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Modulation of Tyramine Signaling by Osmolality in an Insect Secretory Epithelium

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Modulation of Tyramine Signaling by Osmolality in An Insect Secretory Epithelium

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Abstract

The control of water balance in multicellular organisms depends on absorptive and secretory processes across epithelia. This study concerns the effects of osmolality on the function of the Malpighian tubules (MTs), a major component of the insect excretory system. Previous work has shown that the biogenic amine tyramine increases transepithelial chloride conductance and urine secretion in *Drosophila* MTs. This study demonstrates that the response of MTs to tyramine, as measured by the depolarization of the transepithelial potential (TEP), is modulated by the osmolality of the surrounding medium. An increase in osmolality caused decreased tyramine sensitivity, whereas a decrease in osmolality resulted in increased tyramine sensitivity; changes in osmolality of ±20% resulted in a nearly 10-fold modulation of the response to 10 nM tyramine. The activity of another diuretic agent, leucokinin, was similarly sensitive to osmolality, suggesting that the modulation occurs downstream of the tyramine receptor. In response to continuous tyramine signaling, as likely occurs in vivo, the TEP oscillates, and an increase in osmolality lengthened the period of these oscillations. Increased osmolality also caused a

decrease in the rate of urine production; this decrease was attenuated by the tyraminergic antagonist yohimbine. A model is proposed in which this modulation of tyramine signaling enhances the conservation of body water during dehydration stress. The modulation of ligand signaling is a novel effect of osmolality and may be a widespread mechanism through which epithelia respond to changes in their environment.

the homeostatic control of internal osmotic and ionic conditions is essential for the survival of all organisms. In multicellular animals, such osmoregulatory processes are largely carried out by the secretion and absorption of ions and water across epithelia because these tissues lie at the interface between the internal and external environments. To function effectively, secretory epithelia must be able to modulate their activity in response to changing environmental conditions, either by directly sensing such parameters as osmolality, temperature, and ion concentration, or by responding to hormonal signals originating in another part of the organism. Alterations in osmolality have been shown to have many direct effects on epithelia, including changes in ion transport (15, 21, 29, 49, 56), release of signaling molecules (25, 48, 53), and induction of gene expression (18, 26, 31).

Osmoregulation in insects is accomplished through the function of the excretory system, which consists primarily of two epithelial organs, the Malpighian tubules (MTs) and the rectum (8, 40). The MTs are blind-ended epithelial tubes that empty into the gut and are responsible for the production of primary urine. In the fruit fly *Drosophila melanogaster*, urine is produced by the main segments of the four MTs (16); these segments consist of two cell types, the principal cells and the stellate cells (50, 55). Urine secretion in *Drosophila*, as in other insects, is driven by the coupled action of a V-type proton ATPase and a proton/alkali metal antiporter in the apical membrane of the principal cells, resulting in the net active transport of potassium into the tubule lumen and the generation of a lumen-positive transepithelial potential (TEP) (3, 17, 34, 38). Chloride ions and water move passively into the lumen following their electrochemical and osmotic gradients, and the resulting urine is approximately isoosmotic with the surrounding hemolymph (28, 34, 36). The precise mechanism of chloride transport in the *Drosophila* MT remains unclear but is regulated by intracellular calcium levels in the stellate cells; treatment of tubules with agents that cause an increase in cytoplasmic calcium concentrations in the stellate cells result in a rapid increase in transepithelial chloride permeability, a depolarization of the TEP, and an increase in urine secretion (36).

I have previously shown that the biogenic amine tyramine is a potent stimulator of chloride transport and a diuretic agent in the *Drosophila* MT (7). Application of nanomolar doses of tyramine causes a rapid depolarization of the TEP and an increase in transepithelial chloride conductance, as shown by an increased sensitivity of the TEP to peritubular chloride concentration. Tyramine is synthesized in isolated tubules from tyrosine present in the peritubular bath through the action of the enzyme tyrosine decarboxylase in the principal cells. Tyramine then acts on the tubule with a pharmacology very similar to that of a cloned, G protein-coupled tyramine receptor. The response of MTs to tyramine requires an increase in intracellular calcium levels in the stellate cells (6); it is likely that, like the diuretic peptide leucokinin, tyramine acts by stimulating the release of calcium from intracellular stores in the stellate cells (42). This study shows that the sensitivity of the tubule to tyramine is modulated by the osmolality of the peritubular fluid. This modulatory mechanism could potentially play a role in protecting the insect from dehydration stress. These data demonstrate a novel mechanism for the osmotic control of epithelial function and raise the possibility that such a pathway might function in other secretory epithelial tissues.

MATERIALS AND METHODS

Fly maintenance and solutions.

Wild-type *Drosophila melanogaster* (Canton S) were maintained on cornmeal-molasses-agar food mixture at 24°C on a 12:12-h light-dark cycle. All experiments were performed on posterior MTs acutely dissected from adult female flies 6–8 days posteclosion.

Two solutions were used in these studies, depending on the type of experiment being performed. The first was a dissecting/recording saline containing (in mM) 85 NaCl, 20 KCl, 3 $CaCl_2$, 12 $MgSO_4$, 7.5 $NaHCO_3$, 4 NaH_2PO_4, 15 glucose, and 10 HEPES, pH 6.75. This saline was used for studies involving acute application of secretagogues. The second solution, SBM, was a 1:1 mixture of Schneider's *Drosophila* medium (Invitrogen, Carlsbad, CA) and a diluting saline containing (in mM) 36 NaCl, 21 KCl, 15 $MgCl_2$, 5 $CaCl_2$, 4.8 $NaHCO_3$, 2 NaH_2PO_4, 11.1 glucose, and 15 HEPES, pH 6.75. The composition of SBM, based on the compositions of the diluting saline and Schneider's medium, is the following (in mM): 36 NaCl, 21 KCl, 5.2 $CaCl_2$, 7.5 $MgSO_4$, 7.5 $MgCl_2$, 4.8 $NaHCO_3$, 1.3 KH_2PO_4, 3.4 sodium phosphate, 7.5 HEPES, 0.7 α-ketoglutaric acid, 11.1 d-glucose, 0.43 fumaric acid, 0.38 malic acid, 0.42 succinic acid, 2.9 trehalose, 2.8 β-alanine, 1.15 l-arginine, 1.5 l-aspartic acid, 0.25 l-cysteine, 0.21 l-cystine, 2.7 l-glutamic acid, 6.15 l-glutamine, 1.67 glycine, 1.29 l-histidine, 0.58 l-isoleucine, 0.58 l-leucine, 4.5 l-lysine HCl, 2.7 l-methionine, 0.45 l-phenylalanine, 7.4 l-proline, 1.19 l-serine, 1.47 l-threonine, 0.25 l-tryptophan, 1.38 l-tyrosine, 1.32 l-valine, and 1,000 mg/l yeastolate. SBM, which is thought to approximate insect hemolymph in composition, was used for studies of chronically stimulated tubules. The osmolality of both the recording saline and SBM was 255–270 mosmol/kgH_2O. All osmolality measurements were performed with a vapor-pressure osmometer (Wescor, Logan, UT).

Electrophysiology.

Tubules were dissected under saline and placed in a tissue culture dish, in which a 100-μl drop of 0.125 mg/ml poly-l-lysine had been dried (34). Where appropriate, the bathing saline was then replaced with SBM. The TEP was recorded immediately after dissection by impaling the tubule lumen with a sharp electrode (R > 25 MΩ) pulled from theta-glass and filled with 3 M KCl. Potentials were amplified (Axopatch 200B, Axon Instruments, Union City, CA), digitized at 100 Hz and stored online. Recording and analysis were conducted using pCLAMP software (Axon Instruments). The peritubular bath was continuously perfused during recording.

Urine secretion assays.

Urine secretion rates were measured as described previously (16). Tubules were dissected under SBM and placed in a 15-μl droplet of medium under mineral oil. One branch of the tubule was pulled out of the droplet and wrapped around a pin such that the cut end of the ureter and lower section of the other branch were out of the aqueous droplet. At intervals of 12–20 min, the secreted urine droplet was removed from the ureter with a glass rod and its diameter was measured with an ocular micrometer. The volume of the urine droplet was calculated assuming spherical geometry. Solution changes were made immediately before the removal of the urine droplet for the second interval by twice removing 12 μl of medium and replacing it with 12 μl of new solution.

Data analysis.

The response index, a measure of the TEP response to pulses of drugs, was calculated as previously described (7). The area under the voltage curve was calculated for a period beginning 15 s after drug application began and ending 15 s after drug application ended, using −10 mV as the baseline (drug area). For the same period, the area was calculated under a line extrapolated from the voltage trace for the 30 s immediately preceding drug application (control area). The response index was calculated as (control area − drug area)/control area. Records in which the control response to tyramine had a response index of <0.03 were discarded from further analysis.

The average period of the TEP oscillations was calculated using a fast Fourier transform nonlinear least-squares algorithm as previously described (6). Records were analyzed beginning 60 s after each solution change.

Data were plotted and tests for statistical significance were performed with the use of Origin software (OriginLab, Northampton, MA).

Materials.

Except where noted, all reagents were purchased from Sigma (St. Louis, MO). Leucokinin IV was purchased from Bachem (King of Prussia, PA).

RESULTS

Acutely isolated MTs were bathed in saline, and the TEP was recorded with a sharp electrode. Under these control conditions, the TEP is lumen-positive and does not vary with time. Application of tyramine caused a rapid and reversible depolarization of the TEP, which has been shown to result from an increase in the transepithelial chloride conductance (7). The magnitude of this tyramine response was sensitive to changes in the osmolality of the peritubular bath; an increase in osmolality (by addition of sucrose) resulted in a decrease in the response to tyramine, whereas a decrease in osmolality (by addition of water) resulted in an increased response (Fig. 1). Increasing the osmolality of the bath solution by addition of osmolytes other than sucrose (trehalose or sodium chloride) also decreased the tyramine response, while lowering the osmolality of the bath without decreasing the ionic strength (by removing sucrose that had been added to a low-osmolality saline) also increased tyramine sensitivity (data not shown). In the absence of applied tyramine, changes in osmolality had only small effects (<10 mV) on the amplitude of the TEP (Fig. 1, *A* and *B*). As shown in Fig. 1*C*, the sensitivity of the tubule to an acute application of tyramine varied smoothly as a function of peritubular osmolality, resulting in a >10-fold modulation of the response to 10 nM tyramine over the osmotic range tested. At higher doses of tyramine, hyperosmotic stimulation elicited a similar decrement in the response, whereas hypoosmotic stimulation had no effect (Fig. 2). This latter finding is likely the result of the highly nonlinear relationship between the TEP and chloride conductance; at a certain level of chloride conductance, the TEP response will saturate at the Nernst equilibrium potential for chloride (approximately −10 mV), and further increases in conductance will have no effect on the TEP. Because of this nonlinearity, it is difficult to determine precisely how the dose-response curve of the tubule to tyramine is affected by changes in osmolality.

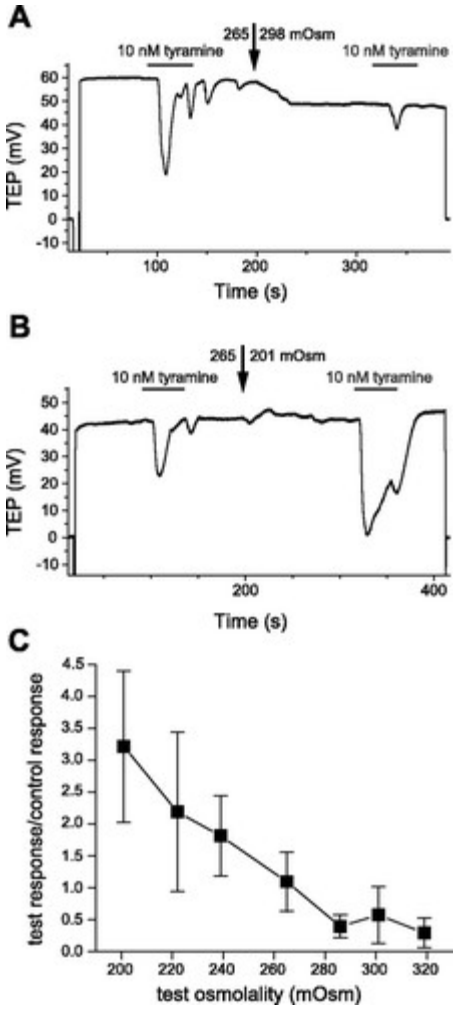

Fig. 1. Modulation of the tyramine response by osmolality. *A* and *B*: transepithelial potential (TEP) recordings of tubules in saline challenged by applications of 10 nM tyramine as indicated by the horizontal bars. At the arrow, the osmolality of the bath solution was changed by addition of sucrose (*A*) or water (*B*). *C*: data from recordings as in *A* and *B*, showing the ratio of the amplitudes of the second tyramine response over the first tyramine response as a function of the osmolality of the bath during the second response. *n* = 6–9 recordings/value, error bars in this and all other figures represent standard deviations. There was no significant variation among the groups in the amplitude of the first tyramine response, at an osmolality of 265 mosmol/kgH_2O (1-way ANOVA, *P* = 0.81). In contrast, paired *t*-tests showed a significant difference between the first and second tyramine responses at all test osmolalities, except 265 and 300 mosmol/kgH_2O (*P* values, in mosmol/kgH_2O: 201, 0.00000060; 222, 0.0076; 239, 0.014; 265, 0.96; 286, 0.0026; 300, 0.069; 319, 0.00068).

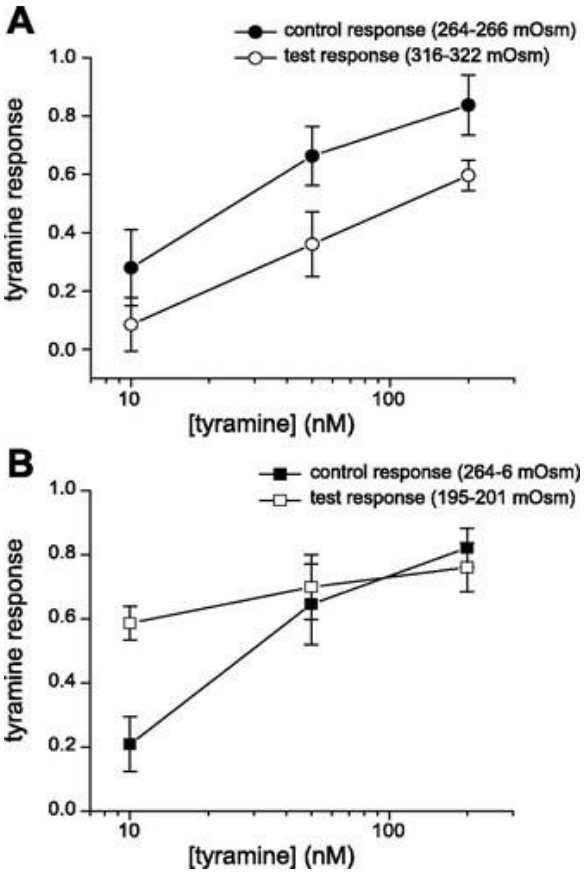

Fig. 2. Effect of osmolality on the dose-response relationship of tubules to tyramine. Individual tubules were held in saline and challenged with two applications of tyramine, first at 264–266 mosmol/kgH_2O, then at either 316–322 mosmol/kgH_2O (*A*) or 195–201 mosmol/kgH_2O (*B*), as in Fig. 1. Response index values for each tyramine response are plotted on the *y*-axis. *n* = 4–8 tubules/point.

To determine whether the changes in osmolality were acting at the level of or downstream of the tyramine receptor, the effect of osmolality on the response of tubules to leucokinin was examined. Leucokinin is a peptide diuretic agent that also stimulates chloride conductance (36, 39). The responses to tyramine and leucokinin are physiologically indistinguishable and exhibit cross-desensitization (6), suggesting that the two secretagogues act on a common signaling pathway. As shown in Fig. 3, an increase in peritubular osmolality causes a reduction in the leucokinin response. Also shown in Fig. 3 is the reduction in the tyramine response to a hyperosmotic stimulus of the same magnitude (data from Fig. 1); it is evident from this comparison that the tyramine and leucokinin responses are similarly modulated by changes in osmolality. This result argues that osmolality is acting at a common step downstream of the separate tyramine and leucokinin receptors.

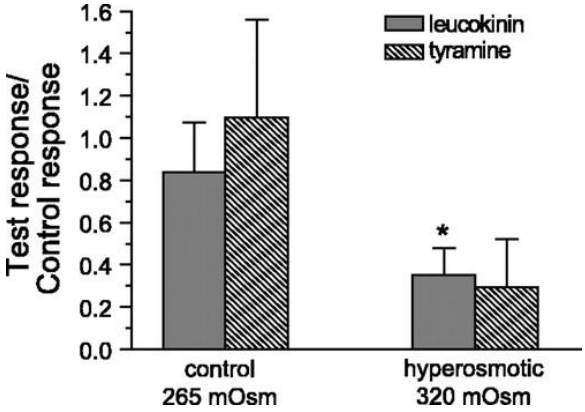

Fig. 3. Effect of osmolality on the response of tubules to leucokinin. Leucokinin data are from records as in Fig. 1, except tubules were challenged with two doses of 500 nM leucokinin IV; tyramine data from Fig. 1 are shown for comparison. The figure shows the ratio of the amplitudes of the second response, at either 265 or 320 mosmol/kgH_2O, to the first response at 265 mosmol/kgH_2O; *n* = 6–9 tubules/condition. Exposure of tubules to hyperosmotic medium resulted in a significant decrease in the ratio of the leucokinin responses (unpaired *t*-test, *P* = 0.0012). The initial leucokinin response amplitudes did not differ between the two groups (unpaired *t*-test, *P* = 0.13). See Fig. 1 legend for statistics on the tyramine data.

Because insect hemolymph contains significant levels of tyrosine, the tyraminergic signaling pathway of the tubules is likely to be constitutively activated in the intact fly (14, 41, 57). Therefore, it was of interest to examine the response of tubules to changes in osmolality while bathed continuously in SBM, which resembles hemolymph in composition and contains tyrosine. The tyrosine in the SBM is converted by the tubules into tyramine, and all actions of tyrosine on isolated tubules are mediated by the tyramine receptor (7). Electrophysiological responses are shown in Fig. 4. At the control osmolality, tubules exhibited pronounced oscillations in TEP, as previously reported (6). As the osmolality of the peritubular bath was increased, the period of the TEP oscillations lengthened. In contrast, there was no significant change in the period of the oscillations in response to a decrease in osmolality. Another assay of tubule function, the measurement of urine secretion rates, gave results consistent with the electrophysiological data. As shown in Fig. 5, tubules exposed continuously to tyrosine responded to an increase or decrease in osmolality with a decrease or increase in urine secretion rate, respectively. However, only the decrease in secretion rate in response to hyperosmotic stimulation is dependent on tyramine signaling; this is shown by the addition of the tyraminergic antagonist yohimbine to the bathing droplet. Yohimbine completely eliminates the electrophysiological response of the MTs to tyramine and blocks ~60% of urine secretion, as previously reported (7). However, if secretion rates are normalized to the values at the control osmolality, it is evident in Fig. 5*C* that a drop in osmolality results in the same relative increase in secretion rate, regardless of the presence of yohimbine, whereas the decrease in secretion rate caused by an increase in osmolality is almost entirely blocked by yohimbine. Interestingly, the increase in urine secretion rate following a drop in osmolality is noticeably larger than that predicted by the Boyle-Van't Hoff relationship, which assumes constant ion flux and production of a urine that is isoosmotic to the bathing solution (Fig. 5*C*). In contrast, the decrease in secretion after an increase in osmolality in the presence of yohimbine is very close to that predicted by theory.

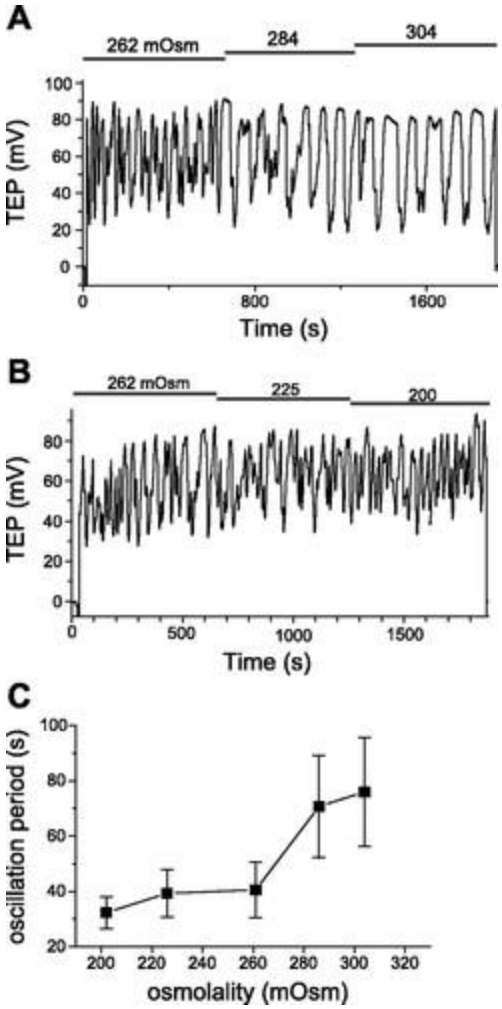

Fig. 4. Effect of osmolality on TEP oscillations. *A* and *B*: recordings from tubules held in SBM and challenged with increasing (*A*) or decreasing (*B*) osmolality. *C*: average periods of TEP oscillations from records such as those shown in *A* and *B*. Individual tubules were exposed to either an increase or decrease in osmolality from the control value. At least 5 min of data were analyzed at each osmolality. *n* = 7–8 tubules for each condition.

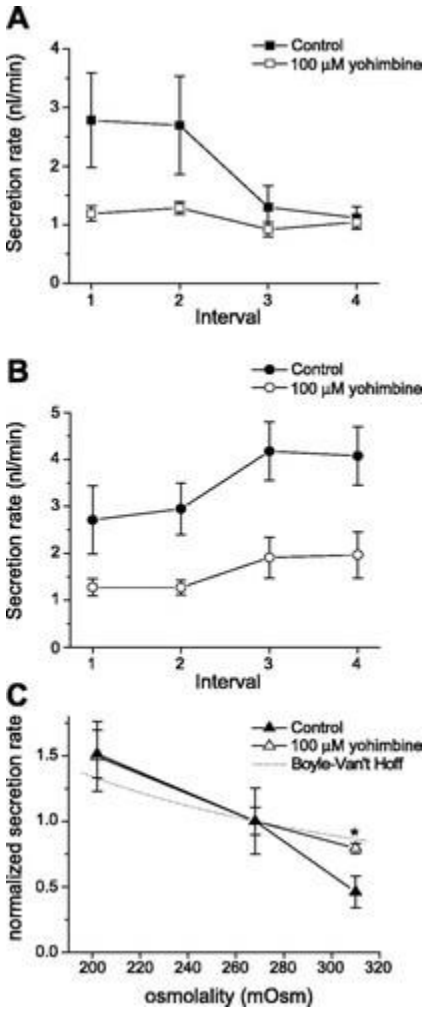

Fig. 5. Effect of osmolality on urine secretion rates. *A* and *B*: urine secretion rates were measured for tubules bathed in SBM in the presence or absence of 100 µM yohimbine. Urine droplets were collected, and secretion rates were calculated, at intervals of 12–20 min. Immediately preceding the second collection, the SBM in the bathing droplet was replaced with either SBM plus sucrose (~310 mosmol/kgH_2O) (*A*) or SBM plus water (~200 mosmol/kgH_2O) (*B*); *n* = 5–7 tubules/condition. *C*: secretion rates from *A* and *B* are normalized to the initial average rate for each condition and plotted as a function of osmolality. The relative response of tubules to hyperosmotic shock was inhibited by yohimbine (*P* = 0.000084, unpaired *t*-test). Initial secretion rates at the control osmolality for each group of tubules were 2.74 nl/min (SD 0.81) (0 yohimbine, hyperosmotic), 2.78 nl/min (SD 0.63) (0 yohimbine, hypoosmotic), 1.24 nl/min (SD 0.11) (100 yohimbine, hyperosmotic), and 1.29 nl/min (SD 0.15) (100 yohimbine, hypoosmotic). The solid line on the graph indicates the theoretical inverse relationship between osmolality and secretion rate predicted by the Boyle-Van't Hoff equation (normalized rate = 268 mosmol/kgH_2O/test osmolality).

DISCUSSION

The modulation of tyramine signaling in the *Drosophila* MT represents a novel effect of osmolality on the function of a secretory epithelium: specifically the modulation of sensitivity to an extracellular ligand. Previous work (25, 53) has demonstrated many other changes in epithelial function following osmotic shock, including an increase in the release of ATP, which then activates G protein-coupled receptors on the epithelial cells, activation of stretch-sensitive ion channels (12, 15, 46), which can directly cause an increase in intracellular calcium levels, alterations in transcellular ion transport (32, 37), and changes in paracellular conductance

(13, 22, 58). Such effects result in rapid modulation of epithelial function by osmolality, just as in the MTs, but the MTs respond to changes in osmolality in a fundamentally different way then has been reported for other epithelia. It is intriguing to note the numerous examples of secretory epithelia that are under the control of biogenic amines and other hormones (2, 10, 11, 20, 30, 47, 52). The current data raise the possibility that some of these signaling pathways might also be modulated by osmolality just as is tyramine signaling in the MTs.

There has been one previous report of osmotic modulation of the period of an oscillatory signal. Reetz and coworkers (45) observed that in cultured rat astrocytes, ATP induced calcium oscillations through the activation of a P2 purinergic receptor, and the frequency of these oscillations was sensitive to osmolality. As in the *Drosophila* MTs, hypertonic media reduced the frequency of the oscillations, although the astrocyte responses were more variable than those of the MTs. No mechanism or functional consequence of this modulation was proposed.

The mechanism through which osmolality acts on tyramine and leucokinin signaling in the MTs remains unknown; however, the data are most easily explained by an effect on either inositol trisphosphate production or intracellular calcium dynamics. In the *Drosophila* MT, leucokinin stimulates the production of inositol trisphosphate and release of calcium from intracellular stores in the stellate cells (42). Tyramine, because it displays cross-desensitization with leucokinin, is presumed to act on the same pathway (6). The subsequent rise in intracellular calcium levels triggers either an increase in the chloride conductance of the stellate cells or an increase in paracellular chloride conductance. Generally speaking, changes in osmolality could act either on the first part of this pathway, between receptor activation and calcium release, or on the second part, between calcium release and the increase in chloride conductance. However, the current study shows that in the continuous presence of tyrosine, changes in osmolality alter the period of the TEP oscillations; these oscillations are hypothesized to reflect oscillations in intracellular calcium levels due to periodic release from and reuptake into intracellular stores (6). An effect of osmolality on oscillation period is most easily explained by a change in either inositol trisphosphate levels or calcium dynamics; a direct effect of osmolality on chloride conductance would more likely alter the amplitude of the TEP oscillations and not the period. Changes in osmolality are known to affect intracellular calcium dynamics in many systems (24), and modulation of inositol trisphosphate-mediated calcium release has been demonstrated (9, 23). Future experiments measuring intracellular calcium levels will be needed to test this hypothesis directly.

Although the effects of tyramine have not been reported in the MTs of any other insect species, leucokinin signaling has been extensively studied in the MTs of the mosquito *Aedes aegypti* (4). Leucokinin increases transepithelial chloride conductance in the MTs of both *Aedes* and *Drosophila*; however, the mechanistic details of the pathway appear to differ significantly between the two species. In *Aedes*, stellate cells are not involved in leucokinin signaling, and the resulting chloride conductance is entirely paracellular (39, 60). Nonetheless, in both *Aedes* and *Drosophila* MTs, leucokinin causes production of inositol trisphosphate and an increase in intracellular calcium levels (59), and *Aedes* MTs can also display oscillations in chloride conductance (5). It will be extremely interesting, therefore, to see whether leucokinin signaling in the *Aedes* MT is also modulated by peritubular osmolality.

It is noteworthy that whereas the response of the MT to acute applications of tyramine was modulated by both increases and decreases in osmolality, when the tyraminergic signaling pathway was continuously activated, the MT responded only to hyperosmotic stimulation. This asymmetric response to changes in osmolality was seen both electrophysiologically, where a drop in osmolality had no effect on the TEP oscillations, and with urine secretion, where inhibition of tyramine signaling by yohimbine did not affect the response of the MTs to a drop in osmolality. Without a direct knowledge of the biochemical pathways responsible for tyramine signaling and the TEP oscillations, one cannot provide any explanation for this difference. However, it is plausible that there exists a lower limit on the period of the TEP oscillations, possibly due to kinetic constraints on processes such as

calcium release from and reuptake into intracellular stores, such that an increase in the effective concentration of tyramine caused by a drop in the osmolality would not result in any further acceleration of the oscillations. Whatever the mechanism, it seems likely that in the intact fly, where the tubule is bathed continuously in a hemolymph that contains tyrosine, the modulation of tyramine signaling causes a drop in urine secretion when hemolymph osmolality rises but is not functionally important when hemolymph osmolality falls. The increase in urine secretion that does occur following a drop in osmolality is greater than that predicted by the Boyle-Van't Hoff relationship, meaning either that the rate of ion flux across the epithelium is not constant or that the urine does not remain isoosmotic to the bathing solution. A similar result was reported for the New Zealand alpine weta, in which the flux rates of both potassium and chloride were greatly enhanced by peritubular hypoosmolality (33). This increase in flux was hypothesized to occur, at least in part, due to a drop in intracellular chloride activity, thereby reducing the electrochemical gradient opposing the movement of chloride across the basolateral membrane. In the MTs of *Rhodnius*, in contrast, the rate of ion flux appears to be invariant across a wide range of peritubular osmolality (27). Thus the *Drosophila* MTs seem to behave more like those of the alpine weta, and further studies of urine composition and osmolality as well as intracellular ion activities are necessary to identify the mechanisms underlying the response of the MTs to hypoosmolality.

What is the role of the osmotic modulation of MT function in the intact fly? One hypothesis is that it allows for enhanced survival under dehydration stress. Under normal conditions, there is a high flux of water through the insect excretory system, with the large majority of the water that is secreted by the main segments of the tubules being reabsorbed, primarily in the rectum but also in the lower tubules and anterior hindgut (8, 35). It has been hypothesized that this flux is important in the diuretic clearance of toxins from the hemolymph (40). During desiccation stress, *Drosophila* can lose a large percentage of their total body water; this water loss is associated with a loss of hemolymph volume and an increase in hemolymph osmolality (1, 19). Indeed, dehydrated *Drosophila* can tolerate increases of >15% in their hemolymph osmolality (1); such an increase is more than sufficient to inhibit tyramine signaling in the MTs. In the intact fly, the tyramine signaling pathway should be constitutively active due to the relatively high concentration of tyrosine in the hemolymph (14, 41). Thus, as a fly becomes dehydrated, tyramine signaling should decrease, leading to a reduction in the rate of urine secretion by the tubules. This, in turn, will reduce the flux of liquid moving into the rectum. I hypothesize that a decreased flow of liquid into and through the rectum will result in a more efficient reabsorption of water into the hemolymph and concentration of the feces. The argument that downregulation of urine secretion by the MTs is an important step in water retention is strengthened by the observation that MT antidiuresis has been observed in other insect species under dehydration stress (51, 54). Thus the sensitivity of the tubule to osmolality would result in an excretory system that combines a high resting flux of water with an enhanced ability to conserve water during dehydration stress.

A final question is why the control of MT secretion rate by osmolality is indirect, acting through the modulation of the frequency of tyramine-dependent TEP oscillations. One possible explanation stems from an analysis of the control of the salivary gland in the blowfly *Calliphora erythrocephala* (43). In that epithelium, secretion is stimulated by serotonin; serotonin causes oscillations in the TEP of the isolated salivary gland, and the frequency of the oscillations increases with serotonin concentration. Rapp and colleagues (44) demonstrated that the conversion of an analog parameter (serotonin concentration) into the frequency domain allowed for a more stable control of secretion rate in the presence of small fluctuations in serotonin levels. Similarly, in a *Drosophila* MT that is constitutively activated by tyramine, the conversion of osmotic information into the frequency domain would then allow for a more stable control of excretory function in the presence of small fluctuations in hemolymph osmolality. This modulatory pathway provides an example of the unexpected complexity of insect renal function and its regulation.

GRANTS

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FOOTNOTES

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