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Localization of a *Drosophila* DRIP-like aquaporin in the malpighian tubules of the house cricket, *Acheta domesticus*☆

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Abstract

Malpighian tubules (Mt) are the primary excretory and osmoregulatory organs of insects, capable of rapidly transporting extraordinary volumes of fluid when stimulated by diuretic factors. In the house cricket, *Acheta domesticus*, the Mt are composed of three morphologically distinct regions (proximal, mid, and distal). Unlike the dipteran Mt, which have both primary and stellate cells, each region of the *Acheta* Mt consists of a morphologically uniform cell type. The mid and distal regions are both secretory in function and increase secretion rate in response to dibutyryl cAMP (cAMP). Achetakinin-2, while acting synergistically with cAMP on the mid-Mt, inhibits secretion by the distal Mt, and the effects can be reversed by cAMP. Using an antibody to the water-specific *Drosophila* aquaporin (DRIP), we demonstrated that DRIP-like immunoreactivity was found in both the distal and mid-Mt. The distribution of the aquaporin altered in response to stimulation and was consistent with the secretory data. The regulation of secretion in *Acheta* Mt is quite different from that of *Drosophila*, with both cation and anion/water transport occurring in the same cells. This is the first demonstration of the presence of an insect aquaporin, namely DRIP, in the Mt of an order other than the Diptera.

Keywords: Aquaporin; AQP; Cricket; DRIP; Insect; Malpighian tubule; Secretion

1. Introduction

In insects, the Malpighian tubules (Mt) are the primary excretory and osmoregulatory organs, analogous to the vertebrate renal tubule. The Mt are a monolayer tubular epithelium surrounded by a tough basement membrane, usually encircled by several spiral bands of muscle and at least one tracheal branch. Numbers of Mt are species-specific and range from two to several hundred. Fluid transport is nominally isosmotic and the elusive "common cation transporter" driving active K^+ and Na^+ secretion has been revealed to be an apical V-type H-ATPase which is presumed to drive Na^+/H^+ and K^+/H^+ antiports (Maddrell and O'Donnell, 1992; Wieczorek et al., 1991, 1999). Water and Cl^- move passively via a low resistance or "shunt" pathway, the precise nature of which is the source of some debate. In the fruit fly, *Drosophila*, Cl^- movement is transcellular, via the stellate cells, whereas in the mosquito, *Aedes*, Cl^- movement is paracellular (Beyenbach, 2003; O'Donnell et al., 1998).

In many insects, particularly the fluid feeders, the Mt are capable of prodigious feats of transport, with secretion rates increasing by as much as a thousand-fold within seconds of the commencement of feeding (Maddrell and O'Donnell, 1992). Regulation of secretion rate is controlled by a suite of neuropeptides, including the biogenic amines such as serotonin (Gäde et al., 1997; O'Donnell and Spring, 2000). In general, the two most important families of neuropeptides appear to be the corticotropin releasing factor-like diuretic peptides (CRF-DPs), named for their homology to the vertebrate CRF/sauvagine/urotensin family of peptides, and the myokinins, which were isolated on the basis of their ability to promote muscle contraction in the hindgut of the cockroach, *Leucophaea*

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maderae (Holman et al., 1987). The CRF-DPs act via cAMP to stimulate the V-ATPase directly, and this enhanced cation transport elevates the secretion rate. The myokinins act via Ca^{++} on the low-resistance shunt pathway, enhancing passive Cl^{-} and water movement. Although capable of increasing fluid secretion on their own, the myokinins also act synergistically with other diuretic factors to promote rapid fluid movement (O'Donnell and Spring, 2000).

Over the last decade it has become well established that the movement of water across membranes is mediated by a subset of the Major Intrinsic Protein (MIP) family of proteins known as aquaporins (AQPs; reviewed by Engel et al., 2000; Verkman and Mitra, 2000; Dow and Davies, 2003). All of the identified AQPs contain six transmembrane domains and two NPA consensus regions which form the actual water pore. The AQPs are further arranged into functional homotetramers within the membrane. Regulation of epithelial permeability may be by removal or insertion of vesicles containing high densities of AQPs, or by *in situ* phosphorylation of the proteins. In the dipteran insects, AQPs have been identified and cloned in the fruit fly, *Drosophila melanogaster*, and two species of mosquito, *Aedes aegypti* and *Anopheles gambiae*. Of the eight known *Drosophila* aquaporins, the *Drosophila* Integral Protein (DRIP) sequence is the most similar to hAQP4, a water-specific AQP with very high transport rates (Chou et al., 1998). Unsurprisingly, DRIP is also closely related to the mosquito AQPs (Kaufmann et al., 2005).

The movement of water across Mt can occur at prodigious rates. In serotonin-stimulated *Rhodnius* Mt, Maddrell and O'Donnell (1992) have calculated that the Mt cells are moving the equivalent of one cell volume every 15 s. This rapid water flux has always been problematic. How can an Mt cell possibly transfer this amount of water transcellularly and still maintain normal tissue function? *Drosophila,* whose Mt are capable of fluid transport at rates exceeding that even of *Rhodnius*, appear to have solved this problem by the spatial separation of function. The large principal cells, with their extensive brush border, are mitochondria-rich and contain both the apical V-ATPase and presumptive H-cation exchangers. It is these cells that respond to the CRF-DPs and other secretagogues by increasing primary cation transport. Interspersed with the principal cells, the smaller and less elaborate stellate cells contain the Cl^{-} channels and AQPs. It is the stellate cells that respond to the myokinins, and in *Drosophila*, DRIP has been localized specifically to these cells (Kaufmann et al., 2005). It would appear that in these insects, the stellate cells operate mainly in a passive mode, providing the low-resistance or shunt pathway for anion and water fluxes.

This is not the situation in other insect orders, however, and the Diptera may be a special case. For example, the house cricket, *Acheta domesticus*, has many more Mt (110 cf 4 in *Drosophila*) and individually the Mt transport fluid more slowly. Although the *Acheta* Mt exhibit regional specialization, cell types within any given region are morphologically uniform (Hazelton et al., 1988). It should be noted however, that morphological uniformity does not necessarily imply that these cells are functionally identical (Sozen et al., 1997). Individual Mt are 6–8 mm in length, and the bulk of this region, known as the mid-tubule, appears to consist of a single cell type, which appears ultrastructurally to be very similar to the principal cell of *Drosphila*. The distal 1–1.1 mm of each Mt, however, is much smaller in diameter, hyaline and comprised of smaller cells that look not unlike stellate cells in cross-section. Furthermore, despite its lack of an extensive brush border and basolateral elaboration, the distal tubule transports fluid at a resting rate 3–4 times that of the mid-tubule, in the same range as the Mt of *Drosophila*. Given these similarities, and the long-standing concern regarding transcellular water flux in primary cells, we considered that the distal tubule of *Acheta* might be the functional analog of the *Drosophila* stellate cell. If this were true, then we would expect to find spatial separation of endocrine response, such that the mid-tubule should respond to CRF-DPs and cAMP, and the distal tubule to achetakinins. Furthermore, we would anticipate that AQPs would be localized primarily in the distal tubule.

The present study was undertaken to address the following questions:

1) Is DRIP, or a closely related AQP, found in the Mt of an insect order other than the Diptera?
2) If DRIP is present, is it localized primarily in the distal Mt?
3) Is the distal Mt the functional equivalent of the dipteran stellate cell?
4) Is the intracellular distribution of AQPs altered in response to endocrine stimulation?

2. Materials and methods

2.1. Insects

Immature *Acheta domesticus* were obtained from Fluker's Farm (Baton Rouge, LA). They were maintained in the laboratory at 28±2 °C on a 14 h light/10 h dark photocycle, and were provided with fresh water and Purina Cricket Chow® *ad libitum*. Only mature females, with fully-developed egg masses, were used in these experiments.

2.2. Solutions

Cricket saline contained the following in mM l^{-1}: NaCl, 95; K_2SO_4, 10; $MgSO_4$, 10; $CaSO_4$, 3.5; D-glucose, 10; glycine, 10; proline, 10; lysine, 4; HEPES, 25. This was adjusted to pH 7.2 with concentrated N_4OH, and the osmotic concentration adjusted to 300–310 mOsm with sucrose. Phosphate-buffered saline (PBS) contained the following in mM l^{-1}: NaCl, 150; NaH_2PO_4, 1.86; Na_2HPO_4, 8.41; pH 7.2. PBT was PBS containing 1% Triton X-100. Hybridization buffer (NTMT) contained the following in mM l^{-1}: Tris, 100; $MgCl_2$, 50; NaCl, 100; levamisole, 1; 0.1% Triton X-100; pH 9.5). Secretagogues were prepared as 10X stocks in cricket saline. Dibutyryl cAMP was used to mimic the actions of Acd-DP as it has been shown to produce a more reproducible response in the Mt (Kim and Spring, 1992). Achetakinin 2 (AK-2) was a kind gift from Dr.