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Review

Anti-diuretic factors in insects: The role of CAPA peptides

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ABSTRACT

Insects have adapted to live in a wide variety of habitats and utilize an array of feeding strategies that present challenges to their ability to maintain osmotic balance. Regardless of the feeding strategy, water and ion levels within the haemolymph (insect blood) are maintained within a narrow range. This homeostasis involves the action of a variety of tissues, but is often chiefly regulated by the excretory system. Until recently, most research on the hormonal control of the excretory tissues has focused on factors known to have diuretic activities. In this mini-review, the current state of knowledge on anti-diuretic factors in insects will be discussed with a particular emphasis on the CAPA peptides in the blood-feeding Chagas' disease vector, *Rhodnius prolixus*.

1. Introduction: the excretory system and its regulation

Insects contain a number of neuroendocrine-derived factors that are responsible for maintenance of fluid and salt homeostasis in the haemolymph over a normal physiological range [15]. This homeostasis allows insects to adapt to a wide array of ecological niches and/or feeding strategies where they may be subjected to extremes in water, ion and nutrient availability as well as intake. In many insects, the excretory system is normally composed of the Malpighian tubules (MTs) and hindgut [70]. Transport of ions into the lumen of the MTs, together with osmotically obliged water, produces a primary secreted fluid that is nearly isosmotic to the haemolymph. Typically, the majority of ion, metabolite and water reabsorption occurs in the insect hindgut. However, during the rapid post-prandial diuresis in *Rhodnius prolixus*, the hindgut does not play a major role in reabsorption of water and ions [16,63]. Instead, fluid secreted by the upper (distal) MTs is modified by the lower (proximal) tubules, where KCl and water is reabsorbed [37,59].

Factors that regulate the excretory system in insects include a variety of peptide families along with biogenic amines [9,10,16,21,31,70,76,102,104]. Diuretic factors that stimulate fluid secretion by the MTs include the biogenic amines tyramine [9,10] and serotonin (5-hydroxytryptamine; 5HT) [73,74], as well as several families of peptides such as the corticotropin-releasing factor (CRF)-related peptides [6,7,33,45,46,84], insect kinins [8,18,38,39,105,110], calcitonin-like peptides [17,34] and the CAPA family of peptides [24,25,47,91]. Generally speaking, few of these have been shown to be true diuretic hormones (i.e. actually shown to be present in the haemolymph at appropriate times). Currently, true diuretic hormones include Locusta DH in the locust, *Locusta migratoria* [84] and 5HT in *R. prolixus* [49]. In contrast to the breadth of factors identified as being diuretic in insects, identification of insect anti-diuretic factors has been limited. Anti-diuresis is of great importance in insects, since this is the normal physiological state sustained by the majority of terrestrial insects, interrupted only occasionally by diuresis associated with increased water intake from dietary or metabolic sources [21].

2. Regulation of anti-diuresis in insects

John Phillips and colleagues in the desert locust, *Schistocerca gregaria*, discovered the earliest factors involved in anti-diuresis in insects. Ionic and osmotic concentrations in the haemolymph of desert locusts are maintained within a narrow range even though the volume of the haemolymph changes significantly during dehydration [12,85–87]. This intricate regulation is accomplished by altering the primary excretory fluid entering the hindgut through changes in the rates of water and ion transport across this epithelium [85–87]. The hindgut in insects is normally divided into an (anterior) ileum and (posterior) rectum, which function in a manner analogous to the proximal and more distal segments, respectively, of vertebrate nephrons [90]. In both of these segments in *S. gregaria*, transport mechanisms for ions and water are driven by an electrogenic Cl^- pump localized to the apical membrane [90]. Factors that regulate transport mechanisms by the locust hindgut have also been investigated. Specifically, three

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distinct factors contributing to an anti-diuretic strategy in locusts are known to act on the hindgut: Cl^- transport stimulating hormone (CTSH), ion transport peptide (ITP) and neuroparsins [32,88,89,100]. Only for the latter two are amino acid sequences available. The stimulatory role of neuroparsins on the locust hindgut is not well understood, and data has been conflicting [21,43]. For example, reciprocal bioassays of corpus cardiacum (a major neuroendocrine organ in insects) extracts from *L. migratoria* and *S. gregaria* on the ileum and rectum of these two locust species have shown similar stimulants are present [54]. Given that neuroparsins have no effect on *S. gregaria* hindgut [43], previous reviews have suggested that further studies are required to clarify the conflicting reports on neuroparsins' prospective role on the locust hindgut [21]. CTSH [100] acts on specific ion transport mechanisms via cAMP [13], and leads to reabsorption of fluid and ions by the rectum. Recently a candidate for CTSH, the heterodimeric hormone consisting of a glycoprotein A (GPA) and a glycoprotein B (GPB) was identified in the fruit fly, *Drosophila melanogaster*, and its receptor is expressed in the hindgut and couples positively with cAMP [99,101]. The third factor known to regulate absorption by the locust hindgut, ITP, acts specifically on the ileum and was originally identified and purified from the locust corpus cardiacum [3,90] and the 72-residue full-length peptide was determined following cDNA cloning [66]. In common with CTSH, ITP is also known to utilize cAMP as a second messenger and this leads to an increase in apical cation conductance and stimulation of an apical electrogenic Cl^- pump, while simultaneously acting via another unidentified second messenger to inhibit apical acid secretion [88].

In cross species studies, a cardioacceleratory peptide from *Manduca sexta* belonging to the CAPA family of peptides (see Section 3 below), ManseCAP2b, was the first factor shown to inhibit fluid secretion by MTs [97] and this peptide was later renamed as ManseCAPA-1 [53]. At the time, this was an unexpected result since CAPA peptides had been previously shown to have diuretic activity in some other insects [24,91]. This discovery indicated that the MTs, like the hindgut, could also be hormonally regulated to elicit antidiuresis. The first endogenous factor capable of inhibiting fluid secretion by MTs was described in the forest ant, *Formica polyctena* [48]; however the identify of this factor has never been determined. Native anti-diuretic factors (ADFs), TenmoADFa and TenmoADFb, have been identified in *Tenebrio molitor*, with potent inhibitory effects on Malpighian tubule (MT) secretion rates, via increases in cGMP levels [29,30]. The ADFs stimulate increases in cGMP that are independent of nitric oxide signaling, and so a soluble guanylate cyclase is unlikely [30]. In addition, cAMP levels stimulated with the native CRF-related DH, *T. molitor* DH_{37}, are decreased in MTs treated with ADFs [30]. Thus, ADFa and ADFb provided the first examples of endogenous peptides, along with their cognate intracellular mediators, which antagonistically regulate fluid secretion of MTs in insects [113]. However, there is no evidence that the TenmoADFa and TenmoADFb neuropeptides are released as neurohormones, since immunohistochemical analysis has not revealed any staining associated with classical neurohaemal storage sites [30,114], although this has been shown for the endogenous diuretic peptide [114]. Specifically, TenmoADFb was localized to two pairs of neurosecretory cells in the protocerebrum, with axons that arborized in a central plexus, however no staining was associated with the corpus cardiacum, which is a major neuroendocrine organ [30]. Interestingly, in cross-species assays, TenmoADFa has been shown to inhibit fluid secretion by MTs via cGMP in *Aedes aegypti* [64], but this peptide has no stimulatory or inhibitory effect on *Acheta domesticus* MTs [20]. Surprisingly, TenmoADFb has been shown to stimulate fluid secretion with activity similar to native kinin-related peptides in *A. domesticus* [20]. Finally, a factor with similar hydrophobicity and presumed molecular weight to *T. molitor* ADFa and ADFb was partially isolated in the Colorado potato beetle, *Leptinotarsa decemlineata* [50], although the sequence of this factor has not been resolved.

All post-embryonic stages of *R. prolixus* are obligate blood feeders, and fifth instars consume a blood meal equivalent to or greater than 10 times their unfed body mass. This increase in body mass leaves the insects more susceptible to predation or detection by their vertebrate hosts. Therefore, beginning immediately upon blood meal engorgement is a rapid diuresis that quickly removes the excess salts and water present in the blood meal (mainly from the plasma) and concentrates the nutritive components (mainly the red blood cells). It is during excretion of this urine that *R. prolixus* transmits the protozoan parasite, *Trypanosoma cruzi*, which is the etiological agent of Chagas' disease, also known as American trypanosomiasis [26]. In *R. prolixus*, it was previously believed that anti-diuresis was facilitated by a reduction in the circulating levels of diuretic hormones in the haemolymph [55]. However, it was subsequently suggested that this mechanism would be inefficient since diuretic hormone titers would be increased as the haemolymph volume declines over the progression of the rapid diuresis [97]. In addition, the mechanical properties of the plasticized cuticle, stimulated by 5HT [75], and the associated abdominal distention leading to activation of stretch receptors [55], would likely not permit a sufficiently precise detection of reduced distention leading to inhibition of diuretic hormone release [97]. Thus, as shown in other insects, it was suggested that *R. prolixus* might also contain endogenous peptides that control the cessation of diuresis [97]. This would ensure that essential water and salts are maintained and desiccation avoided. Of specific importance in this regard is the fact that there is no significant absorption by the hindgut during the rapid diuresis following a blood meal in *R. prolixus* [63]. Thus, any anti-diuretic factor would have to act elsewhere, possibly by having a dual inhibitory role on fluid secretion by MTs and absorption by the anterior midgut. An essential adaptation to hematophagy requires that the actions of the native diuretic and anti-diuretic hormones in *R. prolixus* must coordinate the clearance of the non-nutritive NaCl-rich plasma component during the rapid diuresis over the first few hours immediately following blood meal engorgement. Additionally, further coordination must also accommodate the slow diuresis that ensues over longer periods (several weeks) as the digestion and assimilation of the nutritive blood cells occurs.

3. CAPA gene peptides in insects: peptide consensus sequences and biological actions

CAPA peptides were originally discovered in the lepidopteran, *M. sexta*, based on cardioacceleratory activity [40] and structurally-related perviscerokinins were discovered in the cockroach, *Periplaneta americana*, based on myotropic activity [93]. Renamed recently as ManseCAPA-1 [53], the *M. sexta* peptide was previously known as ManseCAP2b, owing to its activity as a cardioacceleratory peptide (CAP) and having been one of five peptides purified from *M. sexta* ventral nerve chord extracts [107–109]. Eventually, the primary structures of two of these peptides were elucidated by sequencing, which included ManseCAP2b and crustacean cardioactive peptide (ManseCCAP) [14,40].

Genes that encode the CAPA peptides are named *capability* and were identified originally in the fruit fly, *D. melanogaster* [47] and subsequently in the tobacco hornworm, *M. sexta* [53]. The first two peptides derived from the CAPA gene coded precursor peptide typically contain the consensus carboxyl terminal sequence A/PFPRV-NH_2, while the third peptide is referred to as a pyrokinin-related peptide and normally contains the consensus carboxyl terminal sequence G/MWFGPRL-NH_2 (see Table 1). ManseCAPA-1, which belongs to the CAPA peptide family was shown to be a potent inhibitor