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Managing fuels and fluids: Network integration of osmoregulatory and metabolic hormonal circuits in the polymodal control of homeostasis in insects

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Abstract
Osmoregulation in insects is an essential process whereby changes in hemolymph osmotic pressure induce the release of diuretic or antidiuretic hormones to recruit individual osmoregulatory responses in a manner that optimizes overall homeostasis. However, the mechanisms by which different osmoregulatory circuits interact with other homeostatic networks to implement the correct homeostatic program remain largely unexplored. Surprisingly, recent advances in insect genetics have revealed several important metabolic functions are regulated by classic osmoregulatory pathways, suggesting that internal cues related to osmotic and metabolic perturbations are integrated by the same hormonal networks. Here, we review our current knowledge on the network mechanisms that underpin systemic osmoregulation and discuss the remarkable parallels between the hormonal networks that regulate body fluid balance and those involved in energy homeostasis to provide a framework for understanding the polymodal optimization of homeostasis in insects.

KEYWORDS
interorgan communication, insects, metabolism, Neuropeptides, osmoregulation

INTRODUCTION

Animals adjust their internal environment in response to external conditions by engaging physiological responses that actively oppose internal perturbations to maintain homeostasis. The systemic control of salt and water balance is among the most aggressively set homeostatic mechanism in animals as even small osmotic disturbances can have fatal consequences for the organism. In insects, systemic osmoregulation is maintained by the balanced actions of renal (Malpighian) tubule secretion and hindgut reabsorption with both osmoregulatory responses being under complex feedback control by diuretic and antidiuretic hormones (see recent reviews by[1,2]) (Figure 1). Intriguingly, several reports over the past decade have raised the view that the classic osmoregulatory pathways are also implicated in regulating various aspects of metabolism, such as energy intake, uptake, and mobilization.[4–9] Together, these studies point to an emerging model by which insect osmoregulatory circuits and metabolic programs interact intimately to implement the correct homeostatic program in response to different challenges. In this review, we address the hormonal mechanisms by which insects defend against large changes in hemolymph osmotic pressure and highlight how these systems interact with other homeostatic networks. Following a brief overview of the known diuretic and anti-diuretic hormones and their canonical modes of action, we focus on how non-osmotic perturbations (in particular nutrients) modulate these conventional osmoregulatory pathways and how osmotic and non-osmotic cues are integrated to recruit individual effector responses in a manner that optimizes overall homeostasis in insects.

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HORMONAL CONTROL OF ION AND WATER BALANCE IN INSECTS

Insect osmoregulation is under feedback control by several neuropeptides and biogenic amines that are released into circulation from the central nervous system and/or enteroendocrine cells in the gut (Figure 2). So far, four neuropeptides (and one amine) are identified as diuretic hormones and one as an anti-diuretic hormone in the widely used genetic model Drosophila melanogaster; however, this number varies across different species. In this subsection, we describe the functions of these different humoral factors in controlling systemic osmoregulation in D. melanogaster and other insects.

Capa peptides

The Capa peptides belong to the PRXamide family of peptides, which are both structurally and functionally homologous to neuromedin U neuropeptides in mammals. Capa signaling shows a remarkable functional conservation with neuromedin U signaling in mammals, as both systems have been implicated in the stimulation of visceral muscle contractions and waste excretion, modulation of gastric acid secretion, regulation of feeding behavior and energy homeostasis, and stimulation of transepithelial ion transport. However, in insects the Capa peptides were originally classified as cardioacceleratory peptides, and were subsequently found to additionally modulate tubule fluid secretion across a broad range of species.

Calcitonin-like diuretic hormones

Another large diuretic hormone in D. melanogaster is Diuretic Hormone 31 (DH31); a homologue of vertebrate Calcitonin Gene-Related Peptide (CGRP). Originally, this CGRP-like peptide was purified from the central nervous system of the Pacific beetle cockroach Diploptera punctata where it was characterized as a diuretic hormone. In D. melanogaster, DH31-producing cells localize in several distinct populations in the brain where the peptide is released into the circulating hemolymph from the corpora cardiaca. However, in D. melanogaster DH31 is also produced and released from gut enteroendocrine cells situated in close proximity to the tubules in which the DH31 receptor (DH31R) is highly expressed, raising the possibility that this diuretic pathway operates via a paracrine gut-tubule signaling module. Ex vivo application of DH31 in a fluid secretion assay shows a relatively moderate effect on diuresis in D. melanogaster. However, co-application of either DH31 and leucokinin (see "Cokinin peptides") or DH31 and DH44 both demonstrate clear additive effects on fluid secretion. Taken together, these results suggest that the different osmoregulatory pathways play distinct and complementary roles in controlling diuresis in insects.
released into circulation, both Capa-1 and -2 bind to their receptor on tubule principal cells\textsuperscript{[26,42]} upon which they stimulate fluid secretion through activation of several intracellular signaling pathways. Capa receptor activation induces a strong $\text{Ca}^{2+}$ response\textsuperscript{[26,27,43]} which stimulates nitric oxide synthase and the production of nitric oxide. This nitric oxide signal subsequently activates a soluble guanylate cyclase to increase cGMP production, which results in stimulation of fluid secretion from the tubules.\textsuperscript{[38,44]} Paradoxically, Capa-1 has also been argued to have an antidiuretic role in \textit{D. melanogaster} by acting on the basolateral $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter Ncc69 to impair chill tolerance in this species,\textsuperscript{[45,46]} which points to a potential biphasic role of Capa peptides in regulating tubule secretion in flies.

Capa signaling has also been reported to act as an anti-diuretic signal in other species. For example, in the red flour beetle \textit{Tribolium castaneum} an orthologue of the Capa receptor is highly enriched in the renal tubules\textsuperscript{[19,48]} and fluorescently labelled archetypal Capa peptides show specific and displaceable binding to tubules,\textsuperscript{[37]} implying a functional role of Capa signaling in tubule physiology. Indeed, in vitro application of cGMP or synthetic homologues of Capa-1 peptide on tubules from the mealworm \textit{Tenebrio molitor} dramatically reduces fluid secretion.\textsuperscript{[37,38]} These data suggest that Capa acts as an anti-diuretic signal in beetles in a manner similar to that shown for the kissing bug \textit{Rhodnius prolixus}\textsuperscript{[49]} and the yellow fever mosquito \textit{Aedes aegypti}.\textsuperscript{[50–52]} Together, these data strongly imply that Capa signaling exerts an inhibitory effect on tubule secretion, and is an important regulator of in whole-animal water balance in beetles and other insects.

**Leucokinin peptides**

The leucokinin family of peptides is an ancient signaling system evolutionarily restricted to protostomian groups such as insects, tardigrades, crustaceans, nematodes, ticks, and mollusks.\textsuperscript{[53–56]} In \textit{D. melanogaster}, leucokinin is a short peptide hormone (15 amino acids) that is encoded...
FIGURE 3  Global overview of the DH31/DH31R signaling pathway in Drosophila melanogaster. Whole body dissection of 6-day-old D. melanogaster mated female expressing mCD8-GFP (Bloomington Drosophila Stock Center, #5137) under the control of the DH31-GAL4 driver (kind gift by Shu Kondo) immunostained with anti-GFP (ThermoFisher, #A11120, 1:500 dilution) and anti-DH31 (obtained from Dr Jing Wang, 1:500 dilution) antibodies and counterstained by DAPI and phalloidin. CA, corpora allata; EEC, enteroendocrine cell; MTs, Malpighian tubules; VNC, ventral nerve cord. Scale bar, 200 µm.

by the gene pp (named for its diuretic effect), and is one of the most potent diuretic systems in insects[37]; apart from beetles that appear to have secondarily lost this signaling system entirely[9]. This hormone is expressed and released from several distinct populations of neurons in the brain and ventral nerve cord of the fly[57,58] (Figure 2). Intriguingly, while the brain leucokinin neurons are sensitive to hyperosmotic stimuli as part of water-seeking memory circuit,[59] the leucokinin-producing neurosecretory cells in the ventral nerve cord are activated by signals related to water ingestion to remotely control tubule secretion.[60] Thus, whilst the two clusters of leucokinin-producing neurons are both osmosensitive, they are activated by opposing osmotic stimuli in adult flies. The leucokinin receptor is
broadly expressed in the animal, but is most highly expressed in the secondary ‘stellate’ cells of the tubules.\textsuperscript{[61]} Leucokinin receptor activation induces an increase in intracellular Ca\textsuperscript{2+}-levels, which leads to activation of the chloride shunt conductance and the transepithelial movement of water.\textsuperscript{[37,62–64]}

The biogenic amines tyramine and serotonin (5-hydroxytryptamine)

Interestingly, the biogenic amine, tyramine, functions in a manner that is indistinguishable to that of leucokinin, and the tyramine receptor (TyrR) is similarly expressed in stellate cells of the tubule,\textsuperscript{[65,66]} implying that these signaling pathways converge on the same downstream target.\textsuperscript{[65,67]} Yet, whereas leucokinin acts as a long-range signal, tyramine likely functions as a paracrine signal as one of the tyrosine decarboxylase (TDC) enzymes, TDC1, necessary for converting tyrosine to tyramine, is highly expressed in the large principal cells; the predominant cell type in tubules that neighbors the smaller stellate cells.\textsuperscript{[68]} In addition to tyramine, another biogenic amine, serotonin (i.e., 5-hydroxytryptamine), is implicated in the hormonal control of water balance in insects. Serotonin is a potent diuretic factor initially reported from the blood sucking bug \textit{R. prolixus} where it is part of post-prandial diuretic program that helps to stimulate fluid secretion rate a thousand-fold above resting levels. This rapid activation allows the insect to eliminate excess water and ions at a rate equivalent to its original body weight every 20–30 minutes following the ingestion of a large blood meal.\textsuperscript{[69–71]} Later, serotonin was reported to also elicit diuretic activities across a range of other insect species.\textsuperscript{[72,73]} The cellular responses to serotonin is similar to leucokinin in the tubules of the house cricket \textit{Acheta domestica}, as serotonin also increases intracellular Ca\textsuperscript{2+} concentrations.\textsuperscript{[72]}

In contrast, in other species such as \textit{R. prolixus} and \textit{A. aegypti}, serotonin also stimulates an increase of cAMP levels besides altering intracellular Ca\textsuperscript{2+} concentrations.\textsuperscript{[74]} These species-specific differences may be explained by different serotonin receptors expressed in the tubules or different downstream effectors of serotonin signaling in different species.

Neuroparsins, Ion Transport Peptides (ITPs) and other anti-diuretic hormones

Neuroparsins are the first anti-diuretic hormones found in insects. They were originally identified from the corpora cardiaca of the migratory locust \textit{Locusta migratoria} as anti-diuretic hormones due to their ability to stimulate reabsorption of water from the hindgut.\textsuperscript{[75,76]} However, because of the secondary loss of homologous genes in the genetic model \textit{D. melanogaster}, the molecular and cellular actions of the neuroparsins are not well studied. In contrast, ion transport peptides (ITPs), which were originally identified and characterized to promote hindgut water reabsorption in the desert locust \textit{Schistocerca gregaria}\textsuperscript{[77,78]} are widely distributed in insects\textsuperscript{[79,80]} including in \textit{D. melanogaster}.\textsuperscript{[81]} Interestingly, ITPs belong to the crustacean hyperglycemic, moult-inhibiting, and vitellogenesis-inhibiting family of peptides\textsuperscript{[79,80,82–85]} and do not share any sequence homology with the neuroparsins. In \textit{D. melanogaster}, ITP is recognized as a thirst-promoting and anti-diuretic hormone as well as having anorexigenic effects.\textsuperscript{[86]} However, the molecular mechanisms underpinning the physiological actions of ITP, including the deorphanization of the ITP receptor, remain to be solved. Finally, in addition to the previously discussed anti-diuretic roles of Capa peptides, there are several other neuropeptide systems functionally implicated in inhibiting fluid secretion by the tubules in several species. For example, the anti-diuretic factors (ADFs), which were originally identified from head extracts of the \textit{T. molitor}, were shown to potentiate fluid secretion by beetle tubules by increasing cGMP levels in a dose-dependent manner.\textsuperscript{[87]} Similar observations were also made when applying ADFs from \textit{T. molitor} on tubules from \textit{A. aegypti}\textsuperscript{[88]}, suggesting that this system might be broadly available in insects. However, the molecular mechanisms of ADFs are poorly defined, and to date no other gene sequences is available for these peptides in other insect Orders, implying that these peptides are likely beetle-specific. In \textit{D. melanogaster}, Neuropeptide F (NPF) and short neuropeptide F (sNPF) have also been shown to inhibit resting fluid secretion rates, consistent with both peptides acting through Gi to inhibit adenylate cyclase.\textsuperscript{[89]} Interestingly, the receptors of these peptides, NPF receptor and sNPF receptor, are conspicuously enriched only in adult male tubules, which suggests that their impact on tubule function is sex-specific.\textsuperscript{[90]} Although the male-specific roles of these peptides are incompletely understood, NPF was recently shown to inhibit water seeking behavior and to reduce drinking in males.\textsuperscript{[91]} Moreover, a part of the NPF-expressing neurons also co-express ITP.\textsuperscript{[92]} Together, these observations imply that these two systems are part of a larger osmoregulatory network in males.

INTEGRATION OF METABOLIC SIGNALS BY OSMOREGULATORY CIRCUITS

As we have just outlined, several diuretic and anti-diuretic hormones are implicated in the homeostatic control of water balance in insects. Yet, even though these diuretic and anti-diuretic hormones were originally characterized (or at least recognized) in relation to osmoregulatory functions, recent studies have been shown that these hormones also function as neuropeptides involved in metabolism and behavior.\textsuperscript{[93]} For instance, several neuropeptide pathways regulate diuresis as well as feeding behavior and intestinal contractions,\textsuperscript{[5,6,37,59,60,94]} suggesting that the homeostatic control of osmoregulation and metabolism may have been co-opted during evolution. Here, we define the environmental conditions that induce hormone release as well as describe the downstream effector responses they mediate during different environmental challenges.
DH44

The DH44-producing neurons are activated in response to nutritive sugars in *D. melanogaster*.[15,95] (Figure 2). Dus et al. showed that the six DH44-producing neurons in the pars intercerebralis of the brain sense nutritive sugars directly to activate two separate pathways: one in which secreted DH44 promotes the consumption of nutritive sugars via positive feedback regulation, and another in which DH44 stimulates gut peristalsis to promote waste excretion.[5] In addition to nutritive sugars, the DH44-producing neurons also directly sense three amino acids, L-glutamate, L-alanine, and L-aspartate (but not the other 17 L-amino acids); ingestion of these three amino acids rapidly promote food consumption.[7] Taken together, these studies suggest that DH44-producing neurons are sensitive to specific macronutrients and that the DH44 pathway plays a major role in the coordination of feeding behavior in flies. In addition to nutritional inputs, the neuronal circuits controlling circadian rhythms are also connected to the DH44-producing cells to regulate daily diurnal cycles (Figure 2). Hyperactivation or ablation of the DH44-producing neurons in the pars intercerebralis is sufficient to eliminate daily activity rhythms, suggesting that DH44 is a potent modulator of circadian rhythms.[96] Similarly, DH44-R1 is also expressed in Hugin-positive neurons to regulate rest/activity rhythms. The Hugin gene encodes a pre-prohormone that is processed to produce two mature neuropeptides, hug-γ and pyrokinin-2,[97] which also show functional homology to mammalian neuromedin U.[27,98] Both hug-γ/pyrokinin-2 and neuromedin U are known to affect growth and feeding behavior in insects and mammals, respectively, and further share a high degree of sequence homology.[27,34,98,99] Finally, hug-γ has been shown to function as a circadian output molecule and to regulate locomotor activity.[100]

DH31

In *D. melanogaster*, DH31 secretion from gut enteroendocrine cells is sensitive to protein-rich food consumption in a sex-dependent manner.[41] In males, gut-derived DH31 acts within minutes on neurons in the brain that carry DH31-R to reduce protein feeding and to prioritize courtship by regulating allatostatin C and corazonin expressing neurons, respectively.[41] Thus, the acute activation of a DH31-dependent gut-brain signaling module, to control a behavioral switch from feeding to courtship in male flies, provides a key example of how animals arbitrate between different behavioral programs.[41] Intriguingly, the DH31-positive enteroendocrine cells are also in close proximity to the DH31-R expressing principal cells in the tubules (Figure 3), suggesting a potential local gut-tubule axis that regulates systemic water balance via DH31/DH31R signaling. The functional significance and relative importance of this and other gut-derived peptides in the systemic control of water balance, however, remains unresolved.

Besides nutrition, DH31 secretion is also regulated by the circadian clock (Figure 2). DH31 acts as a circadian "wake-promoting signal", and DH31-producing cells also integrate information from other regulators of the circadian rhythm such as pigment-dispersing factor (PDF) signaling. PDF secretion from the subset of circadian clock neurons acts on DH31-expressing dorsal clock neurons to promote DH31 release before dawn. Activation of PDF receptors in the DH31-positive neurons specifically affects sleep and has no effect on circadian rhythms, thus constituting a dedicated locus for circadian regulation of sleep.[24] Together, these observations raise the possibility that DH31 could mediate the diurnal control of tubule secretion in a manner analogous to that seen for the mammalian kidney.[100] Indeed, *D. melanogaster* displays clear diurnal fluctuations in excretory behavior (see "Circadian clock-governed diuretic and anti-diuretic hormones" for details), as evidenced by quantitative differences in the number, size, and shape of deposits excreted over the course of 24-hour-period (Figure 4) as we will discuss in a following section. The circadian clock-governed brain DH31 pathway also mediates reproductive dormancy by controlling juvenile hormone production in the corpora allata (Figure 2). The DH31-producing neurons innervate the corpora allata that expresses DH31-R. Activation of the neuronal circuit leads to an increase in intracellular cAMP in the corpora allata, which results in decreased juvenile hormone biosynthesis.[101] Knocking down Dh31 in the corpora allata projecting neurons or DH31-R in the corpora allata causes a failure in reducing juvenile hormone concentrations, which results in abnormal yolk accumulation in the ovaries under reproductive dormancy-inducing conditions.[101] Thus, given that reproductive dormancy is under influence of day-light length and temperature, DH31 production is indirectly influenced by these two environmental stimuli.

Capa

Capa secretion is primarily sensitive to internal water abundance and dietary sugar levels in *D. melanogaster*. Although Capa secretion is suppressed under desiccation, rehydrating of desiccated flies is sufficient to induce Capa secretion from the Capa-expressing ventroabdominal neurons.[27,28,103] (Figure 2). Yet, this effect of rehydration on Capa secretion is transient.[28] In contrast, the effect of sugar consumption on Capa secretion from the Capa-producing ventroabdominal neurons persists for extended periods, suggesting that hemolymph sugar levels are potent regulator of Capa secretion. Besides these nutritional regulators, Capa-secretion is also sensitive to temperature-fluctuations. When adult males are exposed to non-lethal cold stress, Capa peptides are retained in the Capa-producing ventroabdominal neurons, but are released at high rates following cold shock recovery.[27,46] These data suggest that Capa-induced regulation of tubule function is a key mechanism by which insects survive cold stress.[104] At this point it is unclear whether cues related to these environmental stimuli are sensed directly or indirectly by the Capa-producing ventroabdominal neurons. However, a potential mechanism with which the Capa neurons integrate environmental signals to control hormone release could be via central corazonin neurons,[105] which directly innervate the Capa neurons and have previously been linked with regulating responses associated with metabolic and osmotic stresses.[105–107]
Capa signaling also activates gut peristalsis and promotes nutrient absorption, which impacts the homeostatic control of metabolism. Furthermore, Capa influences the secretion of adipokinetic hormone (AKH), which is a functional analogue of mammalian glucagon. Capa inhibits secretion of AKH from the AKH-producing cells, which additionally influences the homeostatic control of metabolism given that AKH is a key regulator of energy mobilization. Capa signaling thus occupies a central node in a homeostatic program that is essential to coordinate internal water and metabolic homeostasis in flies.

Leucokinin

The leucokinin-producing neurons localize as several distinct clusters in the central nervous system of adult D. melanogaster. The brain leucokinin-positive clusters promote the formation of water memories in response to hyperosmotic stimuli (Figure 2). The leucokinin-positive clusters in the ventral nerve cord have been suggested to remotely control tubule secretion. In addition to sensing osmolarity, it is known that the leucokinin neuron activity is sensitive to glucose. Furthermore, leucokinin signaling is required for proper circadian rhythms. Based on a large-scale RNAi screen, both leucokinin and its receptor were found to be required for normal diurnal rhythms, even though the leucokinin-producing neurons and leucokinin receptor expressing cells are not clock neurons. The circadian control of the leucokinin-producing neurons appears to be mediated by two different pathways: one is through indirect effects on PDF signaling, and the other is through the DH44-producing neurons. In addition to these two pathways, leucokinin signaling also affects sleeping behavior by acting on the insulin producing cells. Silencing leucokinin-producing neurons abolishes the suppressive effect of starvation-mediated sleep, and leucokinin receptor expression in the insulin-producing cells is required for appropriate sleep control, suggesting that leucokinin-dependent modulation of insulin activity is necessary for the integration of metabolic cues in sleep regulation. Leucokinin signaling also modulates feeding behavior. Animals in which the gene encoding for leucokinin or its receptor has been knocked down show an increase in meal size and reduction in feeding frequency, albeit without affecting the total caloric intake. Because leucokinin receptor-positive neurons in the brain and ventral ganglia innervate the foregut, failure in regulating food intake is likely due to impaired communication between gut and brain that is necessary to terminate feeding.

ITP

ITP belongs to the crustacean hyperglycemic hormone family of peptides and was, based on its ability to stimulate hindgut ion and fluid reabsorption, originally described from locusts as an anti-diuretic hormone. More recently, ITP was shown to act as a thirst-promoting signal in flies as well as to repress excretion across a range of insect species, implying that the anti-diuretic function of ITP is evolutionary highly conserved (Figure 2). Intriguingly, ITP release in D. melanogaster is likely governed by circadian pacemaker neurons, where from it is secreted in a rhythmic manner from projection terminals in the dorsal protocerebrum. Consistent with this notion, the rhythmical secretion of ITP is reduced in a clock mutant
A NETWORK PERSPECTIVE ON INSECT OSMOREGULATION AND METABOLISM

Diurnal control of diuretic and anti-diuretic hormones

As described above, all diuretic and anti-diuretic hormones are either directly or indirectly controlled by hormonal networks that are implicated in maintaining diurnal rhythms in insects. These reports strongly imply that insect excretion and water homeostasis are influenced by the circadian clock. In fact, analyzing the fecal output profiles of female flies over a 24-hour period show that both the number, size and intensity of excreta are influenced by diurnal cycles (Figure 4). At the onset of daytime, flies excrete an increasing number of larger, fluid-rich deposits. Yet, as night-time begins the deposits become fewer and smaller with a high dye intensity, suggesting that “night-time excreta” contain less water. Taken together, these observations suggest that the excretory behavior of flies is likely governed by the circadian clock as described for mammals. The relative contribution of the different osmoregulatory circuits in modulating the diurnal control of excretion as well as the physiological importance of such a mechanism would be highly interesting to study.

Network integration of osmotic and non-osmotic signals in insect homeostasis

Why are there so many hormonal systems dedicated to controlling systemic osmoregulation and why are they also modulated by non-osmotic signals? One explanation might be that different osmotic perturbations likely require different combinations of osmoregulatory responses. For instance, both DH44 and DH31 influence sleep and feeding, but only DH31 affects courtship behavior and reproductive dormancy. Similarly, Capa and ITP— but not DH44, DH31, and leucokinin— are known to affect AKH secretion to mobilize energy reserves. Taken together, these observations suggest that under different environmental challenges, different combinations of hormones are necessary to implement the correct homeostatic program. For example, although both leucokinin and Capa affect food intake, leucokinin is important for appropriate regulation of meal size, whereas the action of Capa is to facilitate nutrient absorption by modulating gut motility. Importantly, both meal size and the regulation of gut peristalsis also affect systemic water balance, further emphasizing the intimate interactions between metabolic and osmoregulatory networks. Indeed, DH44 and Capa both potent regulators of systemic energy balance, while DH31 is necessary to arbitrate between energy storage and reproduction. We argue that it is necessary to adopt a network perspective when studying insect homeostasis by examining the combined hormonal cocktail released during a given environmental condition to characterize the dynamic interplay between osmoregulation, metabolism, and behavior.

WHAT IS METABOLIC WATER AND DOES IT MATTER?

For most insects, water lost to the environment is readily recovered by drinking; however, in animals experiencing permanent or intermittent periods of water deprivation, different physiological responses are engaged to maintain water balance. For example, classic studies have shown that the metabolic rate of animals exposed to dry conditions is markedly higher than at normal humidities, implying that some insects may adjust metabolic (oxidative) water production to offset an increase in evaporation. In the desiccation tolerant T. molitor, the oxidation of internal energy reserves (fat and glycogen) increased in starved animals at lower relative humidities compared to high, so that the organismal water levels remained constant. Similarly, studies on the tsetse fly Glossina morsitans and the cockroach Periplaneta americana found that the mobilization of energy reserves from the fat body increased when exposed to dry air in order to maintain a constant hemolymph osmotic pressure. Together, these reports suggest that some insects actively regulate metabolic water production as part of a homeostatic mechanism that acts to oppose the effects of desiccation. However, such a model depends on molecular mechanisms capable of sensing water availability and that cues related to water stress are integrated by metabolic pathways to promote internal water production. Consistent with this notion, work on D. melanogaster has shown that genetic inhibition of water-taste neurons was sufficient to cause an upregulation of AKH signaling, which promote lipid mobilization from the fat body. Specifically, loss of the critical water sensor, pickpocket 28 (ppk28), in gustatory neurons triggered a metabolic switch that promotes AKH release from the corpora cardiaca, which subsequently stimulates internal water production to compensate for the perceived lack of water availability. Conversely, Capa-producing neurosecretory cells in the ventral-abdominal ganglia are activated by hypoxic stimuli to systemically release Capa hormones to inhibit AKH release during periods of fluid excess. Taken together, these studies imply that both neuronal and hormonal circuits converge on the AKH-producing cells, and that they may act to bidirectionally control metabolic water production to maintain water balance. Interestingly, central release of vasopressin (also known as anti-diuretic hormone; enhances water reabsorption in the kidney) was recently shown to stimulate glucagon...
secretion in mammals,[128] raising the possibility that stimulation of metabolic water production during periods of internal water stress is an evolutionary conserved mechanism. Together, there is thus emerging evidence that metabolic water plays a physiologically relevant role in the overall water budget of animals—particularly for species that live permanently in dry surroundings—and so further work needs to be done to elucidate the molecular, cellular and network mechanism controlling metabolic water production. For example, it would be intriguing to test if insects alter metabolic strategies to optimize metabolic water production when exposed to desiccation as described for desert-dwelling mammals.[129]

MOLECULAR IDENTITY OF NUTRIENT SENSORS AND OSMORECEPTORS

The fact that there is feedback control on metabolic and osmoregulatory responses,[12,129] implies the existence of sensory mechanisms that can detect internal deviations in these nutrients. Recent studies provided clear evidence that the D. melanogaster central nervous system possesses direct sensors of circulating sugars like glucose and fructose,[48,84,105,106,130] as well as sensors of metabolic cues, like hugging and AKH.[94,130] Similarly, the insect central nervous system also contains neurons whose activity is regulated by extracellular osmolality and thus have the ability to report changes in internal water abundance.[8,130] However, the molecular identity of the nutrient and osmotic sensors that confer sensitivity to these internal states remain largely unknown. Recently, Miyamoto et al. reported that a highly conserved gustatory receptor, Gr43a, is expressed in distinct neurons of the D. melanogaster brain, where it functions as a fructose receptor. Artificial activation of Gr43a-expressing neurons during nutrient-deprived states promotes feeding, whereas neuronal activation during nutrient-replete states suppresses feeding. These data suggest that Gr43a+ neurons control ingestion behavior in a satiation-dependent manner.[106] Similarly, the glucose transporter Glut1 mediates the activation of a subset of sNPF expressing neurosecretory cells in the D. melanogaster brain to antagonistically control insulin and AKH secretion to maintain glucose homeostasis.[105] Finally, the glucose transporter, Sut2, underpins nutrient-dependent release of gut NPF to restrict AKH release and promote protein appetite in mated females.[131] Sugar sensors thus lie at the heart of internal nutrient sensing and play critical roles in activating organ-specific responses and behavioral outputs based on internal needs.

In addition to these sugar-only sensing pathways, other neurons in the fly brain are able to integrate signals related to both sugar and water. For example, two pairs of interoceptive neurons process homeostatic needs for both sugar and water and were shown to reciprocally regulate food and water consumption to promote balance.[131] Large-scale behavioral screens identified the AKH receptor as well as the osmosensitive transient receptor potential channel Nanchung as underlying these responses. Interestingly, such reciprocal regulation of food and water intake have also been observed in mammals suggesting that this might be a conserved mechanism.[133] Other distinct populations of neurosecretory cells, such as the DH44- corazonin- and Capa-producing neurons, are also modulated by both circulating sugar levels and internal osmolality although the molecules underpinning these responses remain unknown.[5,9,103,105] Together, these reports suggest that the convergence of internal signals of nutrient and water availability onto the same neuronal effectors may be a conserved, widespread mechanism by which the nervous system weighs competing needs to regulate internal homeostasis. More work needs to be undertaken to dissect the molecular mechanisms underpinning the cellular responses to nutrient and osmotic stress and how these interact in response to internal state.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Here, we have summarized our current understanding of insect osmoregulatory circuits and highlighted how classic osmoregulatory pathways have been co-opted to additionally coordinate metabolic responses, such as nutrient intake, uptake, and storage. The emerging view that cues related to both water and nutrient availability activate the same systematic signals now raises several questions that need to be addressed. First, what is the relative contribution of the different osmoregulatory pathways during different osmotic challenges and how do the various systems impact the metabolic profile of the animal? One possible explanation is that different types of osmotic perturbations require different combinations of physiological responses and thus the release of a certain combination of systemic signals to optimize fitness. A more global view of the organ-specific actions controlled by each system as well as a detailed understanding of the intrinsic cues that activate will help resolve this question. Second, what is the molecular identity of the nutrient and osmotic sensors that underpin activation of individual circuits and what are their key physiological triggers? Although the mechanisms underlying nutrient and osmosensing are active areas of research, very little is known about how they work at the molecular level. The unparalleled molecular and genetic toolbox available in D. melanogaster may prove particularly useful in addressing this question. Third, studies are required to define precisely how osmotic and metabolic signals are integrated to recruit a distinct combination of effector responses. The cellular and network interactions that underlie the polymodal optimization of homeostasis remain largely unexplored, yet understanding how these networks dynamically interact will undoubtedly unmask basic principles of how higher organisms maintain osmotic and metabolic homeostasis.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.
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