Ca$^{2+}$ and cAMP signaling pathways interact to increase the diuretic effect of serotonin in Malpighian tubules of the kissing bug. Focus on “Serotonin triggers cAMP- and PKA-1-mediated intracellular calcium waves in Malpighian tubules of *Rhodnius prolixus*”

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IT’S BEEN OVER TWO DECADES SINCE a captivating search for what powers ion transport in the insect Malpighian tubule culminated in the discovery that the vacuolar-type H$^+$ ATPase, and not the Na$^+$-K$^+$-ATPase, drives transepithelial transport of electrolytes (8, 18) and probably also organic solutes in this organ. The H$^+$ gradient generated by the vacuolar-type H$^+$ ATPase, in turn, drives one or more cation/H$^+$ antiporters (NHAs), which, along with a varied assortment of apical and basolateral membrane ion transporters and water channels, leads to transepithelial ion fluxes and water secretion or absorption in various insect epithelia.

Serotonin has long been known to regulate fluid and ion transport in insect epithelia, such as the salivary gland and the Malpighian tubule (2). In fact, many of the pioneering studies on the role of phosphoinositides as regulators of cell function were carried out in the insect salivary gland (1). Other signal transduction pathways associated with serotonin include alterations in intracellular cyclic nucleotide and Ca$^{2+}$ concentrations. Increases in cyclic AMP and changes in cytoplasmic Ca$^{2+}$ likely lead to the posttranslational modifications of membrane proteins that bring about maximal rates of transepithelial electrolyte and fluid secretion by apparently synergistic mechanisms.

*Rhodnius prolixus*, the blood-sucking “kissing bug” that carries the protozoan *Trypanosoma cruzi*, which causes Chagas disease, undergoes rapid diuresis following a blood meal. Diuresis is regulated, at least in part, by a corticotropin-releasing hormone-related peptide and serotonin (9, 16), both of which bind to plasma membrane receptors in the Malpighian tubule and lead to enhanced rates of ion and fluid secretion. Serotonin elicits its effects by way of signaling cascades involving cyclic AMP, Ca$^{2+}$, and phosphoinositides, which in sequence and/or combination bring about diuretic rates of transepithelial solute and water flow.

A recent study by Gioino et al. (7a) in the *American Journal of Physiology—Regulatory, Integrative and Comparative Physiology* provides new insights into the interplay between serotonin-mediated changes in intracellular cyclic AMP and Ca$^{2+}$ and their possible roles in regulating ion and fluid secretion in the Malpighian tubule of *R. prolixus*. Using video microscopy to collect images of changing intracellular Ca$^{2+}$ concentration in cells of this robust and tractable renal tubule [the *R. prolixus* tubule is about 100 μm in diameter and over 40 mm long in 5th instar insects (5)], they discovered that a small number of pioneer cells randomly distributed along the length of the secretory portion of the tubule, respond to serotonin by initiating first an initial large calcium signal followed by Ca$^{2+}$ waves that persist for up to 30 min. Once initiated by serotonin or cAMP, the waves propagate to neighboring cells in both distal and proximal portions of this blind-ended tubule. Ca$^{2+}$ waves occur both asynchronously and heterogeneously, and both the frequency and amplitude of serotonin-stimulated Ca$^{2+}$ wave propagation occur in a concentration-dependent manner. Significantly, the Ca$^{2+}$ waves are blocked by the intracellular Ca$^{2+}$ chelator BAPTA-AM, but not by EGTA (the extracellular Ca$^{2+}$ chelator), indicating a pronounced role for intracellular sources of Ca$^{2+}$ in wave production, and little or no role for extracellular Ca$^{2+}$. Moreover, the chelation of intracellular Ca$^{2+}$ by BAPTA reversed the effects of serotonin by 1) decreasing the Na$^+$ concentration and increasing the K$^+$ concentration in fluid secreted by the tubules, and 2) reducing the transepithelial flux of both cations fivefold. Reciprocal changes in the concentrations of Na$^+$ and K$^+$, concomitant with the significant reduction in transepithelial Na$^+$, K$^+$, and fluid secretion, suggest that the Ca$^{2+}$ waves target, in part, the transepithelial secretion of Cl$^-$, the counterion of Na$^+$ and K$^+$.

Ca$^{2+}$ waves initiated by cAMP suggest the role of PKA. Indeed, H-89, which is known to inhibit PKA, blocks the Ca$^{2+}$ waves triggered by serotonin and cAMP. Accordingly, serotonin triggers Ca$^{2+}$ waves via cAMP and PKA. The work of Gioino et al. (7a) builds on prior studies and suggests that serotonin binding to apparently a single serotonin receptor in *R. prolixus* Malpighian tubules, activates a complex series of cellular outputs. As demonstrated here, attenuation of the Ca$^{2+}$ waves leads to lower serotonin-mediated Na$^+$, K$^+$, and fluid secretion, indicating that Ca$^{2+}$ waves likely are required for maximal fluid and ion transport rates mediated by serotonin.

As to the physiological role of Ca$^{2+}$ waves, serotonin is known to increase transepithelial voltage oscillations in *R. prolixus* Malpighian tubules (9). After an initial spike, the transepithelial voltage begins to oscillate in a regular fashion not unlike the Ca$^{2+}$ wave oscillations observed in the study by Gioino et al. (7a). The ion producing these transepithelial voltage oscillations is likely Cl$^-$, which passes across Malpighian tubules by transcellular pathways (11) and/or paracellular pathways (4, 14). Because Ca$^{2+}$ activates transepithelial Cl$^-$ conductive pathways in Malpighian tubules...
of the yellow fever mosquito *Aedes aegypti* (20) and in the salivary gland of the blowfly *Calliphora vicina* (3), the Ca²⁺ waves observed by Gioino et al. (7a) may direct cyclical changes in transepithelial Cl⁻ conductance as part of the diuretic response of the tubule to serotonin. Consistent with this hypothesis, the transepithelial voltage and resistance oscillations triggered by another diuretic peptide (leucokinin) in Malpighian tubules of *Aedes aegypti*, are dependent on Ca²⁺ signaling and on extracellular Cl⁻ (4, 20). In addition, Ca²⁺ may signal to the mitochondria located in close proximity to the V-ATPase in microvilli of the apical membrane, thereby increasing both mitochondrial potential and local ATP levels, as shown in *Drosophila* Malpighian tubules (17). Elevated ATP levels are expected to fuel the increased transport activities of the V-ATPase and the proton/cation antiporters NHA1 and NHA2 to mediate diuretic rates of transepithelial cation (Na⁺ and K⁺) secretion (19).

As to a physiological role of cAMP in secretory insect epithelia, serotonin increases transepithelial ion and fluid secretion in salivary glands of the blowfly *Calliphora vicina* by promoting the translocation of V₁ subunits from the cytoplasm to the apical membrane of epithelial cells, consistent with the assembly of the V-ATPase holoenzyme in the apical membrane and the stimulation of proton and cation secretion into the tubule lumen (15). In addition, cAMP appears to sensitize the InsP₃/Ca²⁺ signaling pathway to serotonin, thereby promoting the generation of Ca²⁺-dependent voltage oscillations (7).

The sudden substantial increase in urine excretion triggered by diuretic hormones and peptides has long been associated with synergistic transport mechanisms in Malpighian tubules. Maddrell et al. (10) first proposed that the rapid onset of the blood meal-triggered diuresis in *R. prolixus* is caused by the simultaneous release of serotonin and peptide diuretic hormone acting synergistically on Malpighian tubules. Similarly, Coast (6) found low concentrations of a CRF-related peptide and locustakinin to act cooperatively and stimulate fluid secretion by more than the sum of their separate responses in Malpighian tubules of the locust. In their review of synergism in fluid secretion in Malpighian tubules, O’Donnell and Spring presented the general rule that secretion is controlled by two or more hemolymph-borne diuretic factors, such as the CRF-related peptides and kinins, involving one or more second messenger systems that target cation- and anion-selective transport pathways (13). Moreover, they suggested the functional separation of signaling pathways in different cells of Malpighian tubules of, for example, *Drosophila* where 1) cAMP increases tranacellular cation transport in principal cells of the tubule where the V-ATPase is located, and 2) Ca²⁺ increases the Cl⁻ conductance of stellate cells (11, 12). The study by Gioino et al. (7a) provides evidence for synergistic mechanisms residing in the single epithelial cell type of the secretory portion of the *R. prolixus* Malpighian tubule. Moreover, the synergism appears to stem from serotonin binding to a single serotonin receptor that activates two interacting signaling pathways. By activating both cAMP and Ca²⁺ second messenger pathways, serotonin controls both transepithelial cation and anion secretion at multiple sites in the cell. Ca²⁺ supports diuretic rates of transepithelial Na⁺ and K⁺ secretion by 1) providing transepithelial routes for Cl⁻ that have yet to be identified in *R. prolixus* Malpighian tubules, and 2) activating apical mitochondria for supporting ATP-dependent transport by the V-ATPase. Cyclic AMP may support diuretic rates of transepithelial Na⁺ and K⁺ secretion by 1) increasing the number of proton pumps (V-ATPase) at the apical membrane, and 2) sensitizing the InsP₃/Ca²⁺ pathway to serotonin, thereby increasing transepithelial Cl⁻ conductance. Thus, the two second messenger pathways—cAMP and Ca²⁺—mutually reinforce each other to increase the transepithelial secretion of cations (Na⁺ and K⁺) and anion (Cl⁻). The oscillating nature of 1) the intracellular Ca²⁺ signal and 2) the transepithelial voltage suggest the pulsatile transepithelial flow of cations, Cl⁻, and water when the tubule secretes electrolytes and water at high, diuretic rates.

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