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Electrochemical gradients across Malpighian tubules of the house cricket, *Acheta domesticus*, and the mode of action of kinin and CRF-related diuretics

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Abstract: Double-barrelled ion-selective microelectrodes were used to measure Na^+, K^+ and Cl^- activities in the principal cells of cricket Malpighian tubules, allowing electrochemical gradients across apical and basal membranes to be calculated. At the basal membrane, K^+ and Cl^- gradients support passive exit into the bathing medium. A thermodynamic analysis shows both ions can be taken up by $Na^+/K^+/2Cl^-$ cotransport driven by a favourable Na^+ gradient. At the apical membrane, cations are actively transported into the lumen whereas Cl^- moves passively. Evidence is presented to show that diuretic kinins open an apical membrane Cl^- conductance whereas Achdo-DH acts via cAMP to stimulate the basal membrane NKCC.

Keywords: insect, diuretic hormones, ion transport, fluid secretion

INTRODUCTION

Malpighian tubules (MT) are the major excretory organs of insects, and generate a flow of primary urine that is subsequently modified by transport processes in the hindgut before the final urine is eliminated from the anus. Active cation (K^+ and/or Na^+) transport is the "prime mover" in the secretion of primary urine, with anions entering the lumen passively [1]. Water movement is osmotically coupled to the net transepithelial transport of KCl/NaCl. Cation transport is driven by a Bafilomycin A-sensitive V-type H^+ ATPase in the apical membrane of MT principal cells (PC) [2]. The ATPase pumps protons into the

lumen, generating a large electrochemical potential gradient for their return to the cell via cation/H^+ antiports in the apical membrane. Chloride moves passively into the lumen down a favourable electrochemical gradient. Movement of ions across the PC basal membrane involves ion channels, a ouabain-sensitive Na^+/K^+ ATPase, and cation/Cl^- cotransporters. To understand the relative importance of these different routes for anion and cation entry, membrane voltages and intracellular ion activities have been measured using double-barrelled ion-selective microelectrodes (dbISE), which allows electrochemical gradients to be calculated.

MT are not innervated and the secretion of primary urine is controlled by circulating diuretic and antidiuretic hormones, DH and ADH, respectively [3]. The former include the diuretic/myotropic kinins, corticotropin releasing-factor (CRF)-related DH, calcitonin (CT)-like DH, cardioacceleratory peptide 2b (CAP_{2b}) and tachykinin related peptides. CAP_{2b} acts as an antidiuretic on tubules of the blood sucking bug *Rhodnius prolixus*, and two additional, but unrelated ADHs have been identified in the beetle *Tenebrio molitor*. This study focuses on the house cricket, *Acheta domesticus*, and on two DH, a kinin (Achdo-KII) and a CRF-related DH (Achdo-DH). Achdo-KII acts via a Ca^{2+}-dependent process to stimulate Cl^- transport, whereas Achdo-DH acts primarily via cAMP to stimulate cation transport.

MATERIAL AND METHODS

The rearing of crickets and techniques used to measure fluid secretion and ion concentrations in the secreted fluid, and to record transepithelial (V_t) and basal membrane (V_b) voltages have been described elsewhere [4]. The methods used to construct and calibrate dbISE followed those described in detail by [5]. Likewise, calculations of intracellular ion activities, electrochemical gradients and the thermodynamic evaluation of ion transporters were as described in [5].

RESULTS AND DISCUSSION

A K^+ conductance dominates the properties of the PC basal membrane, but a smaller Cl^- conductance is revealed when this is blocked with 6 mM Ba^{2+}; there is no evidence for a Na^+ conductance. Sodium ions secreted by MT must therefore cross the PC basal membrane (the only cell type present in the main segment of cricket MT) via a combination of cation/Cl^- cotransporters and Na^+/organic

solute transporters. Removing proline and glucose from the bathing medium has no effect on V_b (data not shown), and Na^+ uptake along with organic solutes is unlikely to be important. In contrast, bumetanide and furosemide reduce fluid secretion (data not shown), consistent with basal membrane $Na^+/K^+/2Cl^-$ (NKCC) cotransport.

Measurements made with dbISE show that in unstimulated tubules the electrochemical gradients across the basal membrane for K^+ ($\Delta\mu_K{}^b$) and Cl^- ($\Delta\mu_{Cl}{}^b$) favour movement of both ions into the bathing medium whereas there is a substantial gradient ($\Delta\mu_{Na}{}^b$) for the uptake of Na^+ (Table 1). The net electrochemical gradient for the three ions is consistent with NKCC cotransport driven by $\Delta\mu_{Na}{}^b$, although Na^+/Cl^- cotransport cannot be ruled out. The Na^+ gradient is maintained by the extrusion of Na^+ from PC across apical and basal membranes. At the apical membrane, $\Delta\mu_{Cl}{}^a$ favours Cl^- movement into the lumen, whereas $\Delta\mu_K{}^a$ and $\Delta\mu_{Na}{}^a$ favour movement in the reverse direction, and cation secretion occurs by secondary active transport via amiloride-sensitive cation/H^+ antiports.

Table 1. Intracellular ion activities (a^i) and electrochemical gradients across basal ($\Delta\mu_i{}^b$) and apical ($\Delta\mu_i{}^a$) membranes before and after stimulation with 1 nM Achdo-K and 1 mM 8bromo-cAMP

	Unstimulated			1 nM Achdo-K			1 mM 8bromo-cAMP		
	a^i	$\Delta\mu_i{}^b$	$\Delta\mu_i{}^a$	a^i	$\Delta\mu_i{}^b$	$\Delta\mu_i{}^a$	a^i	$\Delta\mu_i{}^b$	$\Delta\mu_i{}^a$
	mM	mV	mV	mM	mV	mV	mM	mV	mV
Na^+	22	-67	-69	22	-67	-42	36	-60	-66
K^+	80	+2	-76	80	+2	-48	-	-	-
Cl^-	28	+6	+30	28	+6	+1	-	-	-

Stimulation with Achdo-KII depolarises V_t and, since V_b is unchanged (<±2 mV), the voltage across the apical membrane (V_a) falls [4]. The values of $\Delta\mu_{Na}{}^b$, $\Delta\mu_K{}^b$ and $\Delta\mu_{Cl}{}^b$ are unchanged, while $\Delta\mu_{Cl}{}^a$ declines towards zero i.e. approaches equilibrium (see Table 1). Despite the fall in $\Delta\mu_{Cl}{}^a$, Cl^- movement into the lumen increases two-fold, and the Cl^- conductance of the apical membrane must therefore be increased by Achdo-KII. The fall in V_a reduces $\Delta\mu_K{}^a$ and $\Delta\mu_{Na}{}^a$, and reduces the proton electrochemical gradient ($\Delta\mu_H{}^a$) against which the V-type H^+ ATPase operates. A combination of these factors will account for the non-selective increase in cation transport by Achdo-KII. A model for kinin action on cricket PC is shown in Figure 1. In unstimulated MT, Cl^- movement into the lumen limits the rate of KCl/NaCl secretion and much of the Cl^- entering

PC via NKCC cotransport returns to the bathing medium. Achdo-KII increases the Cl^- conductance of the apical membrane, which directs more Cl^- towards the lumen and thereby accelerates KCl/NaCl secretion along with osmotically obliged water.

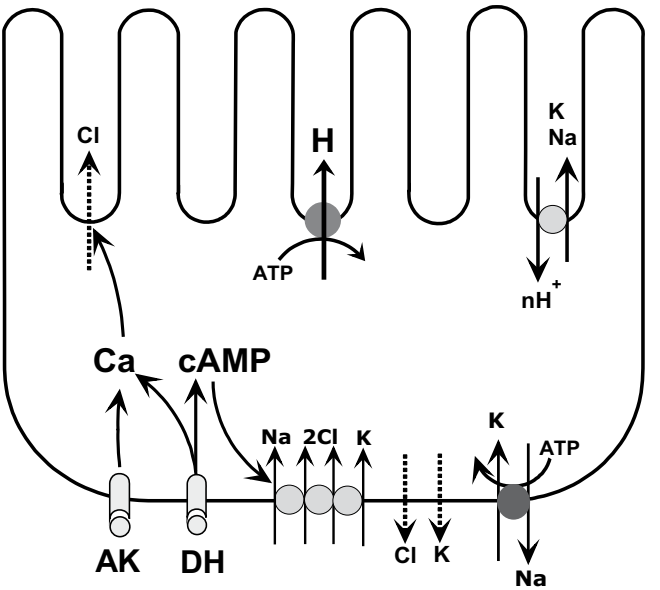

Figure 1. A model for ion transport processes in PC and the actions of Achdo-KII (AK) and Achdo-DH (DH). Dotted arrows indicate the direction of ion movement along electrochemical potential gradients through conductive pathways (ion channels), while dark circles indicate primary active transport processes. NKCC cotransport is driven by the favourable gradient for Na^+ entry, which is maintained by the basal membrane Na^+/K^+ ATPase. In unstimulated tubules, the Cl^- conductance of the basal membrane exceeds that of the apical membrane and much of the Cl^- returns to the bathing medium. Achdo-KII acts via Ca^{2+} to open the apical Cl^- conductance allowing more Cl^- into the lumen, which depolarises V_a and V_t. Achdo-DH may also target the apical Cl^- conductance, but in addition it acts via cAMP to stimulate NKCC cotransport, bringing more Na^+ into the PC. The resultant increase in a_{Na}^i means that Na^+ competes more effectively with K^+ for export to the lumen via apical membrane cation/H^+ antiports driven by a proton gradient established by the V-type H^+ ATPase.

In contrast to Achdo-KII, Achdo-DH increases Na^+ transport relative to K^+ and the secreted fluid $[Na^+]:[K^+]$ ratio rises from ~0.25 to unity. A similar effect is obtained with 8bromo-cAMP, which was used in place of Achdo-DH for much of this study. Sodium enters PC via basal membrane NKCC cotransport (see above) and this transporter is likely activated by cAMP since bumetanide and furosemide attenuate the diuretic activity of both the 2nd messenger and Achdo-DH. In support of this, all three ions (Na^+, K^+ and Cl^-) are needed to support stimulated rates of secretion [3].

Sodium ions entering PC via NKCC cotransport either return to the bathing medium via a ouabain-sensitive Na^+/K^+ ATPase or move into the lumen via apical membrane cation/H^+ antiports. In cricket tubules, Na^+ and K^+ appear to have equal affinity for the antiports [3]. Unstimulated tubules secrete K^+ rich fluid because K^+ is more plentiful in the cytoplasm, with much of the Na^+ that enters the PC being returned to the bathing fluid by the Na^+/K^+ ATPase. DbISE measurements show the intracellular Na^+ activity (aNa^i) of PC is increased following stimulation by 8bromo-cAMP, which is consistent with Na^+ uptake by NKCC cotransport exceeding what can be exported by the Na^+/K^+ ATPase. The increase in aNa^i means Na^+ competes more effectively with K^+ for cation/H^+ antiports and more Na^+ is therefore pumped into the lumen.

Stimulation of NKCC cotransport brings more K^+ and Cl^- into PC much of which enters the lumen since 8bromo-cAMP and Achdo-DH stimulate KCl secretion in addition to NaCl. Although cation transport is increased, V_t depolarises by ~19 mV and, since V_b is unchanged, V_a falls by a similar amount. There are two probable reasons for the fall in V_a. Firstly, more cations are transported into the lumen via cation/H^+ antiports that are electrogenic with a stoichiometry of 1 cation/nH^+, so there is net movement of positive charge into the cell. Secondly, the additional Cl^- entering PC via NKCC cotransport mostly moves into the lumen since V_b is unchanged. Interestingly, preliminary data show Achdo-DH depolarises V_a more than 8bromo-cAMP and it may open the apical membrane Cl^- conductance targeted by Achdo-KII. This would explain why 8bromo-cAMP acts synergistically with Achdo-K whereas Achdo-DH does not [6].

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REFERENCES

[1] Ramsay J.A., J. Exp. Biol., 1954, 31, 104-113.
[2] Maddrell S.H.P., O'Donnell M.J., *ibid.*, 1992, 172, 417-429.
[3] Coast G.M., Orchard I., Phillips J.E., Schooley D.A., Adv. Insect Physiol., 2002, 29, 279-409.
[4] Coast G.M., Nachman R.J., Schooley D.A., J. Exp. Biol., 2007, 210, 3979-3989.
[5] Ianowski J.P., Christensen R.J., O'Donnell M.J., *ibid.*, 2002, 205, 1645-1655.
[6] Coast G.M., Kay I., *ibid.*, 1994, 187, 225-243.