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

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Received 3 May 2006; received in revised form 4 December 2006; accepted 6 December 2006
Available online 16 December 2006

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.

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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 corticotropic releasing factor-like diuretic peptides (CRF-DPs), named for their homology to the vertebrate CRF/sauvagine/urotensin family of peptides, and the myokmins, which were isolated on the basis of their ability to promote muscle contraction in the hindgut of the cockroach, *Leucophaea*...
madera (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\(^{2+}\) 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 AQ 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 Drosophila. 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 longstanding 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 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: NaCl, 95; K2SO4, 10; MgSO4, 10; CaSO4, 3.5; D-glucose, 10; glycine, 10; proline, 10; lysine, 4; HEPES, 25. This was adjusted to pH 7.2 with concentrated NaOH, and the osmotic concentration adjusted to 300–310 mOsm with sucrose. Phosphate-buffered saline (PBS) contained the following in mM: NaCl, 150; NaH2PO4, 1.86; Na2HPO4, 8.41; pH 7.2. PBT was PBS containing 1% Triton X-100. Hybridization buffer (NTMT) contained the following in mM: Tris, 100; MgCl2, 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.
G. Mark Holman, Texas A and M University. Final concentrations were: db-cAMP, $10^{-3}$M; AK-2, $10^{-8}$M.

2.3. Secretion experiments

Secretion experiments were performed using our standard protocols (Kim and Spring, 1992; Spring and Kim, 1995). Briefly, Mt masses were dissected under saline aerated with hydrated carbogen (95%O$_2$:5%CO$_2$). Individual Mt were transferred to 50 µl droplets of saline, located in preformed wells of Sylgard® (Dow Corning, Midland, MI) in 6-cm diameter Petri dishes. The wells were filled with water-saturated mineral oil to prevent evaporation of the saline droplet. To measure secretion by whole Mt, the proximal end of a tubule was drawn out of the droplet with fine forceps and anchored in a slit in the wall of the Sylgard well. The basement membrane was carefully nicked with a sharp glass micropipette and the resulting urine droplet was measured at intervals, using a calibrated graticule ocular. Secretion rates were calculated as π mm$^2$ min$^{-1}$, based on the measured length of the immersed Mt. To measure the secretion rate by the distal Mt alone, the proximal tubule was drawn from the droplet until only the distal region remained immersed and then anchored to the well wall. To measure the secretion rate of the mid-Mt alone, both distal and proximal regions were carefully removed from the droplet so that only a length of mid-Mt remained immersed. The Mt was always nicked at the proximal end. Tubules were allowed to stabilize for at least 60 min to obtain a basal secretion rate. To test a secretagogue, immediately following the last control reading, 5 µl of saline was withdrawn and replaced with 5 µl of 10X stock solution.

2.4. Light microscopy

Drosophila Mt were prepared for in situ mRNA hybridization experiments following our standard protocols (Kauffman et al., 2005). Labeling was visualized using 4.5 ng ml$^{-1}$ 4-nitro blue tetrazolium chloride and 1.75 ng ml$^{-1}$ 5-bromo-4-chloro-3-indolyl-phosphate in NTMT. Freshly dissected Acheta Mt masses were immersed in cricket saline containing 0.5% methylene blue for 15 min. Tubules were washed for 3×15 min in saline, then individual Mt transferred to coverslips and photographed using a Micromaster compound light microscope and digital camera.

2.5. High resolution scanning electron microscopy

These experiments were performed using established protocols (Townsend et al., 2000; Hazleton et al., 2002b). Briefly, Mt were fixed in 2.5% cacodylate-buffered glutaraldehyde at 4°C for 18 h. Following primary fixation, Mt were post-fixed in 1% OsO$_4$, run through a concentration series to 50% DMSO to prevent ice crystal formation, and freeze fractured in a liquid nitrogen slush. After thawing, specimens were maintained in 0.1% OsO$_4$ for 3–6 days to extract the cytosol, then treated with 1% tannic acid (1 h) followed by 1% OsO$_4$ (1 h). Mt were then treated with a graded ethanol dehydration series, mounted on aluminum stubs and gold-coated. Tubules were viewed with a JEOL 6300-F field emission scanning electron microscope at an accelerating voltage of 20 kV.

2.6. DRIP localization studies

The DRIP primary antibody was prepared against the C-terminal peptide sequence CIIFKVRGKDDETDSYDF. The C-terminal sequence antigen was utilized because it is not conserved among the other seven Drosophila AQPs and it should be exposed in the cytosol, rather than embedded in the membrane. The peptide was synthesized in bacteria as a glutathione-S-transferase fusion protein and applied to a glutathione agarose (Sigma) column. GST-peptide was eluted from the column using free glutathione (Sigma). Protein concentration was estimated using the BioRad Protein Assay Reagent (BioRad). Free peptide was also conjugated to keyhole limpet hemocyanin by Pocono Rabbit Farm and Laboratory, Inc. A 1:1 (w/w) mixture of GST-DRIP and KLH-DRIP peptides was used as the immunogen in a rabbit (Pocono Rabbit Farm and Laboratory, Inc.) following established protocols. The anti-DRIP serum binds a doublet just below 56 kDa (DRIP dimer) or resolves as a single band at 28 kDa on a Western blot from D. melanogaster. The reason for this variability is not yet clear, but could arise from different conditions used during sample preparation (N.K., M.L. Zeidell, and J.L.B., unpublished results).

To perform Western blots with anti-DRIP antisera, Mt masses were harvested from adult female Acheta, frozen in liquid nitrogen, and stored at −80°C. Mt masses were suspended by pipetting in 1% SDS in PBS with protease inhibitors. Soluble material in the supernatant fraction was removed after 5000 RPM 5 min centrifugation (Biofuge pico, Haereus) and total protein was precipitated by the addition of 50% trichloroacetic acid (TCA) to a final concentration of 10%. Precipitation was allowed to proceed for 30 min on ice. Precipitated protein was collected by centrifugation at 13,000 RPM for 10 min and lipids were removed with an acetone wash. Protein was resuspended in SDS Sample Buffer with protease inhibitors and electrophoresed on a 12.5% SDS polyacrylamide gel (approximately 1/4 Mt mass per lane). Protein was transferred to nitrocellulose, and non-specific binding was blocked by a pre-incubation with 5% nonfat milk. Blots were incubated in 1:5000 preimmune serum or anti-DRIP serum with 5% nonfat milk overnight at 4°C. Blots were washed 3×15 min in TBS and incubated for 4 h at room temperature in 1:5000 horseradish peroxidase-conjugated donkey anti-rabbit antibody (GE Healthcare). After three 15 min washes, the blots were developed with the SuperSignal West Pico Chemiluminescent Substrate (Pierce).

Preliminary experiments revealed that the antibodies could not readily penetrate the basement membrane, resulting in preparations with only non-specific surface staining. This necessitated the removal of the basement membrane, following established methodologies (Townsend et al., 2000; Hazleton et al., 2002b). DRIP localizations were performed using the following protocol: Five to eight Mt masses were dissected and
transferred to a bath of cricket saline aerated with hydrated carbogen as described above. After 60–75 min, two to three Mt masses were transferred to aerated saline containing 4 mg ml⁻¹ elastase (Type I, from porcine pancreas; Sigma) plus the appropriate treatment for 30 min. Tubule masses were fixed in 3.7% formaldehyde at 0 °C for >1 h. Removal of the protective basement membrane makes the Mt very friable. Accordingly, individual tubules were removed and transferred to coverslips previously coated with poly-1-lysine (300 kD; 10 mg ml⁻¹). All subsequent treatments were performed with the Mt affixed to the coverslips to minimize damage to the tissue. The Mt were rinsed 3 × 10 min in PBT, then placed in 5% normal goat serum in PBT at 20 °C for 1 h to block non-specific antibody binding. Antibody, diluted 1:50,000 with PBT was added and the preparations incubated at 4 °C overnight. Removal of the primary antibody was followed by 3 × 15-min washes in PBT. Alexa 488 goat anti-rabbit secondary antibody, diluted 1:500 in PBT was added and the Mt were incubated in the dark at 4 °C for 24 h. The secondary was removed with 3 × 15 min washes with PBT. Glass spacers were glued to microscope slides and the coverslips mounted using Prolong® Antifade mounting medium (Molecular Probes). Specimens were examined using a Biorad® MRC 1024 confocal laser scanning microscope.

3. Results

Fig. 1 illustrates some of the structural differences between the D. melanogaster and A. domesticus Mt. The Drosophila Mt

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Fig. 1. A. Light micrograph of Drosophila Mt with the stellate cells visualized (arrow) using in situ mRNA hybridization with anti-DRIP probes. Scale bar = 50 μm. B. SEM of Acheta Mt showing the differences in surface appearance and diameter between the distal and mid-Mt. Note the abrupt transition between the two regions. 200×. C. Light micrograph of Acheta Mt at the distal-mid junction (arrow). The distal cells are hyaline, mid-Mt cells are opaque due to the large amounts of CaPO₄ and urate they contain. Scale bar = 50 μm. D. Light micrograph of distal Mt tip showing the squamous epithelial cells and the cluster of knob-like anchoring structures. Scale bar = 50 μm. E. Distal Mt showing the hyaline cells and the open lumen. L, lumen. Scale bar = 50 μm. F. HR-SEM of mid-Mt. The paired nuclei and well-developed brush border can be clearly seen. The hemispherical structures are the pockets left after the spherites were dislodged during sectioning. Note the extensive amount of endomembrane. 3000×. L, lumen; N, nucleus.
(Fig. 1A) shows the thin stellate cells (darkly stained) interspersed with the larger, more highly elaborated principal cells. The distribution of stellate cells is more-or-less constant along the length of the Mt. Fig. 1B is an SEM of the distal region of the *Acheta* Mt at the distal-mid junction. The distal region is much narrower in diameter and there is an abrupt change in cell type at the distal-mid junction, visible at both the light and EM levels. In Fig. 1C, the transition in cell type at the distal-mid junction can be readily seen at the light level, with the distal region appearing hyaline, in contrast to the yellowish or whitish mid-Mt. The terminus of the distal Mt is always covered with a handful of small, knob-like cells (Fig. 1D), which are presumed to anchor the tubule to other tissues (Hazleton et al., 1988). The *Acheta* distal Mt is also composed of an apparently uniform cell type and shows a clearly defined lumen (Fig. 1E). The cells of the mid-Mt are large, bi-nucleate and have greatly elaborated basolateral infolds as well as the extensive brush border characteristic of transporting epithelia. These cells also contain tremendous amounts of endomembrane (Fig. 1F; Hazleton et al., 2002b).

The cells of the distal tubule (Fig. 1 D, E) are much smaller and less elaborate than those of the mid-Mt and have few of the structural features normally associated with rapid fluid transport. Visually and ultrastructurally, the cells of the distal Mt are very similar to the dipteran stellate cells. They are squamous, with few mitochondria and none of the brush border or basolateral elaborations expected of transporting epithelia (Hazleton et al., 1988). For them to maintain a rapid flow of fluid, it would appear that the ion gradient must be developed elsewhere.

Fig. 2 is a Western blot of *Acheta* Mt extract treated with pre-immune and anti-DRIP sera. The antiserum recognizes one prominent protein at the size predicted for aquaporin dimer (∼50 kDa). It is common for the AQPs to run as multimers, even under reducing conditions, presumably due to the high hydrophobicity of the protein (Van Hoek et al., 1995). In any event, these data clearly indicate antiserum specificity.

The effects of db-cAMP on *Acheta* Mt are illustrated in Fig. 3A. Initially, the distal tubule was secreting fluid at a rate more than three times that of the mid-Mt. Over first 60 min post-stimulation, there was a 2 to 4-fold increase in secretion rate by the mid-tubule, after which the rate stabilized for the duration of the experiment (at least 3 h, unpublished observations). In contrast, for the distal region there was 30 min time lag, followed by a slight (20–25%) increase in secretion rate, which again was sustained for the duration of the experiment. When AK-2 was added to the bath (Fig. 3B), the mid-tubule showed a
slower, but steady increase in secretion rate over the 60-min measurement interval, and the addition of cAMP increased the secretion rate still further, demonstrating the synergistic effects of the two compounds. In the distal tubule, however, AK-2 produced a steady decline in secretion rate. The addition of cAMP rescued the distal Mt, not merely restoring secretion to control rates but elevating the rate to where one would expect it to be following the addition of cAMP alone (cf Fig. 3A).

Fig. 4A is a confocal image of the unstimulated mid-Mt, with neither primary or secondary antiserum present. Autofluorescence is always observed in *Acheta* Mt (Hazeldon et al., 2002a). Control (unstimulated) Mt showed some DRIP staining, associated primarily with the cell surface (Fig. 4B). When the Mt were treated with cAMP, there was a noticeable increase in DRIP staining (Fig. 4C). There was no clear localization of the DRIP; however, staining was diffuse throughout the cytoplasm. In contrast, treatment with AK-2 showed intense staining on both the apical and basal membranes, with much less cytoplasmic staining (Fig. 4D). Note that with both cAMP and AK-2, the Mt lumen was visibly distended, characteristic of rapid fluid transport in the absence of the confining basement membrane.

Fig. 5 shows the results for the distal Mt. Again, there was some degree of autofluorescence in the absence of antiserum (Fig. 5A). Unstimulated Mt showed rather diffuse DRIP staining, although there were indications that staining was more intense along the basal and apical surfaces. When treated with cAMP (Fig. 5C), the bulk of the DRIP concentrated along the basal membrane. In contrast, AK-2 staining was primarily cytosolic, and there was very little reaction along the apical or basal membranes (Fig. 5D). The appearance of the tissue suggests that DRIP is concentrated in intracellular vesicles.

4. Discussion

The present study is the first to demonstrate the presence of an insect AQP, specifically a DRIP-like protein, in the Mt of an order other than the Diptera. Furthermore, we have shown that distribution and regulation of DRIP in *Acheta* are very different than those observed in fruit flies and mosquitoes.

The separation of primary ion transport and counter ion and water movement in the Mt of *Drosophila* represents a satisfying and elegant solution to the vexing issue of how insect Mt manage to move such large volumes of water without...
completely disrupting normal cell function. As such, it is tempting to believe that this might be a widespread phenomenon amongst the insects. The appearance of the stellate cells in dipteran Mt and the cells of the distal tubule in Acheta is very similar. Along with this histological evidence, we know that the Acheta distal Mt transports fluid very rapidly, despite the apparent lack of features that would support ion-driven water transport. It would seem reasonable to assume that the Acheta distal Mt functions much like the dipteran stellate cells, providing a passive low-resistance pathway for water and anion movement, driven indirectly by primary cation transport in the mid-Mt. Functional differences would be through regional specialization, rather than by having multiple cell types within a single tissue.

Although DRIP was cloned more than a decade ago (Dow et al., 1995), its biophysical characteristics have only recently been described (Kaufmann et al., 2005). It is a water-specific channel, most closely related to human AQP4 (44% homology). DRIP has a much higher homology to the other known dipteran AQPs, isolated from the mosquitoes, A. aegypti (65%) and A. gambiae (64%). As these are the only sequenced insect AQPs, the degree of conservation within the Insecta is open to speculation, although one would expect it to be considerably higher than across phyla. The C-terminal peptide sequence is not highly conserved among the other Drosophila AQPs, but is limited to the water-specific AQP. The available BLASTP data also suggest that at least a portion of this epitope, restricted to the water-specific AQPs, is conserved across the Insecta.

Accordingly, we believe that DRIP-positive immunoreactivity should be a good indicator of the presence of water-specific AQPs in Acheta. This is supported by our Western blot data (Fig. 2) which show a single major band of immunoreactivity at the size predicted for an aquaporin dimer (~ 50 kDa). As noted previously, the AQP’s often run as multimers even under reducing conditions. This is presumed to be due to the high
hydrophobicity of the protein (Van Hoek et al., 1995), and is consistent with our prior observations with DRIP in Drosophila extracts (Kaufmann et al., 2005).

The secretion experiments (Fig. 3), however, do not support the hypothesis that the distal Mt may represent a spatially distinct shunt pathway for water and anions. Cyclic AMP increases fluid transport in both the distal and mid-Mt, although its effects in the distal region are less pronounced. Acheptakinin-2, which affects Cl− and water permeability, exerts its stimulatory effects on the mid-Mt, and dramatically reduces fluid transport by the distal region. Cyclic AMP and AK-2 act synergistically on the mid-tubule, and CAMP has the capacity to completely reverse the effects of AK-2 on the distal Mt. It thus appears that both the CRF-DPs and the myokinins are operating on the same cell type in the mid-tubule and that in Acheta at least, there is nothing comparable to the dipteran shunt pathway. It may still prove that the distal region provides a low resistance pathway for water and Cl− movement in unstimulated Mt, but this begs the question of why transport would shut down in the presence of AK-2, but not cAMP. Clearly, whatever mechanisms are operative, they are not comparable to those in the stellate cells.

The localizations of DRIP are consistent with the secretion data. Unstimulated mid-Mt show some immunostaining on the basolateral surface (Fig. 4). In cAMP-stimulated mid-tubules, however, although there is more tissue staining, it is diffuse and appears to be confined mostly to internal membranes. In sharp contrast, AK-2 produces intense staining of both the apical and basolateral membranes. Again, these data are consistent with other observations. Cyclic-AMP stimulation initiates a profound and rapid vesiculation of the mid-Mt. In some cases, more than 50% of the cell volume is occupied by these vesicles (Hazelton et al., 2001). The function of these vesicles has been puzzling and we have speculated that they may be a source of AQP s (Hazelton et al., 2002a). In this case, it would appear that while cAMP may be involved in the activation or synthesis of AQP s associated with the ER, it is not causing their insertion into the plasma membranes.

The action of AK-2 is quite the converse. Relatively little staining is seen in the ER, but the apical and basolateral membranes stain intensely. This is consistent with our observations regarding AK-stimulated fluid movement. It is unusual to find a single AQP localized in both apical and basolateral domains. One might expect to find a constitutive AQP in one domain, and a hormonally regulated one in the other, as in the mammalian collecting duct (Sasaki et al., 1998). The Mt of Acheta are unusual in this regard, however. In a series of experiments designed to track fluid and membrane movement in the stimulated Mt, we showed that for all our markers, the basal, apical and endomembrane systems behaved as though they were a single compartment, even when the experiments were performed at 4 °C (Hazelton et al., 2002b). Clearly the cells exhibit asymmetry; the Mt could not function otherwise. On the other hand there is a dynamism and exchange of material among compartments that is not found in other, particularly vertebrate, systems. In this instance, it appears that DRIP may indeed be hormonally regulated in both apical and basolateral membranes.

In the distal Mt, we see an apparent reversal of hormone function. As in the mid-Mt, the unstimulated controls show some degree of staining, mostly along the basal surface. Cyclic AMP increases the intensity of this basal staining without appreciably changing intracellular levels. The effect of AK-2 on the distal Mt is very similar to that of cAMP on the mid-Mt. There is a reduction in the amount of basal staining with the bulk of the fluorescence now being found internally, presumably in vesicles. The distribution of DRIP staining is consistent with our secretion data.

When Acheta Mt are maintained in vitro, they will continue to secrete for several hours at a steady rate. During this steady-state interval, secretion by the distal Mt is roughly 3–4 times that of the mid-Mt. Cyclic-AMP mediated pathways increase the rate of secretion in both segments, however the effect is much more dramatic in the mid-Mt. In contrast, AK-2, while increasing fluid transport in the mid-Mt synergistically with cAMP, inhibits fluid transport by the distal region. This means that unlike Drosophila, both primary cation and anion/water transport are occurring within the same cells in the mid-Mt and both hormone pathways are operative within the same cells. Again we are faced with the conundrum of how the Mt cells manage to translocate such large volumes of water without completely disrupting normal cell function.

In the distal Mt, the results of this study are more puzzling. Why, when the Mt are being challenged to secrete fluid rapidly, does the distal region shut down? The fact that the distal Mt can be rescued by cAMP-mediated processes suggests that this response can be overridden when necessary. Is there some advantage to the cricket in promoting fluid movement through the cells of the mid-Mt? We know that under appropriate dietary conditions, Acheta stores large amounts of CaPO4 in the mid-tubule in the form of spherites, which can occupy nearly half of the cell’s internal volume. Cyclic AMP causes rapid mobilization of the spherites, some being dissolved in situ, others being physically ejected into the tubule lumen (Spring and Felgenhauer, 1996). It may be that transcellular water movement is part of this process, a necessary adjunct to the mechanism of storage excretion.

One aspect of this study that deserves mention is that it clearly demonstrates that although Drosophila is an exemplary model system, primarily due to its genetic tractability, it is not necessarily an inclusive model for the Insecta. With regard to fluid transport, there are clearly multiple solutions to the universal problem of salt and water balance. The present study of DRIP immunolocalization in Acheta, and the changes in distribution produced by endocrine stimulation, strongly suggest that water permeability, as well as primary ion transport are being hormonally regulated. However, many fundamental issues, such as the way in which the mid-Mt cells cope with such large transcellular water fluxes, remain to be answered.

Acknowledgments

We would like to thank Ms. Ann Hume for her assistance in the use of the University of Louisiana Microscopy Center. We
would also like to thank Dr. John Hamlin for critical reading of the manuscript. We are grateful to Drs. Roy Brown and Betty Lemmon for generously providing equipment, time and thoughtful conversation. We continue to be indebted to Dr. Mark Holman for the gift of the achetakinins.

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