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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™ 1.4 L or Mainstays™ 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.

2.2. Malpighian tubule secretion assay

Soldier bugs have four MTs the anatomical arrangement of which is very similar to that of stink bugs. Insects were pinned ventral side uppermost in a dissection dish, covered with *Oncopeltus fasciatus* saline [5], and the ventral abdominal sterna cut away. The gut was then carefully pulled to one side so as to expose the MT. No attempt was made to remove intact MTs, because their blind distal ends are tightly wrapped around one another and are closely associated with the heart. Instead, proximal segments were removed by gripping the ureter using a pair of watchmaker's forceps, cutting it free from the gut, and then carefully teasing the MT free of tracheal connections with a glass rod. Once a length of 1.5–2 cm had been freed in this way it was severed from the distal end and transferred to a 10 μL drop of *O. fasciatus* saline resting on the Syllgard-lined base of a petri dish containing water-saturated paraffin oil. Both ends of the MT segment were pulled out from the saline and wrapped seperately around minutin pins set close to the drop of bathing fluid. Generally, segments of all four MT could be removed from an insect in <10 min.

Preliminary experiments (data not shown) using MT from *P. maculiventris* adults showed they were packed with a white crystalline material, probably uric acid, which blocked the free flow of tubule fluid. All data reported in this paper were therefore obtained using MT from fifth instar nymphs. The tubules secreted spontaneously in *O. fasciatus* saline, with fluid almost invariably escaping from the cut proximal end. After a 40 min equilibration period the bathing fluid was changed and the secreted fluid that had accumulated was discarded. Droplets of secreted fluid were then collected at 30 min intervals before and after the addition of test compounds to the bathing fluid. The diameter (D) of the droplets was measured with an eyepiece micrometer as they rested on the non-wettable Syllgard base of the assay plate beneath paraffin oil. The volume (V) in nanoliters (nL) was calculated assuming the droplets to be perfect spheres ($V = D^3\pi/6$), and rates of secretion (in nL min^{-1}) obtained from dividing by the time interval over which the droplet was collected. The MT of *A. hilare* were handled in a similar fashion.

2.3. Peptides and chemicals

Compounds tested for effects on MT secretion were as follows: serotonin (Sigma–Aldrich, St. Louis, MO); a kinin from the house cricket *A. domesticus* (Achdo-K2); the CRF-related DH of the termite *Z. nevadensis* (Zoone-DH, a gift from David Schooley); the calcitonin (CT)-like DH from the Pacific beetle cockroach *Diploptera punctata* (Dippu-DH$_{31}$, a gift from David Schooley); a tachykinin-related peptide (TRP) from the Madeira cockroach *Leucophaea maderae* (Leuma-TRP-I); an antidiuretic factor (ADF) from the meal worm *T. molitor* (Tenmo-ADFb, a gift from David Schooley); and a CAP$_{2b}$ identified in *A. hilare* (Acrhi-CAP$_{2b}$-2). The Acrhi-CAP$_{2b}$-2, Achdo-K2 and Leuma-TRP-I were synthesized as previously described [5]. All other chemicals were obtained from Sigma.

2.4. Data handling

Graphs were prepared using GraphPad Prism 5.02 (GraphPad Software Inc., La Jolla, CA) and appropriate statistical tests performed with GraphPad InStat 3.06. A value for $P < 0.05$ was accepted as significant.

3. Results

3.1. Effect of the CRF-related Zoone-DH and serotonin

Serotonin and the CRF-related Zoone-DH stimulate maximum secretion by the MT of *R. prolixus* [38] and were therefore tested on tubules removed from fifth instar *P. maculiventris* nymphs. Basal secretion by *P. maculiventris* MT was vary variable, ranging from 0.02 to 1.00 nL min^{-1}, with a mean of 0.24 ± 0.03 nL min^{-1} ($N = 46$). Serotonin was tested at 1 μmol L^{-1}, which stimulates maximum secretion by the MT of *R. prolixus*. Rates of secretion measured over 30 min intervals before and after the addition of serotonin are shown in Fig. 1A and do not differ significantly ($P = 0.42$; $N = 8$; paired t-test). In contrast, 100 nmol L^{-1} Zoone-DH, which maximally stimulates *R. prolixus* MT, accelerated secretion by soldier bug tubules almost 6-fold (572 ± 65%; $N = 6$) within 30 min, and the rate of secretion was nearly 10-fold (974 ± 274%; $N = 6$) higher after 60 min (Fig. 1B).

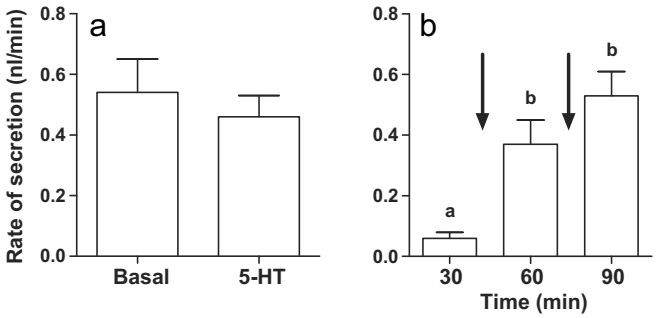

Fig. 1. (A) Rates of secretion measured before (basal) and after the addition of 1 μmol L^{-1} serotonin (5-HT). Bars represent the mean and vertical lines +1 S.E.M. of 8 replicates. Serotonin has no significant effect on tubule secretion. (B) The CRF-related Zoone-DH (100 nmol L^{-1}) stimulates secretion over 30 min, and the rate increases further over a subsequent 30 min period. Bars represent the mean and vertical lines +1 S.E.M. of 6 replicates. The times of addition of Zoone-DH are shown by the vertical arrows. Different letters are used to indicate values that differ significantly in paired t-tests.

3.2. Effect of Acrhi-CAP2b on the response to Zoone-DH

Peptides of the CAP$_{2b}$ family have antidiuretic activity on MT from *R. prolixus* [29,31] and *A. hilare* [5]. A native CAP$_{2b}$ from *A. hilare* (Acrhi-CAP$_{2b}$-2) was therefore tested at 10 μmol L^{-1} on *P. maculiventris* tubules stimulated with 100 nmol L^{-1} Zoone-DH. After measuring basal secretion, MTs were challenged with Zoone-DH alone for 30 min and then with Zoone-DH plus Acrhi-CAP$_{2b}$-2 for 30 min. The results are shown in Fig. 2. Secretion was

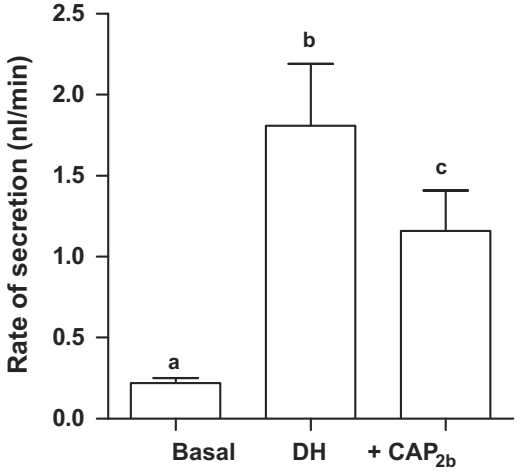

Fig. 2. The effect of 100 nmol L^{-1} Zoone-DH (DH) alone and then with the addition of 10 μmol L^{-1} Acrhi-CAP$_{2b}$-2 (+CAP$_{2b}$). Bars represent the mean and vertical lines +1 S.E.M. of 6 replicates. Fluid secretion was stimulated by Zoone-DH and this was partially reversed by Acrhi-CAP$_{2b}$-2. Different letters are used to indicate values that differ significantly in paired t-tests.

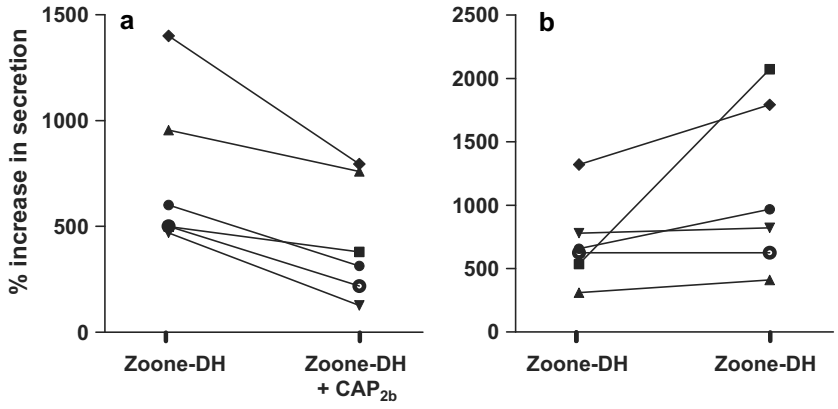

Fig. 3. A comparison of the response of individual tubules stimulated in the first experimental period by 100 nmol L^{-1} Zoone-DH and then challenged for 30 min with either (A) 100 nmol L^{-1} Zoone-DH plus 10 μmol L^{-1} Acrhi-CAP_{2b}-2 (data from Fig. 2) or (B) 100 nmol L^{-1} Zoone-DH alone (data from Fig. 1B). Data points show the percentage increase in secretion over the basal rate for the same tubule. Lines connect values obtained from one tubule.

increased 7-fold by Zoone-DH, but decreased when Acrhi-CAP_{2b}-2 was added to the bathing fluid. The difference between rates of secretion measured before and after adding Acrhi-CAP_{2b}-2 was -0.67 ± 0.16 nL min^{-1}, which is very significant ($P < 0.01$; paired *t*-test). Fig. 3 compares the response of individual tubules from this experiment to those challenged only with Zoone-DH (data from Fig. 1B). In all six tubules tested, Zoone-DH-stimulated secretion was reduced following the addition of Acrhi-CAP_{2b}-2, whereas it either increased further or remained constant in the continued presence of Zoone-DH alone.

3.3. Effects of other known diuretic and antidiuretic peptides

Calcitonin-like DH and kinins are diuretics in a number of insects [32], but have little or no effect on secretion by *R. prolixus* MT [35,38]. Examples of both peptide families, the CT-like DH of *T. molitor* (Tenmo-DH_{31}) and a kinin from *A. domesticus* (Achdo-K-2), were tested for activity on soldier bug MT at concentrations shown to stimulate maximum tubule secretion in conspecific assays. Following the measurement of basal secretion, tubules were first challenged with 100 nmol L^{-1} Tenmo-DH_{31} then, after 30 min, this was washed off and replaced with 10 nmol L^{-1} Achdo-K2. Finally, 30 min later, Achdo-K2 was replaced with 100 nmol L^{-1} Zoone-DH to test the viability of the MT. The results are presented in Fig. 4A. Tenmo-DH_{31} and Achdo-K-2 had no effect on secretion ($P = 0.55$; one-way ANOVA), but it was increased 4-fold by Zoone-DH.

In a second experiment, two other peptides with reported diuretic and/or antidiuretic activity were tested on *P. maculiventris* MT. Tachykinin-related peptides (TRPs) stimulate secretion by MT from the tobacco hornworm, *Manduca sexta* [33], and the locusts, *L. migratoria* and *Schistocerca gregaria* [18]. A TRP from *L. maderae* (Leuma-TRP-1) was therefore tested over 30 min on soldier bug tubules at 100 nmol L^{-1}, sufficient to stimulate maximum secretion by MT from *M. sexta*, *L. migratoria* and *S. gregaria*. The same batch of MTs was also used to test the effect of a *T. molitor* antidiuretic factor Tenmo-ADFb, which decreases fluid secretion by mealworm tubules stimulated with the CRF-related Tenmo-DH_{37} at sub-nanomolar concentrations (EC_{50} = 240 pmol L^{-1}) [12], but which has diuretic activity on cricket tubules (EC_{50} = 1.5 μmol L^{-1}) [4]. After washing off the TRP, soldier bug MTs were challenged with 5 μmol L^{-1} Tenmo-ADFb, a concentration likely to have a diuretic effect, first alone and then after 30 min together with 100 nmol L^{-1} Zoone-DH. Fig. 4B shows that neither Leuma-TRP-1 nor Tenmo-ADFb stimulated secretion at the concentrations tested ($P = 0.95$; one-way ANOVA). Tenmo-ADFb was subsequently tested over 30 min intervals for antidiuretic activity at 1 nmol L^{-1} and 100 nmol L^{-1} on MT that had first been stimulated for 30 min with 100 nmol L^{-1} Zoone-DH. Fluid secretion was increased 4-fold by Zoone-DH, and was unchanged following the addition of 1 and 100 nmol L^{-1} Tenmo-ADFb in the continued presence of the DH (Fig. 5; $P = 0.85$; one-way ANOVA).

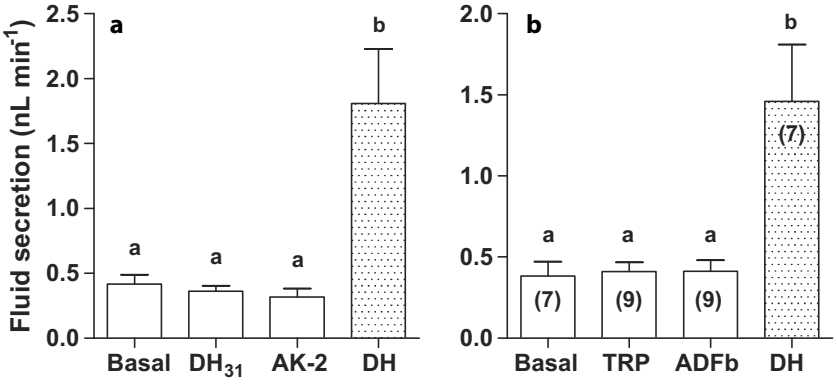

Fig. 4. (A) Rates of secretion measured before and after the addition of first 100 nmol L^{-1} of the CT-like Dippu-DH_{31} (DH_{31}) and then with 10 nmol L^{-1} of the kinin, Achdo-K-2 (AK-2). Zoone-DH (100 nmol L^{-1}) was added at the end of the experiment to test tubule viability (DH; stippled bar). Bars represent the mean and vertical lines +1 S.E.M. of 8 replicates. Neither the CT-like DH nor the kinin had any effect on fluid secretion. Different letters are used to indicate values that differ significantly. (B) Rates of secretion measured before and after the addition of first 100 nmol L^{-1} of the tachykinin-related peptide Leuma-TRP-I (TRP) and then 5 μmol L^{-1} Tenmo-ADFb (ADFb). Zoone-DH (100 nmol L^{-1}) was added at the end of the experiment to test tubule viability (DH; stippled bar). Bars represent the mean and vertical lines +1 S.E.M. for the number of replicates shown in parentheses. Leuma-TRP-I and Tenmo-ADFb had no effect on secretion. Different letters are used to indicate values that differ significantly.

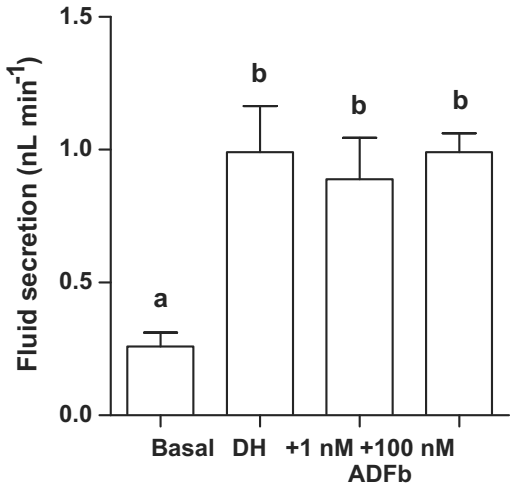

Fig. 5. The effect of Tenmo-ADFb on Zoone-DH-stimulated secretion. Tubules were initially stimulated with 100 nmol L^{-1} Zoone-DH alone (DH) and then together with first 1 nmol L^{-1} and then 100 nmol L^{-1} Tenmo-ADFb. Bars represent the mean and vertical lines +1 S.E.M. for the number of replicates shown in parentheses. Tenmo-ADFb had no effect on Zoone-DH-stimulated secretion. Different letters are used to indicate values that differ significantly.

3.4. Comparative studies with MT from A. hilare

A previous study on the control of secretion by MT of the stink bugs *A. hilare* and *N. viridula* did not examine whether CT-like DH, tachykinin-related peptides, and Tenmo-ADFb influenced the process. To allow comparisons with *P. maculiventris*, we therefore examined their actions on the MTs of *A. hilare*. Fig. 6A shows the effect of 100 nmol L^{-1} Leuma-TRP-I, 100 μmol L^{-1} Tenmo-ADFb and 100 nmol L^{-1} Dippu-DH_{31} on fluid secretion. A high concentration of Tenmo-ADFb was used to determine whether it stimulated MT secretion, because the EC_{50} for its diuretic activity on cricket MT is in the micromolar range (1.5 μmol L^{-1}) [4]. Each test substance was washed off after 30 min using two changes of saline before adding the next peptide and, at the end of the experiment, the MT were challenged with 100 nmol Zoone-DH to confirm their viability. Leuma-TRP-I, Tenmo-ADFb and Dippu-DH_{31} had no effect on fluid secretion (Fig. 6A; $P = 0.86$; one-way ANOVA), but it was accelerated 3-fold by Zoone-DH. A second experiment was performed to determine whether Tenmo-ADFb had antidiuretic activity. Tubules were stimulated with 100 nmol L^{-1} Zoone-DH and after 30 min they were challenged over two further 30 min periods with 1 nmol L^{-1} and then 100 nmol L^{-1} Tenmo-ADFb in the continued presence of the CRF-related DH. The concentrations of Tenmo-ADFb chosen were based upon its potent antidiuretic activity on MT from *T. molitor* (see above). Fluid secretion was increased >3-fold by Zoone-DH and was unchanged ($P = 0.98$; one-way ANOVA) by the addition of 1 and 100 nmol L^{-1} Tenmo-ADFb (Fig. 6B).

4. Discussion

At the concentrations tested, only the CRF-related peptide Zoone-DH and a CAP_{2b} from *A. hilare* (Acrhi-CAP_{2b}-2) influenced fluid secretion by MT from the spined soldier bug, *P. maculiventris*, with the former eliciting a diuretic response, which was reduced by the latter, an antidiuretic effect. Serotonin had no effect on secretion, neither did the neuropeptides Achdo-K-2, Leuma-TRP-1, Dippu-CT-like DH, and Tenmo-ADFb. These findings replicate those obtained previously with MTs from the stink bug *A. hilare* [5] that have been expanded upon in the present study. The similar results obtained with MTs from phytophagous and zoophagous pentatomid bugs probably reflect the fact that although they exploit different food resources their feeding strategies are very similar. Indeed, when prey is scarce *P. maculiventris* will feed on plant material [10].

Serotonin is used as a DH in the blood-sucking bug *R. prolixus* [23], but it had no effect on secretion by MT from pentatomid bugs, which may reflect their different feeding habits. In *R. prolixus*, serotonin controls a number of physiologically important process immediately before and after feeding commences, which enable nymphs to imbibe 10-times their body weight of blood in just 15 min [28]. Circulating levels of serotonin increase within a minute of the start of feeding [19], and stimulate the transfer of fluid from the AMG and into the upper MT [22]. This concentrates nutrients in the lumen of the AMG and simultaneously initiates a rapid diuresis during which >50% of the imbibed salt and water load are voided over about 3 h. In contrast, *P. maculiventris* necessarily feeds more slowly, because the tissues of the prey must first be liquidized by hydrolytic enzymes in the saliva before they can be sucked into the AMG. Thus although predaceous bugs may consume up to 80% of prey species several times larger than themselves, this takes about 2 h. Moreover, the liquefied food is nutrient-rich and therefore does not require to be concentrated by fluid withdrawal from the AMG. Hence they do not need to accelerate MT secretion immediately

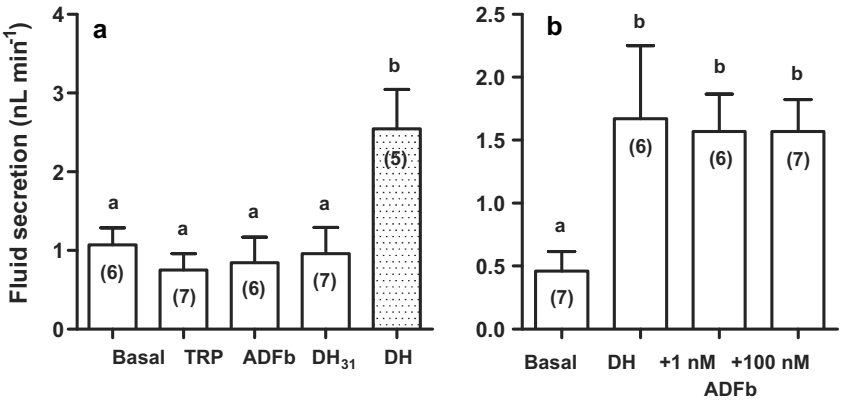

Fig. 6. Experiments with MT from the stink bug *A. hilare*. (A) Rates of fluid secretion measured before and after separate additions of 100 nmol L^{-1} Leuma-TRP-I (TRP), 100 μmol L^{-1} Tenmo-ADFb (ADFb), and 100 nmol L^{-1} Dippu-DH_{31} (DH_{31}). At the end of the experiment 100 nmol L^{-1} Zoone-DH (DH; stippled bar) was added to check tubule viability. Bars represent the mean and vertical lines +1 S.E.M. for the number of replicates shown in parentheses. (B) The effect of Tenmo-ADFb on Zoone-DH-stimulated secretion. Tubules were initially stimulated with 100 nmol L^{-1} Zoone-DH alone (DH) and then challenged with first 1 nmol L^{-1} and then 100 nmol L^{-1} Tenmo-ADFb in the continued presence of Zoone-DH. Bars represent the mean and vertical lines +1 S.E.M. for the number of replicates shown in parentheses. Tenmo-ADFb had no effect on Zoone-DH-stimulated secretion. Different letters are used to indicate values that differ significantly.

after feeding commences, which is consistent with the lack of effect of the biogenic amine on MTs from *P. maculiventris*.

The diuretic activity of the Zoone-DH on *P. maculiventris* MT was unsurprising, because peptides of this family have previously been shown to stimulate secretion by MTs from the heteropteran bugs *R. prolixus* [38] and *A. hilare* [5]. Fluid secretion by soldier bug MTs was increased up to 6-fold by 100 nmol L^{-1} Zone-DH, which is somewhat greater than the 2–3 fold increase observed in *A. hilare* [5]. However, the response of pentatomid MTs to Zoone-DH is small compared with the 1000-fold increase (equivalent to the effect of serotonin) in *R. prolixus* MT [38], which is consistent with the blood-sucking bug needing to rapidly void much of the imbibed volume load in order to recover some maneuverability.

The native CAP_{2b} of *A. hilare* and *N. viridula* (Acrhi-CAP_{2b}-2) reduced secretion by Zoone-DH-stimulated MT from *P. maculiventris*, as shown previously with stink bug MTs [5], and consistent with the antidiuretic activity of Rhopr-CAP_{2b}-2 in *R. prolixus* where it decreases secretion by serotonin-stimulated MT [30]. Interestingly, Rhopr-CAP_{2b}-2 is not thought to reduce secretion by *R. prolixus* MT stimulated with the recently identified native CRF-related peptide Rhopr-DH, which suggests the latter may act via both cAMP-dependent and cAMP-independent pathways (Paluzzi and Orchard, unpublished observations). In contrast, a CAP_{2b} (Manse-CAP_{2b}) from *M. sexta* decreases secretion by MTs of the beetle *T. molitor* that had been stimulated with the native CRF-related peptide Tenmo-DH_{37}, although its potency (EC_{50} = 85 nmol L^{-1}) is considerably less than that of the native antidiuretic factors Tenmo-ADFa (EC_{50} = 10 fmol L^{-1}) and Tenmo-ADFb (EC_{50} = 0.24 nmol L^{-1}) [11,12,40].

Other peptides that were tested had no effect on tubule secretion, although they were employed at concentrations previously shown to exert diuretic and/or antidiuretic activity. That Achdo-K-2 has no effect on secretion by *P. maculiventris* MT is consistent with previous work showing kinins have no effect on secretion by the MTs of *A. hilare* [5] and *R. prolixus* [38]. It is none the less surprising, given that peptides belonging to this family have diuretic activity in both hemimetabolous (Orthopteran and Dictyoptera) and holometabolous (Diptera and Lepidoptera) insects [32]. Kinin-like immunoreactive material is present in the brain and MTGM of *R. prolixus* [36], and the genome of a hemipteran insect, the green peach aphid, *Myzus persicae* (Sternorrhyncha: Aphidoidea) encodes several kinins [1], although it should be noted that aphids are unusual in not having MTs. It is worth remembering, however, that negative results from cross-species assays can be misleading, as for example in the housefly, *M. domestica*, where kinins from *L. maderae* and *A. domesticus* had little or no effect on MT secretion when tested at 1 μmol L^{-1} [16], whereas the native peptide (Musdo-K) increased secretion by 3–4-fold with an EC_{50} of 0.65 nmol L^{-1} [15]. On the other hand, in *R. prolixus*, even partially purified native kinin-immunoreactive material had no effect on MT secretion [38]. Nevertheless, kinins could contribute indirectly to the control of diuresis by stimulating contractions of the AMG [34] and hindgut [38], thereby reducing unstirred layers at both the luminal and hemolymph surfaces of the transporting epithelia.

Peptides belonging to the family of CT-like DH show a high degree of sequence conservation and stimulate secretion by MT from dictyopteran (*D. punctata*), orthopteran (*L. migratoria*), and dipteran (*D. melanogaster*, *Anopheles gambiae*, *Aedes aegypti*) insects [3,6,13]. However, the native CT-like DH of *R. prolixus* (Dippu/Rhopr-DH_{31}) had minimal diuretic activity when used in a conspecific assay, and it had no effect on secretion by MTs from *P. maculiventris* and *A. hilare* when tested at 100 nmol L^{-1}, which is considerably greater than EC_{50} values reported for activity in *D. punctata* (9.8 nmol L^{-1}) and *L. migratoria* (0.56 nmol L^{-1}) [13]. As with kinins, the CT-like DH probably contributes to the excretory process by stimulating contractions of the AMG and hindgut, and by improving the circulation of hemolymph through increasing the frequency of contractions of the dorsal vessel [34,39].

The actions of tachykinin-related peptides (TRPs) and Tenmo-ADFb, one of the two antidiuretic factors present in *T. molitor*, on MT secretion have been investigated in very few species. TRPs from *L. migratoria* (Locmi-TRP-I) and *L. maderae* (Leuma-TRP-I and IV) stimulate secretion by MT from pharate adult *M. sexta* in a dose-dependent fashion at ≥10 nmol L^{-1}, with the maximum response being achieved 30–40 min after peptide addition [33]. In contrast, the maximum response of MT from the desert locust (*S. gregaria*) to 10 nmol L^{-1} Locmi-TRP-I was reached within 5 min, and it is a far more potent diuretic (EC_{50} = 1.2 nmol L^{-1}) in both *S. gregaria* and *L. migratoria* [18]. TRPs have not been tested on *R. prolixus* MT, but 100 nmol L^{-1} Leuma-TRP-I had no effect over 30 min on secretion by tubules from *P. maculiventris* and *A. hilare*, although the potent myotropic actions of TRPs mean they too could contribute indirectly to the excretory process. Tenmo-ADFb is a potent antidiuretic on MTs from *T. molitor* (EC_{50} = 0.24 nmol L^{-1}), and opposes the diuretic activity of the CRF-related Tenmo-DH_{37} [12]. In marked contrast, Tenmo-ADFb had diuretic activity on MT from the cricket, *A. domesticus*, but with considerably lower potency (EC_{50} = 1.5 μmol L^{-1}) [4]. Tenmo-ADFb was tested at concentrations ranging from 1 nmol L^{-1} to 5 μmol L^{-1} on MTs from *P. maculiventris* and *A. hilare*, but it had no discernible diuretic activity and it did not reduce secretion by Zoone-DH-stimulated tubules.

The control of MTs from the predacious soldier bug *P. maculiventris* appears very similar to that reported previously for the phytophagous stink bugs *A. hilare* and *N. viridula* [5], in that fluid secretion is stimulated by a CRF-related DH (Zoone-DH) and inhibited by a member of the CAP_{2b} family (Acrhi-CAP_{2b}-2). The latter is consistent with the antidiuretic activity of Rhopr-CAP_{2b}-2 on *R. prolixus* MT, reinforcing the view that this is a heteropteran feature rather than a specific adaptation to hematophagy. Although the response of MTs from phytophagous and zoophagous pentatomid bugs to Zoone-DH and Acrhi-CAP_{2b}-2 appear similar in fluid secretion assays, it is possible that species differences will emerge from studies of their effects on Na^+ and K^+ transport, reflecting differences in the dietary intake of these ions.

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