AN INVESTIGATION OF THE MIDGUT K⁺ PUMP OF THE TOBACCO HORNWORM (MANDUCA SEXTA) USING SPECIFIC INHIBITORS AND AMPHOTERICIN B

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Summary

Active K⁺ secretion in isolated posterior midguts of Manduca sexta was studied by measuring the short-circuit current. One aim of this study was to verify the postulate from biochemical reports that the cooperative apical arrangement of a vacuolar-type H⁺-ATPase (V-ATPase) and a K⁺/H⁺ antiporter drive the short-circuit current. Hence, we tested several specific inhibitors of the V-ATPase on the in vitro midgut preparation. Nitrate, bafilomycin A₁, 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-Cl) and amiloride all reduced the short-circuit current. This suggests that the H⁺-ATPase is involved in transepithelial K⁺ secretion. However, even at relatively high doses of these inhibitors, the block of the short-circuit current was not complete. Two other agents, thallium ions (Tl⁺, at millimolar concentrations) and trimethyltin chloride (TMT, 50 μmol l⁻¹), did abolish the short-circuit current.

Apical, but not basal, use of the ionophore amphotericin B largely eliminated the short-circuit current. This supports the view that the current-generating source resides in the apical membranes. An apical (and probably intracellular) site of action for NO₃⁻, Tl⁺ and TMT is suggested by the observation that basal amphotericin B is needed for blockage by NO₃⁻ but does not, however, influence the effect of Tl⁺ and TMT. Likely sites of action are the V-ATPase (for nitrate and TMT) and the K⁺/H⁺ antiporter (for Tl⁺).

Introduction

Like other phytophagous lepidopteran larvae, the tobacco hornworm Manduca sexta actively transports K⁺ across its midgut from haemolymph to lumen. When the isolated tissue is bathed with standard high-K⁺ (32 mmol l⁻¹) insect saline on both sides, it generates a lumen-positive potential difference of 70–100 mV, and tissue conductance (Gₜ) ranges between 10 and 15 mS cm⁻². After short-circuiting the potential difference, a short-circuit current (Iₛₛ) of several hundred microamps per square centimetre can be measured, which closely matches the luminally directed net flux of radioactively labelled K⁺ (Wood and Moreton, 1978; Harvey and Wolfersberger, 1979; Cioffi and Harvey, 1981).

Key words: K⁺ secretion, short-circuit current, V-ATPase, nitrate, bafilomycin A₁, trimethyltin chloride, ionophore, tobacco hornworm, Manduca sexta.
According to the present model, K⁺ secretion proceeds as follows: K⁺ enters the electrically coupled goblet and columnar cells (Moffett et al. 1982; Moffett and Koch, 1988) passively via basolateral Ba²⁺-blockable K⁺ channels (Zeiske et al. 1986). An active apical transport step (Dow et al. 1984) extrudes K⁺ into the goblet cavity, which in turn opens through a valve-like structure into the luminal space. This mechanism, the so-called ‘K⁺ pump’ (Harvey, 1980; Harvey et al. 1983), is electrogenic, Na⁺-independent (Harvey and Nedergaard, 1964) and ouabain-insensitive (Jungreis and Vaughan, 1977). On the basis of electron micrographs, it was suggested that the numerous 10 nm particles studding the cytosolic face of the goblet cell apical membrane, the so-called ‘portasomes’, might be the morphological correlate of the K⁺ pump (Harvey, 1980).

Two agents have been claimed to be specific blockers of the Iₑ carried by K⁺ in the midgut of other lepidopteran larvae: thallium (Tl⁺) is thought to affect the apical K⁺ pump in Hyalophora cecropia (Zerahn and Koefoed, 1979; Zerahn, 1982), whereas the mode of action of TMT in Spodoptera littoralis is unclear (Thomas and May, 1984).

It has recently been shown that, in purified goblet cell apical membranes, active K⁺ transport is caused by the concerted action of a V-ATPase that pumps H⁺ and a K⁺/H⁺ antiporter that recycles H⁺ (Wieczorek et al. 1991). Although the diuretic amiloride is known to block both the K⁺/H⁺ antiporter and the V-ATPase, the latter transporter is also sensitive to several other agents, especially the antibiotic bafilomycin A₁ (Wieczorek et al. 1991), but also nitrate (Wieczorek et al. 1986) and the nucleotide-analogue NBD-Cl (Forgac, 1989).

On the basis of microelectrode studies in the M. sexta midgut, an additional active Ba²⁺-sensitive K⁺ transport step was postulated to act in parallel with the basolateral K⁺ channels (Moffett and Koch, 1988, 1991; Chao et al. 1990).

The present study asks several questions. (1) Is it possible to impair the transepithelial K⁺ current in vitro using the drugs used in biochemical studies? (2) Are the proposed mechanisms sufficient to describe Iₑ under all conditions? (3) Do mechanisms other than those mentioned above contribute to the functionally defined active ‘K⁺ pump’? We addressed these problems by testing the effects of the specific inhibitors mentioned above on K⁺ transport in whole isolated midgut preparations. In order to localize the current-generating membrane(s) and, if necessary, the target membrane of a respective inhibitor, we employed the polyene antibiotic amphotericin B. This ionophore renders membranes permeable to small monovalent ions and may be used to ‘functionally remove’ a membrane through elimination of electrical and chemical gradients, a method successfully applied to vertebrate epithelia (see Dawson et al. 1990).

Materials and methods

Insects

Larvae of Manduca sexta (Linnaeus) purchased from Carolina Biological Supply Company (Burlington, NC, USA) were reared on a synthetic diet. Early fifth-instar larvae weighing 3–5 g were used. The posterior midgut was dissected from cold-anaesthetized animals. Adhering Malpighian tubules, tracheae and fat bodies were removed, and the opened tissue was mounted as a flat sheet in a modified Ussing chamber as described
previously (Zeiske et al. 1986). The midgut was superfused on both sides with the bathing solutions, using gravity feed.

Salines and chemicals

The standard saline 32K(Cl) (according to Nedergaard and Harvey, 1968) contained (in mmol l\(^{-1}\)): 30, KCl; 2, KHCO\(_3\); 5, CaCl\(_2\); 5, MgCl\(_2\); 5, Tris (pH 8, aerated) and 166, sucrose. In the saline designated 90K(Cl), 58 mmol l\(^{-1}\) potassium gluconate was added in exchange for 116 mmol l\(^{-1}\) sucrose while [Cl\(^-\)] remained at 50 mmol l\(^{-1}\), and the pH was adjusted to 7.14. In 90K(NO\(_3\)) saline only the Cl\(^-\) of the 90K(Cl) was replaced by NO\(_3\)\(^-\). In some experiments employing apical or basal amphotericin B, the K\(^+\) channel blocker Ba\(^{2+}\) was applied in high doses (up to 16 mmol l\(^{-1}\)). For osmotic and ionic strength balance, the control saline then additionally contained (net) 32 mmol l\(^{-1}\) Tris gluconate [titrated to pH 7.0; 32K32Tris(Cl)], and Ba\(^{2+}\) application was carried out by substituting 1 mmol l\(^{-1}\) barium gluconate for 2 mmol l\(^{-1}\) Tris gluconate. Osmolarity was adjusted by appropriate subtraction of sucrose. The pH change from 8 to 7, where 93% of Tris is cationic, did not affect \(I_{sc}\) under any conditions. Amphotericin B (Sigma), bafilomycin A\(_1\) (purchased from Dr K. Altendorf, Universität Osnabrück, Germany) and NBD-Cl (Merck-Schuchardt, Hohenbrunn, Germany) were added from dimethylsulphoxide (DMSO) stock solutions. At the maximum concentration used in the present study (0.5%) the solvent alone had no effect on \(I_{sc}\) or transmembrane conductance (\(G_t\)). Amiloride (a gift of Merck, Sharp and Dohme, Munich, Germany) was directly dissolved in the salines, and pH was controlled. Thallium (final concentration 2–5 mmol l\(^{-1}\) TINO\(_3\)) and trimethyltin chloride (TMT) were added from aqueous stock solutions.

Electrical measurements

Voltage-sensing calomel electrodes and Ag/AgCl current-passing electrodes were connected to the bathing solution in the half-chambers by 0.2 mol l\(^{-1}\) NaCl–agar bridges (KCl was not used in order to avoid K\(^+\) leakage into the bathing solutions). Tissues were mounted and equilibrated for approximately 30 min before short-circuiting. After an additional 15 min, the \(I_{sc}\) became reasonably stable. \(I_{sc}\) was measured with a voltage-clamp device (VCC 600, Physiologic Instruments, San Diego, CA, USA) and monitored on Linseis recorders (Selb, Germany). The measured solution resistance was approximately 3 Ω cm\(^2\) in 32K(Cl) (for the distance of less than 1 mm between the voltage electrodes), so no correction was made for this (see also Zeiske et al. 1992). Tissue conductance (\(G_t\)) was calculated from current responses to 400 ms voltage pulses (\(\Delta V\)).

Results

Effect of amphotericin B on the apical membranes

In order to assess which of the epithelial membranes, apical or basolateral, could be targeted by the putative K\(^+\)-current-blocking agents, we wanted to employ the membrane permeabilizer amphotericin B. In vertebrate epithelia, the one-sided use of polyelectic antibiotics virtually eliminates the resistance of the adjacent membrane without affecting the membrane on the contralateral side of the tissue (Palmer et al. 1980; Wills, 1981;
Dawson et al. 1990). The efficacy of these ionophores depends on factors such as thickness, sterol content (Marty and Finkelstein, 1975; Kleinberg and Finkelstein, 1984) and accessibility of the respective membrane. We therefore tested whether amphotericin B could be incorporated into the insect midgut membranes. If the ionophore was embedded into the apical membrane, it should, for example, cause a severe disturbance in K+ current, as the goblet cell apical membranes are known to contain the major current-generating source (Dow et al. 1984). A typical experiment can be seen in Fig. 1. It demonstrates the rapid decrease in outwardly directed $I_{sc}$ and increase in $G_t$ after apical addition of amphotericin B. The inset to Fig. 1 summarizes the mean change of these variables for eight preparations: 5 μmol l$^{-1}$ amphotericin B tripled $G_t$ and reduced $I_{sc}$ by more than 80%. In one experiment, $I_{sc}$ fell to zero. Doubling the ionophore dose had almost no additional effect (six preparations). Thus, the severe impact of amphotericin B on $I_{sc}$ and $G_t$ shows convincingly that it is incorporated into insect epithelial membranes.

**Permeabilization of the basolateral membranes**

The drastic apical effect of amphotericin B emphasizes that this membrane not only powers the K+ current but also contributes the major resistance to K+ movement. If amphotericin B were equally effective on the basal membrane, then the already relatively
low basal resistance might even be eliminated by the ionophore. This would open up a way to determine the ratio of apical to basolateral resistances. To determine the extent of the polyene-induced conductance, Ba^{2+} (an effective blocker of the normal basolateral K^+ permeability) was applied to the basal side before and after amphotericin B. A typical experiment is shown in Fig. 2, and the inset summarizes the results of six such experiments. Under control conditions (CTR) mean $I_{sc}$ and $G_t$ are 290±32 µA cm$^{-2}$ and 11.1±1.3 mS cm$^{-2}$ (±1 S.E.M.), respectively. Vertical bars represent ±1 S.E.M.

![Fig. 2. Response of $I_{sc}$ to basal application of 12 mmol l$^{-1}$ Ba^{2+} before and after 5 µmol l$^{-1}$ amphotericin B had been added to the basal bath (ΔV=5 mV). Salines: apical, 32K32Tris(Cl); basal, 32K32Tris(Cl) or a 1:4 mixture of the latter and 32K16Ba(Cl). The inset shows normalized $I_{sc}$ and $G_t$ of six similar experiments. Under control conditions (CTR) mean $I_{sc}$ and $G_t$ are 290±32 µA cm$^{-2}$ and 11.1±1.3 mS cm$^{-2}$ (±1 S.E.M.), respectively. Vertical bars represent ±1 S.E.M.](image)

The ratio between the $I_{sc}$ before and after basal addition of amphotericin B can be expressed as:

$$\frac{I_{sc}}{I_{sc,Ampho}} = \frac{E}{R_a + R_b} \frac{R_{a,Ampho} + R_{b,Ampho}}{E_{Ampho}},$$

(1)
where $E$ is the electromotive force of the tissue and $R_a$ and $R_b$ are the resistances of the apical and basolateral membranes before and after treatment with amphotericin B (Ampho), respectively. $I_{sc,\text{Ampho}}$ is the short-circuit current measured after addition of amphotericin B. Among small monovalent ions, cations can permeate amphotericin pores approximately seven times more easily than can anions (Dawson, 1987). Therefore, it seems reasonable to assume that after basal polyene application the electromotive force of the basolateral membranes ($E_b$) is still governed by the Nernst potential for $K^+$, which is the only small permeant cation available (see legend of Fig. 2 for the experimental conditions). Since, furthermore, basally applied amphotericin is not supposed to affect the apical membranes, we may speculate that $E = E_a + E_b$ remains unchanged. With $E = E_{\text{Ampho}}$, $R_a = R_{a,\text{Ampho}}$ and $R_{b,\text{Ampho}} = 0$, equation 1 simplifies to:

$$\frac{I_{sc}}{I_{sc,\text{Ampho}}} = \frac{R_a}{R_a + R_b} = fR_a.$$  \hspace{1cm} (2)

$fR_a$ is the fractional resistance of the apical membranes and was estimated to be $0.89 \pm 0.02$ (±1 s.e.m., $N=6$).

Inhibitors of the vacuolar-type proton pump

To test the assumption that the apically located V-ATPase exclusively energizes transepithelial $K^+$ secretion in the *M. sexta* posterior midgut, we tested the effect of V-ATPase blockers on $K^+$ current.

Nitrate

Nitrate acts on the cytosolic side of the V-ATPase (Nelson, 1987), where it causes a reversible dissociation of the intracellularly attached $V_1$ sector from the transmembraneal $V_o$ sector (Bowman et al. 1992; Forgac, 1992). An exchange of $30 \text{ mmol} l^{-1} \text{ Cl}^-$ for $\text{NO}_3^-$ on both sides of the intact tissue bathed with $32\text{K(Cl)}$ saline caused a decrease of only $20 \pm 3\%$ in $I_{sc}$ ($\pm 1$ s.e.m., $N=4$). Since this effect might be due to a cessation of $\text{Cl}^-$ stimulation of the basolateral $K^+$ permeability (Zeiske et al. 1992), we permeabilized the basolateral membranes with amphotericin B. Three ionophore-treated preparations were bathed with cytosol-like $90\text{K(Cl)}$ saline, and $\text{NO}_3^-$ was progressively substituted for $\text{Cl}^-$ on both sides. This resulted in a drop of $I_{sc}$ but, at the same time, caused a $60\%$ rise of $G_t$ (Fig. 3A). A high, possibly paracellular, shunt permeability to nitrate has been reported for midgut tissue (Chao et al. 1989), and it seems likely that this was masking the $\text{NO}_3^-$ inhibition of the ‘active’ apical $K^+$ conductance. The decrease in $I_{sc}$ ($\Delta I_{sc}$) caused by increasing $\text{NO}_3^-$ doses was reversible and, as shown in Fig. 3B, can be described by the Michaelis–Menten equation:

$$\Delta I_{sc} = \frac{\Delta I_{\text{max}}}{1 + K_i/[\text{NO}_3^-]},$$  \hspace{1cm} (3)

where $K_i$ is the inhibition constant of $\text{NO}_3^-$ and $\Delta I_{\text{max}}$ is the percentage of $I_{sc}$ which is blocked by an infinite $\text{NO}_3^-$ concentration. Fitting equation 3 to the pooled data yields a $K_i$ value of $20 \text{ mmol} l^{-1}$ and a $\Delta I_{\text{max}}$ value of $78\%$. 
Bafilomycin

Bafilomycin A₁ is known as a specific inhibitor of V-ATPases and affects the purified enzymes in nanomolar doses (Bowman et al. 1988). However, under the conditions of this study, apical concentrations of greater than 1 μmol l⁻¹ were required to inhibit Iₛ反转] by 20% (N=4). Fig. 4 demonstrates that the additional basal application of this agent did not reduce Iₛ反转] further and that, even with 5 μmol l⁻¹ bafilomycin A₁ in the apical bath, the inhibition of Iₛ反转] was far from complete. This dose reduced Gₜ by approximately 20%.

NBD-Cl

NBD-Cl is also a widely used blocker of vacuolar-type H⁺-ATPases (Forgac, 1989). It was tested on two preparations at apical concentrations between 1 and 500 μmol l⁻¹. It caused half-maximal inhibition of Iₛ反转] at 100–200 μmol l⁻¹. However, the relative decrease in Gₜ was always greater than that in Iₛ反转] and both tissues were visibly damaged after application of 500 μmol l⁻¹ NBD-Cl, indicating side effects of this alkylating agent.

Amiloride

Amiloride blocks epithelial Na⁺ channels and Na⁺/H⁺ antiporters (Benos, 1982). It inhibits both the K⁺/H⁺ antiporter and the V-ATPase (at half-maximal values of...
approximately 0.2 mmol l\(^{-1}\) and 0.7 mmol l\(^{-1}\), respectively) in vesicles of purified goblet cell apical membranes (Wieczorek et al. 1991). Although 1 mmol l\(^{-1}\) amiloride in the apical bath had no effect on K\(^+\) transport, 10 mmol l\(^{-1}\) (which is just below the solubility limit) reduced I\(_{sc}\) by approximately 80% (two preparations, Fig. 5). \(G_t\) was not affected.

**Other inhibitors of active K\(^+\) current**

We tested the influence of two substances known effectively to inhibit midgut K\(^+\) secretion in other caterpillars.

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**Fig. 4.** Effect of bafilomycin A\(_1\) (Baf; values in \(\mu\)mol l\(^{-1}\)) on I\(_{sc}\). Both sides were superfused with 32K(Cl) saline. \(\Delta V=5\) mV.

**Fig. 5.** Effect of apical amiloride (Ami; values in mmol l\(^{-1}\)) on I\(_{sc}\). Both sides were superfused with 90K(Cl) saline. \(\Delta V=5\) mV.
Thallium

Thallium(I) blocks K⁺ secretion in the midgut of the American silkworm Hyalophora cecropia (Zerahn and Koefoed, 1979; Zerahn, 1982) and in the M. sexta midgut (see Schirmanns and Zeiske, 1991). In contrast to H. cecropia, where Tl⁺ is half as effective when applied from the basal side as it is when applied apically, the cation eliminated Iₛₑ when applied in millimolar concentrations to either side of the tobacco hornworm midgut (N=6). This inhibition was partly reversible, although long-term (more than 30 min) exposure to Tl⁺ resulted in an irreversible drop in Gᵣ.

Trimethyltin chloride (TMT)

TMT, applied to the basal membrane at a concentration of 10⁻⁹ mol l⁻¹, abolishes K⁺ transport in the midgut of the Egyptian army worm Spodoptera littoralis, whereas apical application requires 1 mmol l⁻¹ for a comparable effect (Thomas and May, 1984). In the midgut of M. sexta, the basal dose causing half-maximal inhibition was approximately 0.2 μmol l⁻¹. Basal application of 50 μmol l⁻¹ TMT always resulted in a complete block of Iₛₑ, while the simultaneous decrease in Gᵣ was 26±6 % (±1 S.E.M., N=5). In six of ten preparations, the apical use of TMT also inhibited Iₛₑ to a comparable degree; two preparations were less affected and two were unaffected. To evaluate whether the basolateral K⁺ transport step is the site of TMT action, as was suggested for S. littoralis (Thomas and May, 1984), we performed three experiments with amphotericin in the basal medium. Permeabilization of the basolateral membranes did not influence the (at low doses reversible) TMT sensitivity of Iₛₑ (Fig. 6A). Thus, TMT also acts apically. To determine whether TMT impairs the basolateral K⁺ permeability, we incorporated amphotericin B into the apical membrane. Basal addition of 50 μmol l⁻¹ TMT still abolished (residual) Iₛₑ. Nevertheless, Gᵣ retained its sensitivity to basal Ba²⁺ (Fig. 6B). Consequently, the K⁺ channels in the basolateral membranes seem to be unaffected by TMT.

Discussion

Resistive and electrical properties of apical and basolateral membranes

As in most other epithelia, the apical membranes of the M. sexta posterior midgut dominate the transcellular resistance. This could be shown through the amphotericin-based evaluation of fᵣₑ. To minimize possible changes in cell volume or cytosolic pH, we used a cytosol-like saline of pH 7.14 (see Chao et al. 1991) in most experiments requiring apical or basal application of amphotericin B. The apical incorporation of the ionophore results in a drastic increase in Gᵣ (Fig. 1). Nevertheless, under these conditions Gᵣ remains sensitive to the basal action of the K⁺ channel blocker Ba²⁺ (see Fig. 6B). Compared with other K⁺ channels (Latorre and Miller, 1983; Zeiske, 1990), high Ba²⁺ concentrations are necessary to obtain a distinct effect. Zeiske et al. (1986) demonstrated that, even at 20 μmol l⁻¹, the Ba²⁺ block of K⁺ current in M. sexta midgut is incomplete. They suggested that the comparably weak inhibition is an intrinsic feature of the basolateral K⁺ channels in this tissue. In addition, the possibility that Ba²⁺ might pass through these
channels, as it does in the Ca\(^{2+}\)-activated K\(^+\) channel (Neyton and Miller, 1988), and thus contribute to \(I_{sc}\), cannot be excluded.

Our data allow us to conclude that the effect of amphotericin B, as in vertebrate epithelia, is restricted to the membrane directly exposed to the ionophore. Since both goblet and columnar cells are electrically coupled (Moffett et al., 1982; Moffett and Koch, 1988), we have no information about the specific site of action of amphotericin B, although the more easily accessible columnar cell brush border should react preferentially. In fact, almost the whole goblet cell apical membrane area is ‘hidden’ in the goblet cavity (Cioffi, 1979) and might be difficult to reach because of the small goblet ‘valve’ access path (Moffett and Koch, 1992) and the probably viscous goblet matrix (Dow et al., 1984). The simultaneous drop in apical resistance and \(I_{sc}\) can be explained by an ionophore-induced shunt of the electrogenicity of the apical ‘K\(^+\) pump’. However, a minor fraction of \(I_{sc}\) is apparently not sensitive to even high concentrations of amphotericin B. There are two alternative explanations for this: either (i) there is a limit to apical ionophore incorporation, so the small amount of charge transferred by the still operating apical K\(^+\) pump is sensed by the voltage electrodes of the clamp device; or (ii) a current source located in the basolateral membranes is now unmasked.

Moffett and Koch (1988) presented evidence that, at basal K\(^{+}\) concentrations of less than 10 mmol l\(^{-1}\), or during extreme hypoxia, the transbasal electrochemical gradient is

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Fig. 6. (A) Time course of changes of \(I_{sc}\) in 90K(Cl) saline. The basal membrane was permeabilized with 5 \(\mu\)mol l\(^{-1}\) amphotericin B. Apical or basal TMT (values in \(\mu\)mol l\(^{-1}\)) inhibit \(I_{sc}\). \(\Delta V=5\) mV. (B) Mean values of \(G_t\) when the apical membranes had been exposed to 5 \(\mu\)mol l\(^{-1}\) amphotericin B and the remaining \(I_{sc}\) had been eliminated by basal 50 \(\mu\)mol l\(^{-1}\) TMT (CTR), and after subsequent basal application of 16 \(\mu\)mol l\(^{-1}\) Ba\(^{2+}\) (\(N=4\)). \(G_t\) was calculated from the current response to 10 mV voltage pulses. Salines: apical, 32K32Tris(Cl); basal, 32K32Tris(Cl) or 32K16Ba(Cl). Vertical bars represent ±1 S.E.M.
unfavourable for passive K⁺ entry. The transepithelial K⁺ current that is observed under these conditions must therefore depend on a second active K⁺ transport mechanism in the basolateral membranes. It was claimed that this transporter is blocked by Ba²⁺ and accounts for most of the basal K⁺ uptake even under standard conditions in 32 mmol l⁻¹ K⁺ saline (Chao et al. 1990; Moffett and Koch, 1991). Basal amphotericin B does permeabilize the basal membrane (Fig. 2), but it does not reduce Iₛ as would be expected if an active and electrogenic basolateral K⁺ transporter were to be shunted by the ionophore. Consequently, either such a transporter is not switched on under the present conditions or it cannot be electrogenic. Therefore, we feel that the electromotive force of the basolateral membranes before, as well as after, basal incorporation of the ionophore reflects the equilibrium potential of the permeant ions. Taking the latter into account, we estimated the fractional apical resistance fRₐ to be 0.89. This value applies to the ‘syncytial’ tissue and it matches very well with the results from microelectrode measurements (0.81 in goblet cells and 0.96 in columnar cells; Blankemeyer and Harvey, 1978).

Inhibition of the ‘K⁺ pump’

All inhibitors of the V-ATPase tested in this study also inhibited Iₛ. NO₃⁻ is relatively ineffective before basal application of amphotericin B, indicating that the ionophore makes the basolateral membranes more permeable to NO₃⁻ and thus creates a pathway to the apical intracellular NO₃⁻-binding site. Provided that an extrapolation of the dose–response relationship to NO₃⁻ concentrations greater than 50 mmol l⁻¹ is valid, the percentage of the maximal Iₛ depression caused by NO₃⁻ does not reach 100 %. An overlapping effect caused by removing Cl⁻, which stimulates the purified V-ATPase (Stone et al. 1990), is unlikely for, in the presence of apical amphotericin B, substitution of Cl⁻ by gluconate does not reduce the (residual) Iₛ (N=6).

The effects of bafilomycin A₁ and amiloride (on the K⁺/H⁺ antiporter and/or the V-ATPase) have so far been tested on only a few intact invertebrate epithelial preparations. In the Malpighian tubules of Formica polyctena, 5 μmol l⁻¹ bafilomycin A₁ is required to halve the fluid secretion driven by V-ATPase (Weltens et al. 1992). This corresponds to the effect of the same dose on Iₛ in our preparation. In Cl⁻-absorbing gills of the crab Eriocheir sinensis (Putzenlechner et al. 1992) and in K⁺-secreting Malpighian tubules of Drosophila hydei (Bertram et al. 1991), bafilomycin A₁ affects K⁺ transport at concentrations as low as 0.1 μmol l⁻¹. 1 mmol l⁻¹ amiloride halves K⁺ secretion in the latter tissue (Bertram, 1989) and almost abolishes it in the Malpighian tubules of Rhodnius prolixus (Maddrell and O’Donnell, 1992). The concentrations of both inhibitors required for a visible effect in the M. sexta midgut are rather higher than these values. As discussed earlier, this could be due to a larger diffusional barrier preventing big molecules from gaining access to the respective sites of action. Figs 4 and 5 demonstrate the slowness of the effects of the drug very clearly. The inhibition of Iₛ by relatively high doses of bafilomycin and amiloride, but also by NBD-Cl (not shown here), was always incomplete and seemed to be non-specific in the case of NBD-Cl. Hence, we cannot yet exclude the possibility that, besides the cooperation of the V-ATPase and the K⁺/H⁺ antiporter, an additional active and electrogenic transport step contributes a minor part to the apical ‘K⁺ pump’ (but see below).
Two other agents, Tl⁺ and TMT, abolish active K⁺ secretion. Tl⁺ does not block, but passes through, the basolateral K⁺ channels (Schirmanns and Zeiske, 1991) and, in the H. cecropia midgut, this cation inhibits K⁺ current competitively with K⁺ (Zerahn, 1982). Consequently, the site of Tl⁺ action seems to be the apical K⁺-translocating step, possibly the K⁺/H⁺ antiporter. TMT belongs to the highly membrane-active triorganotins. These compounds are known to inhibit, among other things, the F₁Fₒ-ATPase (Matsuno-Yagi and Hatefi, 1993), to denature membrane proteins (Zucker et al. 1988) and to mediate electroneutral Cl⁻/OH⁻ exchange (Antonenko, 1990), thereby eliminating chloride and pH gradients across biological membranes (Karniski, 1992). However, the efficiency of triorganotin compounds in causing these phenomena corresponds to the degree of their lipid solubility (Powers and Beavis, 1991), in which TMT ranks last. Since, in the S. littoralis midgut, the more lipophilic tripropyltin chloride was ineffective at a concentration of 1 mmol l⁻¹ (Thomas and May, 1984), a ‘common’ triorganotin effect seems to be unlikely for the caterpillar midgut. Fig. 6 strongly suggests that TMT inhibits only the apical transport step, being more effective from the basal side. TMT may act either indirectly, through the mitochondrial respiratory chain, or directly on transport structures in the apical membranes, e.g. the V-ATPase. Recently, and in support of this view, it was shown for a crab (Eriocheir sinensis) gill epithelium that 0.1 μmol l⁻¹ TMT inhibits one-third of the biochemically assayed V-ATPase activity, whereas a 100-fold higher dose is required for a similar effect on the phylogenetically related F₁Fₒ-ATPase (M. Putzenlechner, personal communication). Thus, TMT might (at least at these low concentrations in invertebrate epithelia) act as a relatively specific inhibitor of the V-ATPase, rather than affecting K⁺/H⁺ antiporter or even mitochondrial power supply.

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References


Insect midgut K⁺ pump block


