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The control of Malpighian tubule secretion in a predacious hemipteran insect, the spined soldier bug *Podisus maculiventris* (Heteroptera, Pentatomidae)

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ABSTRACT

Spined soldier bugs, *Podisus maculiventris*, are heteropteran insects that feed voraciously on other insects, particular the soft bodied larval forms of Lepidoptera and Coleoptera. The response of *P. maculiventris* Malpighian tubules (MTs) to serotonin and known diuretic and antidiuretic peptides has been investigated, and is compared with that of MT from the hematophagous and phytophagous heteropteran bugs *Rhodnius prolixus* and *Acrosternum hilare*, respectively. A CRF-related peptide diuretic hormone (DH) from the termite *Zootermopsis nevadensis* (Zoone-DH) stimulated MT secretion, which was reversed by a member of the CAP_{2b} family of peptides from *A. hilare* (Acrhi-CAP_{2b}-2), an antidiuretic effect. Serotonin had no effect on secretion, neither did a representative calcitonin-like DH, kinin, tachykinin-related peptide, and an antidiuretic factor from the mealworm *Tenebrio molitor* (Tenmo-ADFb) in both *P. maculiventris* or *A. hilare*. Serotonin is a DH in *R. prolixus*, and its lack of effect on MT from *P. maculiventris* and *A. hilare* suggests this is an adaptation to hematophagy. On the other hand, the antidiuretic activity of members of the CAP_{2b} family in all three bugs is consistent with this being a heteropteran feature rather than a specialism for hematophagy.

1. Introduction

Hemipteran insects are characterized by their mouthparts, which are of a piercing and sucking type. They are used by many hemipterans to suck plant sap (e.g. aphids), but the diet of the "true" bugs (Heteroptera) is more varied, with some feeding on vertebrate blood while others are voracious predators of other insects, especially caterpillars and grubs. The varied feeding behaviors and diets of heteropterans places different requirements on the excretory system for the regulation of hemolymph volume and composition, and for the removal of toxic waste, and this might be expected to be reflected in the control of Malpighian tubule (MT) secretion by diuretic and antidiuretic hormones.

The endocrine control of MT secretion has been most intensively studied in *Rhodnius prolixus*, a blood sucking heteropteran. Both nymphs and adults of *R. prolixus* feed infrequently, but when they do so they imbibe blood meals equivalent in volume to 10-times the unfed body weight of a nymph [22], which greatly restricts their maneuverability and poses a considerable threat to hemolymph homeostasis. The blood meal is pumped into an expanded anterior midgut (AMG) and over the next 3 h >60% of the imbibed salt and water are absorbed from the AMG and transferred (along with KCl) into the lumen of the upper region of the four Malpighian tubules (MTs) [22]. Potassium chloride is absorbed from the water impermeable lower region of the MT [24] and NaCl-rich urine is voided from the anus at the same rate it is absorbed from the AMG. This rapid diuresis allows the insect to gain some maneuverability and concentrates the major food component of the meal (blood cells) in the lumen of the AMG, from where it is slowly passed back to the posterior midgut (PMG) for the digestion and assimilation of nutrients over a period of days [41].

During the rapid diuresis, a fifth instar nymph of *R. prolixus* transfers a volume of NaCl-rich fluid from the lumen of the AMG into the MT that is equivalent to 10-times the hemolymph volume [22]. The two processes must therefore be precisely matched so as to avoid acute changes in hemolymph volume and composition, and this is achieved by using the same hormones to control fluid transport by the AMG and the upper MT. The rapid diuresis is triggered by a surge in the hemolymph titer of the diuretic hormone (DH) serotonin, which peaks at 115 nmol L^{-1} after 5 min, before falling back to 22 nmol L^{-1} after 20 min, although levels remain elevated for up to 24 h [19]. The peak titer of serotonin is sufficient to maximally stimulate fluid movement across the AMG and upper MT [2]. As the titer of serotonin declines a corticotropin releasing-factor (CRF)-related DH (Rhopr-DH) is released into the circulation and acts via the same second messenger (cAMP) to sustain the high rates of fluid transport by both the AMG and the upper MT [34,37], possibly by acting synergistically with the

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biogenic amine (V.A. Te Brugge and I. Orchard, personal communication). The volume of blood stored in the AMG declines throughout the postprandial diuresis, gradually removing the stimulus for DH release (abdominal expansion [21]). The hemolymph titer of DH will then fall due to enzymatic degradation and/or removal from the circulation. These processes will terminate the rapid diuresis, but would not ensure the uptake of fluid from the AMG is precisely matched by its secretion into the lumen of the upper MT. To achieve the necessary coordination of these two processes, a member of the cardioactive peptide (CAP) 2b family (Rhopr-CAP_{2b}-2, which is also referred to as Rhopr-CAPA-2) is released into the circulation 2–3 h after feeding [29] and acts to reduce serotonin-stimulated fluid transport by the AMG [17] and upper MT [30].

Compared with the wealth of information available on the control of diuresis in *R. prolixus* little is known of the endocrine control of excretion in other heteropterans. In a recent paper [5], we reported the effects of serotonin, CRF-related DH, CAP_{2b} and insect kinins on the MT of the pentatomid stink bugs, *Acrosternum hilare* and *Nezara viridula*, and compared them with *R. prolixus*. Stink bugs feed on plant tissues employing a macerate-and-flush strategy [14], in which saliva containing digestive enzymes is injected into the parenchyma of the plant to liquefy the tissues [25]. The macerated and partially digested material is then ingested by sucking. Compared with *R. prolixus*, the volume of fluid imbibed is small and is rich in K^+ rather than Na^+. There is therefore no requirement for a rapid postprandial diuresis, and serotonin, which is the immediate stimulus for this in *R. prolixus*, has no effect on MT secretion. Tubule secretion is stimulated by a CRF-related DH from the termite *Zootermopsis nevadensis* (Zoone-DH), but there is only a modest acceleration (2–3 fold) compared with the 1000-fold increase reported for *R. prolixus* MT [38], which is equivalent to the maximum response to serotonin [20]. Given there is no rapid postprandial diuresis in stink bugs, and hence no corresponding withdrawal of fluid from the AMG, it was surprising to find that Acrhi/Nezvi-CAP_{2b}-2 had antidiuretic activity. Peptides belonging to the CAP_{2b} family stimulate secretion by MT of the dipteran flies *Drosophila melanogaster* [9], *Musca domestica* [26] and *Stomoxys calcitrans* [27], and of the orthopterans *Acheta domesticus* and *Locusta migratoria* (G.M. Coast, unpublished observations), and their antidiuretic activity in *R. prolixus* was assumed to be an adaptation to meet the demand of having to coordinate the termination of stimulated rates of fluid transport across the AMG and upper MT.

The antidiuretic activity of CAP_{2b} peptides in stink bugs suggests this is a heteropteran feature rather than a specialism to meet demands associated with hematophagy. To test this hypothesis, we have investigated the control of MT secretion in another pentatomid, the spined soldier bug *Podisus maculiventris*, which belongs to the subfamily Asopinae. The Asopinae are set apart from stink bugs (Pentatominae) because of their predatory behavior. Both nymphs (with the exception of the 1st instar) and adults feed on the soft-bodied larvae of lepidopteran, coleopteran and hymenopteran insects, and *P. maculiventris* has been extensively studied, because it is a voracious feeder and has potential value in the control of outbreaks of Colorado beetle and other economically important foliage-feeding plant pests [10]. Predatory stink bugs use their stylets to pierce and hold their prey while it is macerated from within. The stylets are also used to inject copious amounts of saliva containing a range of digestive enzymes, including proteinases, into the prey [8]. The saliva quickly liquefies the internal tissues of the prey, which are then sucked up through the stylets into the AMG where digestion continues. The insects alternately inject saliva into the prey and suck up the liquefied parts [7]. The process is very efficient and up to 80% on the biomass of the prey, which can be up to 5 times their body weight, may be ingested in 2 h [7,8]. Digestion of the liquefied prey continues in the AMG and the posterior midgut (PMG) where the absorption of nutrients is completed.

Here we show that the actions of serotonin and a number of diuretic peptides on secretion by the MT of *P. maculiventris* are identical to those previously reported for phytophagous stink bugs. We have also extended our investigation of hormonal control in the phytophagous stink bug *A. hilare* by observing the effects of the peptides calcitonin-like DH, tachykinin-related peptide, and an antidiuretic factor from the mealworm *Tenebrio molitor* (Tenmo-ADFb) on MT fluid secretion. Most notably, Acrhi-CAP_{2b}-2 was found to have antidiuretic activity in MT from *P. maculiventris*, which reinforces the suggestion that this is characteristic of heteropterans and not an adaptation to hematophagy.

2. Materials and methods

2.1. Insects

The spined soldier bugs used in this study were obtained through Arbico Organics (Tucson, AZ). Two initial overnight shipments, one of 250 eggs/vial and one of 50 late instar nymphs and adults were received along with an additional shipment of nymphs and adult about 3 weeks later. The eggs were placed in plastic petri dishes (100 mm × 15 mm) containing cotton dental wicks kept wet with deionized water until nymphs hatched and molted to the second instar. The nymphs/adults were placed together in Rubbermaid^{TM} 1.4 L or Mainstays^{TM} 1.0 L round plastic containers (WalMart Stores) with the center of the lids cut out or holes melted in the lid with a metal rod, and escape was prevented with an organdy material. Loosely crumpled paper towels were placed in the containers to reduce interactions between bugs, because of their cannibalistic behavior. Paper towels were replaced, or the bugs were moved to clean containers, depending on the accumulation of feces and prey that had been fed on. Initially, adults were placed in Bug Dorms 1 (BioQuip Products, Rancho Dominguez, CA 90220), but it was more difficult for the adults to locate the prey, so all subsequent rearing was in the round plastic containers. In the Bug Dorm 1, adults laid egg masses of varying numbers on the sides of the cages, on paper toweling hung from the tops, or on the sleeve of the cages. In the plastic containers, adults laid egg masses on the sides of the containers, on the paper toweling, or on the organdy material. Egg masses were removed from substrates and placed in petri dishes as previously described for the egg shipment.

Nymphs and adults were fed larvae of the beet armyworm, *Spodoptera exigua* Hübner, that were obtained as overnight shipments of eggs masses on wax paper oviposition substrates from a laboratory culture maintained at the USDA-ARS, Beneficial Insects Research Unit, Kika de la Garza Research Center, Weslaco, TX. After larvae hatched, they were placed in the same around plastic containers as the spined soldier bugs, except that an artificial rearing medium, Stonefly *Heliothis* Diet (Ward's Natural Science, Rochester, NY) was properly prepared and distributed on the bottom, around the sides, or both to allow greater survival and space for the larvae to feed and develop. Based on availability, different larval instars were fed to the bugs; however, larval size did not seem to matter because multiple small nymphs were observed feeding on the same large larvae. A major advantage of using beet armyworm larvae was that the rearing containers could be placed in a refrigerator and they were suitable as prey for extended periods of time depending on the degree of contamination. In some cases, when the larvae were not used and were allowed to pupate, pupae were removed from the rearing containers and were placed in 4 L glass containers with wax paper, and larvae were collected from these for rearing when the moths had emerged, laid eggs and the larvae hatched.