the values of the three parameters of the equivalent electrical circuit. A limitation of this mode of analysis, as stressed by Helman and Fisher (1977), is that it is based upon the occurrence of a curvilinear, as opposed to a linear, *I*/*V* plot; such occurrence, in any particular tubule, at any particular time, is unpredictable.

### *Experimental protocol*

Twenty-seven tubules were perfused, 13 in $Cl^-$ Ringer and 14 in $SO_4^{2-}$ Ringer. All experiments were conducted at room temperature (18–20 °C). Following initiation of tubular perfusion, $V_t$ was recorded continuously on a chart recorder, and the cable parameters and/or an *I*/*V* plot were obtained at 4–6 min intervals. After attainment of 'equilibrium' (usually within 10–15 min of mounting the tubule), cyclic AMP was added to the bath. The cable parameters and/or an *I*/*V* plot were recorded 1–2 min later. Where the addition of cyclic AMP was followed by the prompt appearance of a transient peak in $V_t$, these recordings were made as close to the time of appearance of the peak as possible. Thereafter, the cable parameters and/or *I*/*V* plots were again recorded at 4–6 min intervals for the following 15–20 min. In one experiment, a second application of cyclic AMP was given a few minutes after the first (see Fig. 2 and Table 3).

### *Statistics*

Results are presented as means ± S.E.M. The slope resistances of the linear regions of the *I*/*V* plots were calculated by

regression analysis. Paired and independent-sample *t*-tests were used to assess differences between means. Where the variances differed markedly (as in comparison of the means obtained in the $Cl^-$ or $SO_4^{2-}$ Ringer's solutions), the Mann–Whitney *U*-test was employed. Differences of $P<0.05$ were regarded as significant.

## Results

### *Morphological transients*

#### *$Cl^-$ Ringer*

Within a few seconds of adding cyclic AMP to the bath, the tubular outer diameter (OD) was seen to increase, while the lumen narrowed, in some instances being virtually obliterated. The cells appeared swollen. The lumen regained its initial width some 2–3 min later, and by 10 min was visibly distended; at this point, the tubular OD was even larger. As measured by ocular micrometer, typical changes in OD were 53 μm before exposure to cyclic AMP, 60 μm a few seconds later, 65 μm at 2 min and 75 μm at 10 min. The irregular contours of the tubule cells prevented precise measurement of changes in luminal diameter.

#### *$SO_4^{2-}$ Ringer*

Exposure to cyclic AMP produced no changes in tubular morphology (or barely discernible changes of the same pattern as above).

### *Cable analysis*

#### *$Cl^-$ Ringer*

The calculated diameter of the tubular lumen (*D*) fell sharply immediately after exposure to cyclic AMP, regained its initial value some minutes later, and then increased to well above control levels (Table 1A). These changes, in conjunction with those in tubular OD (above), confirm the occurrence of transient but marked cellular swelling; thus, the height of the tubular cells, calculated as (OD–*D*)/2, was initially 10.5 μm, about 23 μm at 1–2 min, and about 11 μm at 10–12 min.

The core resistance ($R_c$) varied inversely with the luminal diameter. The transtubular resistance ($R_t$) doubled immediately after exposure to cyclic AMP, before finally falling to less than half its initial value. These changes in resistance were accompanied by slight but transient increases in both the transtubular potential ($V_t$) and the apparent (see Discussion) $I_{sc,v}$.

In one experiment on a tubule bathed in $Cl^-$ Ringer, a second dose of cyclic AMP was added to the bath 7 min after the first. Following each dose, the resultant *I*/*V* and cable transients (including the calculated values of luminal diameter) were reversed in less than 5 min (see Table 3; Fig. 2A,B).

#### *$SO_4^{2-}$ Ringer*

Following exposure to cyclic AMP, both $V_t$ and $I_{sc,v}$ increased over the ensuing 12 min (Table 1B). An early peak in $V_t$ was seen in two of these nine tubules. There were no significant changes in either the luminal diameter (*D*) or $R_c$. $R_t$ fell promptly and then progressively to less than one-third of its initial value, with a concomitant fall in length constant.

### *I/V plots and parameters of the equivalent electrical circuit*

Calculation of serial changes in the parameters of the equivalent electrical circuit, in any individual tubule, is dependent upon the finding of sequential curvilinear *I*/*V* plots before and after exposure to cyclic AMP. These were found before, and 1–2 min and 7–12 min after, exposure to cyclic AMP, in five of 13 tubules bathed in $Cl^-$ Ringer and in five of 14 tubules bathed in $SO_4^{2-}$ Ringer. The $Cl^-$ group was

Table 1. *Cable parameters in tubules bathed in either $Cl^-$ or $SO_4^{2-}$ Ringer, as found immediately before (Pre) and then at approximately 1, 7 and 12 min after exposure to cyclic AMP*

| | $V_t$ (mV) | | | | $R_t$ (Ω cm) | | | | $R_c$ (MΩ $cm^{-1}$) | | | | *D* (μm) | | | | $I_{sc,v}$ (μA $cm^{-1}$) | | | | *L* (μm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre | 1 | 7 | 12 | Pre | 1 | 7 | 12 | Pre | 1 | 7 | 12 | Pre | 1 | 7 | 12 | Pre | 1 | 7 | 12 | Pre | 1 | 7 | 12 |
| A $Cl^-$ Ringer (*N*=10) | | | | | | | | | | | | | | | | | | | | | | | | |
| Mean | –0.5 | 0.9 | 0.3 | 0.3 | 3.4 | 7.0 | 2.8 | 1.5 | 13.2 | 59.9 | 18.0 | 8.5 | 32.0 | 14.6 | 30.1 | 52.5 | –0.11 | 0.10 | 0.23 | 0.98 | 210 | 134 | 175 | 187 |
| S.E.M. | 0.6 | 0.7 | 0.5 | 0.6 | 0.8 | 1.5 | 0.3 | 0.4 | 4.1 | 19.5 | 6.0 | 4.0 | 4.5 | 2.0 | 5.1 | 14.6 | 0.21 | 0.16 | 0.31 | 0.90 | 45 | 20 | 33 | 37 |
| *P*< | – | 0.05 | 0.05 | NS | – | 0.025 | NS | 0.05 | – | 0.025 | NS | NS | – | 0.005 | NS | 0.05 | – | NS | 0.05 | NS | – | NS | NS | NS |
| B $SO_4^{2-}$ Ringer (*N*=9) | | | | | | | | | | | | | | | | | | | | | | | | |
| Mean | 3.0 | 18.7 | 25.0 | 27.5 | 21.4 | 13.0 | 7.6 | 6.4 | 50.3 | 63.5 | 51.8 | 54.0 | 15.8 | 14.3 | 16.2 | 16.1 | 0.22 | 1.86 | 4.48 | 5.31 | 238 | 191 | 164 | 144 |
| S.E.M. | 2.3 | 4.3 | 5.0 | 5.0 | 6.4 | 2.1 | 1.6 | 1.1 | 12.4 | 18.1 | 16.0 | 18.7 | 2.7 | 2.4 | 2.8 | 2.6 | 0.15 | 0.68 | 1.30 | 1.32 | 43 | 45 | 44 | 32 |
| *P*< | – | 0.005 | 0.001 | 0.001 | – | NS | 0.025 | 0.025 | – | NS | NS | NS | – | NS | NS | NS | – | 0.025 | 0.005 | 0.005 | – | 0.05 | 0.025 | 0.005 |

| | $V_t$ | $R_t$ | $R_c$ | *D* | $I_{SC,v}$ | *L* |
|---|---|---|---|---|---|---|
| C $Cl^-$ *versus* $SO_4^{2-}$ Ringer (*P*<) | | | | | | |
| Pre | NS | 0.01 | 0.01 | 0.005 | NS | NS |
| 1 min | 0.001 | 0.025 | NS | NS | 0.025 | NS |
| 7 min | 0.001 | 0.005 | 0.05 | 0.025 | 0.005 | NS |
| 12 min | 0.001 | 0.001 | 0.025 | 0.01 | 0.01 | NS |

$V_t$, transtubular potential (mV); $R_t$, transtubular resistance (kΩ cm); $R_c$, core resistance (MΩ $cm^{-1}$); *D*, luminal diameter (μm); $I_{sc,v}$, virtual short-circuit current (μA $cm^{-1}$); *L*, length constant (μm); NS, not significant.

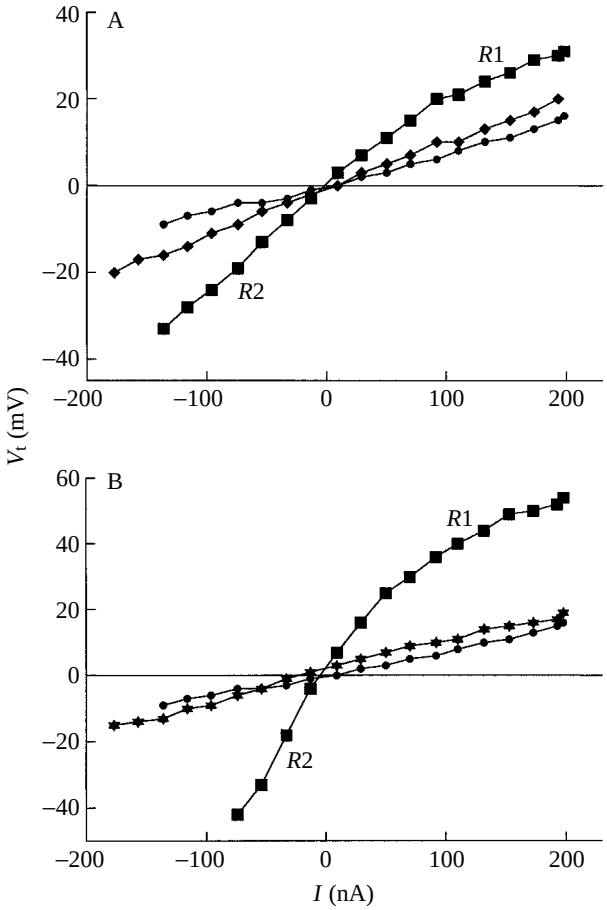


Fig. 2. Representative *I*/*V* plots of a tsetse fly tubule bathed in $Cl^-$ Ringer and exposed twice to cyclic AMP (the second dose was added to the bath 7 min after the first). The accompanying changes in cable parameters are shown in Table 3. (A) *I*/*V* plots before (◆), 1 min after (■) and 4 min after (●) exposure to cyclic AMP. The *I*/*V* plot became transiently curvilinear, with a marked increase in *R*2. (B) *I*/*V* plots 1 min before (●), 1 min after (■) and 3 min after (★) the second dose of cyclic AMP. The *I*/*V* plot again became transiently curvilinear, with an even more marked increase in *R*2. Note that $V_t$ (the potential at zero current input) remained at 0 mV throughout.

incomplete, in that the *I*/*V* plot was linear in one instance before exposure to cyclic AMP, and 10 min thereafter in another. The results were very different in the two groups (Table 2).

### *$Cl^-$ Ringer*

Exposure to cyclic AMP was without significant effect on $V_t$, *R*1, *E*1 and/or $R_{sh}$. The *I*/*V* plot became transiently curvilinear, with a marked increase in *R*2 (Table 2A; Fig. 2). As the apparent values of *E*1 and thus of $R_{sh}$ are erroneously low in tubules bathed in $Cl^-$ Ringer (see Discussion), and as $V_t$ was in any event close to 0 mV, values of $R_{ser}$ could not be calculated.

### *$SO_4^{2-}$ Ringer*

As expected (Isaacson and Nicolson, 1994), the tubules bathed in $SO_4^{2-}$ Ringer displayed much higher values of *R*1, *R*2, *E*1 and $R_{sh}$ than those bathed in $Cl^-$ Ringer (Table 2B; Fig. 3).

Table 2. I/V *transients derived from curvilinear* I/V *plots obtained from tubules bathed in $Cl^-$ or $SO_4^{2-}$ Ringer, as found immediately before (Pre) and then at approximately 2 and 10 min after exposure to cyclic AMP*

| | $V_t$ (mV) | | | *R*1 (kΩ) | | | *R*2 (kΩ) | | | *E*1 (mV) | | | $R_{sh}$ (kΩ) | | | $R_{ser}$ (MΩ) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre | 2 | 10 | Pre | 2 | 10 | Pre | 2 | 10 | Pre | 2 | 10 | Pre | 2 | 10 | Pre | 2 | 10 |
| A $Cl^-$ Ringer | | | | | | | | | | | | | | | | | | |
| *N* | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 5 | 4 | 4 | 5 | 4 | | | |
| Mean | 0.4 | 2.4 | 1.5 | 225 | 336 | 141 | 149 | 369 | 148 | 15.3 | 29.4 | 13.4 | 215 | 377 | 184 | | | |
| S.E.M. | 0.2 | 1.2 | 0.6 | 57 | 135 | 47 | 21 | 98 | 37 | 1.0 | 13.1 | 2.6 | 50 | 114 | 39 | | | |
| *P*< | – | NS | NS | – | NS | NS | – | 0.02 | NS | – | NS | NS | – | NS | NS | | | |
| B $SO_4^{2-}$ Ringer (*N*=5) | | | | | | | | | | | | | | | | | | |
| Mean | 6.2 | 34.8 | 29.2 | 954 | 591 | 482 | 1006 | 700 | 536 | 63.6 | 66.6 | 63.8 | 1139 | 3109 | 927 | 18.67 | 2.04 | 1.93 |
| S.E.M. | 2.2 | 10.7 | 7.4 | 185 | 104 | 169 | 276 | 148 | 158 | 13.8 | 11.8 | 9.7 | 281 | 1852 | 135 | 7.41 | 0.97 | 1.03 |
| *P*< | – | 0.025 | 0.025 | – | 0.025 | 0.005 | – | NS | 0.025 | – | NS | NS | – | NS | NS | – | 0.05 | 0.05 |

| | $V_t$ | *R*1 | *R*2 | *E*1 | $R_{sh}$ |
|---|---|---|---|---|---|
| C $Cl^-$ *versus* $SO_4^{2-}$ Ringer (*P*<) | | | | | |
| Pre | NS | 0.004 | 0.004 | 0.04 | 0.01 |
| 2 min | 0.004 | NS | NS | 0.05 | 0.004 |
| 10 min | 0.004 | 0.02 | 0.01 | 0.01 | 0.01 |

$V_t$, transtubular potential (mV); *E*1, voltage (mV) at the inflection point of the *I*/*V* plot; *R*1 and *R*2, slope resistances (kΩ) above and below the inflection point *E*1, respectively; $R_{sh}$, shunt resistance (kΩ); $R_{ser}$, series resistance (MΩ); NS, not significant.

Note that both *E*1 and $R_{sh}$ are necessarily less than their 'true' values (see Discussion).

A cyclic-AMP-induced peak in $V_t$ occurred in two of the tubules in $SO_4^{2-}$ Ringer. In both, it was accompanied by a transient overshoot in fall of $R_{ser}$ and a rise in $R_{sh}$; $V_t$ then fell, with partial reversal of both these changes in resistance.

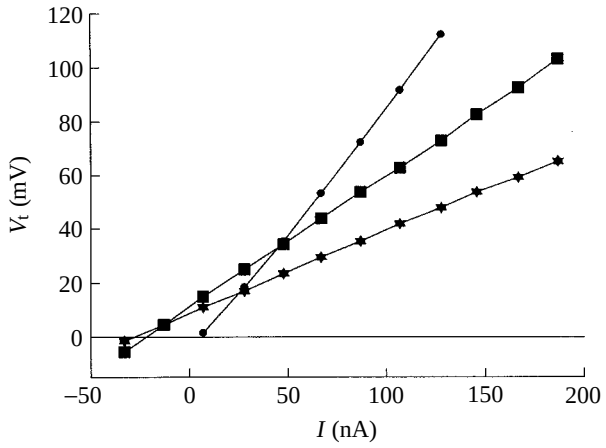


Fig. 3. Representative *I*/*V* plots of a tsetse fly tubule bathed in $SO_4^{2-}$ Ringer immediately before (●), 1 min after (■) and 9 min after (★) exposure to cyclic AMP. *R*2 fell immediately and progressively. Note the increase in $V_t$ (the potential at zero current input).

Exposure to cyclic AMP was followed by the immediate onset of a progressive increase in $V_t$, on two occasions with a short-lived peak, reaching relatively stable and elevated levels at 10 min (see also Fig. 1). *R*1 and *R*2 fell progressively (Fig. 3). *E*1 and $R_{sh}$ remained unchanged. $R_{ser}$ fell sharply.

In terms of the equivalent electrical circuit, the occurrence of a transient peak in $V_t$ could be due to an increase in either *E*1 or $R_{sh}$, to a fall in $R_{ser}$, or to any combination of these. Curvilinear *I*/*V* plots were found before, during and immediately after the $V_t$ peak in the two tubules. In both, the peak coincided with a large fall in $R_{ser}$ and a proportionately lesser increase in $R_{sh}$; the subsequent partial decline in $V_t$ coincided with a large fall in $R_{sh}$ (to less than its initial value) and a relatively slight rise in $R_{ser}$ (to still much less than its initial value).

## Discussion

Tsetse fly Malpighian tubules are apparently unique in that the transtubular potential, $V_t$, is barely detectable in $Cl^-$ Ringer, despite the composition of the latter approximating to that of tsetse fly haemolymph. $V_t$ is revealed on replacing the $Cl^-$ Ringer with $SO_4^{2-}$ Ringer. We have interpreted these findings as reflecting the short-circuiting, in $Cl^-$ Ringer, of the apical transport potential *via* apical $Cl^-$ channels (Isaacson and Nicolson, 1994). It follows that cable analysis of tsetse tubules bathed in $Cl^-$ Ringer will yield incorrectly low values of $I_{sc,v}$ (as $I_{sc,v}=V_t/R_t$). Similarly, the values of *E*1 and $R_{sh}$, as derived from an *I*/*V* plot, will be grossly underestimated; in conjunction with a $V_t$ close to 0 mV, this renders calculation of $R_{ser}$ impossible. We have therefore used both $Cl^-$ and $SO_4^{2-}$ Ringer's solutions in this study, the former primarily for examining the swelling transient and the latter for obtaining meaningful values for $I_{sc,v}$, *E*1, $R_{sh}$ and $R_{ser}$.

### *Electrical transients*

Prior to exposure to cyclic AMP, the results of cable analysis (Table 1) of both the tubules bathed in $Cl^-$ Ringer (the $Cl^-$ group) and those bathed in $SO_4^{2-}$ Ringer (the $SO_4^{2-}$ group), while differing from each other, were essentially identical to those reported previously (Isaacson and Nicolson, 1994).

At 0–3 min, the $Cl^-$ group responded to cyclic AMP with a visible and immediate cellular swelling and a narrowing of the tubular lumen, presumably reflecting cyclic-AMP-induced inflow of NaCl across the basolateral cell membrane, with a resultant osmotic inflow of water. Although impossible to determine in the tubules bathed in $Cl^-$ Ringer (see above), the steep fall in $R_{ser}$ (in the $SO_4^{2-}$ group; Table 2B) is consistent with just such a large increase in basolateral ionic permeability. This interpretation is in agreement with earlier observations: cyclic AMP has been shown to increase basolateral $Na^+$ conductance in the mosquito Malpighian tubule (Sawyer and Beyenbach, 1985), and cyclic AMP depolarises the basolateral potential of tsetse fly tubules (Isaacson and Nicolson, 1994, and see Fig. 4).

Cable analysis (Table 1) confirmed the visually observed narrowing of the tubular lumen, revealing also the resultant increases in core resistance ($R_c$) and tubular input resistance (i.e. $\Delta V_t/\Delta I$, not listed); the latter is reflected in the steeper slope of the *I*/*V* plot (*R*2 in Table 2; Fig. 2A) and also in the increase in transtubular resistance ($R_t$ in Table 1). These findings are in contrast to those of the $SO_4^{2-}$ group (Table 1; Fig. 3), in which cellular swelling and luminal narrowing were not detected; here, both $R_t$ and *R*2 fell (although the fall in $R_t$ was statistically non-significant).

Conceivably, compression of the intercellular spaces secondary to cellular swelling could also contribute to the increase in tubular input resistance. However, no significant change was detected in the apparent (and as pointed out above, far from accurate) value of $R_{sh}$ (Table 2). Furthermore, preliminary electron microscopic examination has shown the intercellular spaces to be barely visible, with adjacent cells tightly apposed to each other, in both control and cyclic-AMP-stimulated tsetse Malpighian tubules (Isaacson and Nicolson, 1994).

At 6–8 min, the lumen was again clearly visible in the $Cl^-$ group. In keeping with this volume regulatory response, $R_t$ and the luminal diameter (and therefore $R_c$) returned towards their initial levels (Table 1). In the $SO_4^{2-}$ group, $R_t$ fell and, as $R_c$ remained constant, so too did the length constant (Table 1). As $R_t$ fell in both groups of tubules, the fall in the $Cl^-$ group was presumably a consequence of both the subsidence of the cellular swelling, the inverse of the response noted at 0–3 min, and, as in the $SO_4^{2-}$ group, a direct effect of cyclic AMP on transtubular resistance (presumably, as argued above, on basolateral ionic conductance).

At 10–12 min, both $R_t$ and *R*2 had fallen in the $Cl^-$ group (Tables 1, 2), the former to less than half its initial value. These changes now paralleled in direction those seen in the $SO_4^{2-}$ group, as expected if the initial increases in $R_t$ and *R*2 were secondary to the now dissipated cellular swelling.

Cable analysis confirmed the visibly discernible luminal distension in the $Cl^-$ group (Table 1), presumably reflecting

Table 3. *Cable parameters of a tubule bathed in $Cl^-$ Ringer, exposed to two consecutive doses of cyclic AMP (see Fig. 2)*

| Time (min) | | $V_t$ (mV) | $L$ (μm) | $R_t$ (Ω cm) | $R_c$ (MΩ $cm^{-1}$) | $R_{in}$ (kΩ) | $D$ (μm) | $I_{sc,v}$ (μA $cm^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| –6 | | 0 | 258 | 3.3 | 4.9 | 127 | 39 | 0 |
| –2 | | 0 | 281 | 2.6 | 3.2 | 91 | 49 | 0 |
| 1 | Cyclic AMP | 1 | 200 | 8.8 | 22.0 | 439 | 18 | 0.1 |
| 5 | | 0 | 287 | 2.4 | 2.9 | 83 | 52 | 0 |
| 8 | Cyclic AMP | 4 | 191 | 10.7 | 29.4 | 561 | 16 | 0.3 |
| 12 | | 3 | 286 | 3.6 | 4.4 | 127 | 41 | 0.8 |
| 15 | | 3 | 338 | 1.6 | 1.4 | 46 | 75 | 1.9 |

Times are in minutes relative to first exposure to cyclic AMP.
$R_{in}$, tubular input resistance (kΩ); remaining parameters and units as in Table 1.

increased secretion of tubular fluid. This suggestion is supported by the progressive increase in $I_{sc,v}$, as seen in the tubules bathed in $SO_4^{2-}$ Ringer. (As pointed out earlier, the attenuated levels of $V_t$ found in tsetse tubules bathed in $Cl^-$ Ringer reduce the calculated values of $I_{sc,v}$, as here, where $V_t$ was indistinguishable from the control level and close to 0 mV.)

The calculated (and necessarily attenuated) values of $E1$ and $R_{sh}$ in the $Cl^-$ group remained indistinguishable from control levels (Table 2A), suggesting that the presumed increase in 'true' $I_{sc,v}$, if it could be measured, would necessarily be due to a fall in $R_{ser}$. This was clearly evident in the $SO_4^{2-}$ group (Table 2B).

### *Cell volume regulation*

Microscopic examination of the tubules bathed in $Cl^-$ Ringer revealed the lumen to be partially obliterated during the first 2 min, but distended by 10 min, after exposure to cyclic AMP. Calculation based upon the typical changes in tubular OD, and on the mean cable estimates of changes in lumen diameter – which paralleled those observed visually – suggested an almost immediate and large increase in cell volume (as indicated by the approximate doubling of apparent cell height), and that this increment in cell volume was gradually dissipated over the ensuing 10 min, with a progressive increase in $I_{sc,v}$ (Table 1). Even greater increases in cell volume have been measured in non-secretory regions of *Rhodnius prolixus* Malpighian tubules bathed in hypo-osmotic saline (O'Donnell and Mandelzys, 1988). Malpighian tubules function normally (as judged by fluid secretion) in a wide range of bathing fluid osmolalities (Maddrell, 1969).

The electrical concomitants of the volume regulatory response in tsetse fly tubules are clearly seen on inspection of the sequential changes in a single tubule subject to two successive applications of cyclic AMP (Table 3; Fig. 2A,B). As cyclic AMP is without effect on $E1$, and as its effect on $R_{ser}$ is persistent (Table 2), these electrical transients reflect only the resistance changes resulting from changes in cell volume.

### *The composite picture*

As pointed out above, measurement of electrical parameters is inaccurate or impossible in tsetse Malpighian tubules bathed in $Cl^-$ Ringer. However, a composite picture of the cyclic-AMP-induced events in these tubules emerges on incorporation of the patterns of change in these parameters in the $SO_4^{2-}$ group, as follows. The immediate response to cyclic AMP is a massive increase in basolateral ionic permeability (to $Na^+$ and $Cl^-$); $R_{ser}$ falls steeply. The resultant influx of solute and water causes a large increase in cell volume, maximal within the first 1–3 min. The cellular swelling encroaches on the tubular lumen, increasing $R_c$ and $R_t$ and also the slope ($R2$) of the $I/V$ plot. A volume regulatory mechanism now comes into play. As the cell volume falls, so too do $R_c$, $R_t$ and $R2$, and the tubular lumen regains its initial diameter. Tubular secretion commences, as judged by the rise in $I_{sc,v}$. At 10–12 min, by which time the cell volume is almost back to normal, the high rate of secretion causes marked distension of the tubular lumen; accordingly, $R_c$ and $R_t$, and therefore $R2$, fall further. In terms of the equivalent electrical circuit, the stimulatory effect of cyclic AMP on tubular secretion is due solely to the induced fall in $R_{ser}$, with $E1$ and $R_{sh}$ being unaffected. This is in agreement with our previous studies at room temperature (Isaacson and Nicolson, 1994), but not at elevated temperatures (Nicolson and Isaacson, 1996).

As the $SO_4^{2-}$ Ringer contained only 8 mmol $l^{-1}$ $Cl^-$, it might be expected that cyclic-AMP-induced events would be less evident in the $SO_4^{2-}$ than in the $Cl^-$ group of tubules. Thus, cellular swelling and narrowing of the tubular lumen, with corresponding increases in $R_c$ and $R2$, were minimal or absent, while $R_t$, in sharp contrast to the initial increase seen in the $Cl^-$ group, tended to fall (so confirming that its rise in the latter was secondary to cellular swelling). As judged by the slower rate of rise in $I_{sc,v}$, and by the absence of luminal distension with a consequent fall in $R_c$, the tubular secretory response was relatively subdued. Further evidence for the dependency on $Cl^-$ comes from the diminished volume response to cyclic AMP of tubules bathed in $Cl^-$ Ringer and exposed to furosemide (L. C. Isaacson and S. W. Nicolson, unpublished observations).

### *The $V_t$ transient*

An exception to the above expectation was the not infrequent appearance of a striking transient in $V_t$ (Fig. 1),

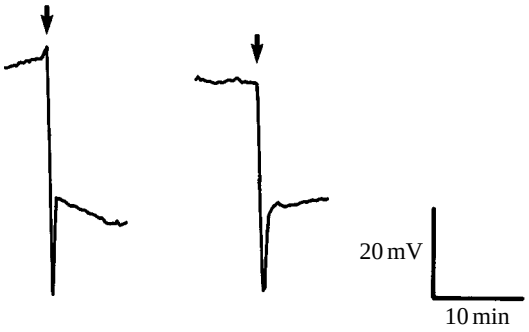


Fig. 4. Change in basal membrane potential of two tsetse fly tubules bathed in $Cl^-$ Ringer and stimulated with cyclic AMP (arrows). Note that the voltage scales differ in Figs 1 and 4.

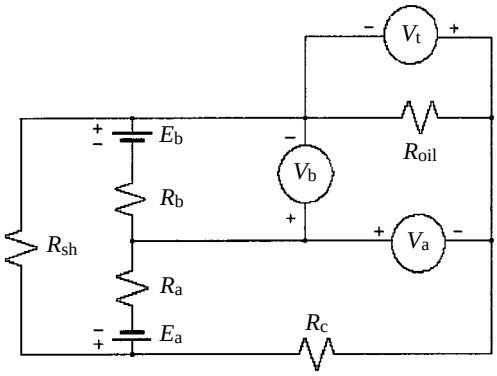


Fig. 5. An equivalent electrical circuit for the oil-gap technique, with conventional placement of voltmeters for measurement of transtubular ($V_t$), basal ($V_b$) and apical ($V_a$) potentials. $R_{oil}$ is the resistance of the extratubular saline layer within the oil barrier; $R_c$ is the resistance of the fluid within the tubular lumen. $E_a$, $R_a$, $E_b$ and $R_b$ are the electromotive forces and resistances across the apical and basal membranes, respectively. $R_{sh}$ represents possible transcellular and paracellular shunts. For simplicity, the open end of the tubule (the non-perfused bath) is assumed not to contain a voltage source (Isaacson and Nicolson, 1989). The values of the various circuit parameters are not at all critical; within wide limits, whatever the values chosen for these parameters, increasing $R_c$ lowers the apparent transtubular voltage, hyperpolarises the apical membrane and is without effect on $V_b$ (Table 4).

largely due to a short-lived overshoot in the cyclic-AMP-induced increase in basolateral conductance (fall in $R_{ser}$); this was accompanied by a proportionately lesser rise in $R_{sh}$. The reason for the latter is not obvious, but is presumably related to the brief inrush of fluid accompanying the fall in $R_{ser}$. Confirmation of this finding can be found in the changes in basal membrane potential of tsetse fly tubules stimulated with cyclic AMP, when a similar short-lived overshoot was sometimes apparent (Fig. 4; L. C. Nicolson and S. W. Isaacson, unpublished observations).

Voltage transients accompanying exposure of a Malpighian tubule to a secretory stimulus have been reported previously: triphasic excursions in both $V_t$ and the apical potential, as detected by the oil-gap technique, were seen following exposure of *Rhodnius prolixus* tubules to 5-hydroxytryptamine (O'Donnell and Maddrell, 1984). In the oil-gap technique, an oil barrier separates the fluids bathing the closed and open ends of the tubule, electrodes are placed in each bath, and $V_t$ is assumed to be the potential detected by the electrode in the bath enclosing the open end. This assumption has, however, been shown to be incorrect (Aneshansley *et al.* 1989; Isaacson and Nicolson, 1989), a major and unavoidable source of error being the conductance of the thin layer of saline coating the outer surface of the tubule within the oil barrier (Fig. 5). The potential detected by the electrodes within the two baths is thus not the transtubular potential, but merely that across this extratubular shunt. The larger the ratio of the resistance of the extracellular shunt to that of the luminal fluid ($R_c$), the closer the measured potential approximates to the actual $V_t$. Or, conversely, the larger the core resistance, the smaller the apparent transtubular potential. Similarly, it can be shown that the apparent apical potential, as measured between an intracellular electrode and another in the unperfused bath (at the open end of the tubule), hyperpolarises as $R_c$ increases (Table 4).

Table 4. *Simulation of the errors inherent in the oil-gap technique*

| | Actual | | | Measured | | |
|---|---|---|---|---|---|---|
| $R_c$ (MΩ) | $V_t$ (mV) | $V_b$ (mV) | $V_a$ (mV) | $V_t$ (mV) | $V_b$ (mV) | $V_a$ (mV) |
| 0.1 | −18.2 | −53.0 | −34.8 | −16.6 | −53.0 | −36.4 |
| 1 | −21.4 | −53.6 | −32.2 | −10.7 | −53.6 | −42.9 |
| 10 | −26.0 | −54.3 | −28.3 | −2.6 | −54.3 | −52.0 |

Errors revealed by the equivalent electrical circuit (Fig. 5), arbitrarily assuming $E_b$=60 mV, $E_a$=0 mV, $R_{sh}$ and $R_{oil}$=1 MΩ, $R_a$=1 MΩ and $R_b$=0.2 MΩ. These values are perhaps not unreasonable for a tubule bathed in $Cl^-$ Ringer. $R_a$ was assumed to be several times larger than $R_b$, as is usually the case in 'tight' epithelia, and as also suggested by the data of O'Donnell and Maddrell (1984). For simplicity, $E_a$ was assumed to be zero.

The 'actual' values (in mV) of $V_t$, $V_b$ and $V_a$, for varying values of $R_c$, are contrasted with those which would be found using the oil-gap technique. Increasing $R_c$ lowers the apparent $V_t$, hyperpolarises $V_a$ and is without effect on $V_b$.

It follows that changes in lumen diameter (and thus $R_c$) accompanying transient perturbations in cell volume – as seen in this study – could readily account for triphasic responses in the potentials detected by the oil-gap technique. As initial cellular swelling, although not commented upon, was presumably also present in stimulated *Rhodnius prolixus* tubules (O'Donnell and Maddrell, 1984), it would appear that the triphasic potential transients which they observed were artefactual and not comparable to the voltage transients reported here for perfused tubules.

## *Conclusion*

Cell swelling was maximal at the start and minimal at the end of the 10–12 min experimental period; evidence of

increased tubular secretion first became apparent at 3–7 min and was maximal at 10–12 min. Fluid secretion by the tsetse Malpighian tubule is driven almost entirely by the active transport of $Na^+$ (Gee, 1976*a*). A similar pattern of volume increase preceding increased transepithelial $Na^+$ transport has been observed in the proximal tubule of the mammalian kidney (Laprade, 1994), following both iso-osmotic and hypo-osmotic volume perturbation (luminal addition of glucose and alanine, or hypo-osmotic shock, respectively). The volume changes required to activate such volume regulatory transport pathways are not necessarily large; the mammalian proximal tubular cell responds to volume changes of less than 3% (Lohr and Grantham, 1986). It may be of particular relevance to this study that the activities of several different volume-sensitive transport pathways are modulated by intracellular $[Cl^-]$ (Parker, 1994). However, little is known of the mechanisms underlying volume regulatory responses (Lang *et al.* 1995; Strange, 1994). Cable analysis, because of its speed and because of the dramatic responses obtained, appears to be an excellent tool for elucidating these mechanisms in the tsetse fly Malpighian tubule.

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