Pestycydy, 2008, (1-2), 17-26.
ISSN 0208-8703

Aspects of the control of diuresis in the blood gorging insect, *Rhodnius prolixus*

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Abstract: Post-feeding diuresis in *Rhodnius prolixus* is neurohormonally controlled by serotonin and at least one peptidergic diuretic hormone (possibly a CRF-related peptide). These appear to be released by two different neurosecretory groups – 5 dorsal unpaired median (DUM) cells and 5-6 lateral neurosecretory cells of the mesothoracic ganglionic mass. The DUM cells co-localize serotonin and the calcitonin-related peptide RhoprDH31. The lateral neurosecretory cells co-localize a CRF-related peptide and a kinin-like peptide. Serotonin and the CRF-related peptide, ZooneDH, each stimulate maximum secretion by the upper Malpighian tubules, whereas only serotonin stimulates KCl reabsorption from the lower Malpighian tubules. RhoprDH31 is only weakly diuretic and Leucokinin 1 has no diuretic activity. Neither stimulates KCl reabsorption. The possible role for multiple diuretic hormones and for co-localization of diuretic factors is discussed.

Keywords: serotonin, diuretic hormones, neuropeptide, Malpighian tubules, DUM cells, neurosecretory cells

INTRODUCTION

Rapid diuresis is initiated in *Rhodnius prolixus* following a blood meal, in order to eliminate a large portion of the water and NaCl that is imbibed [1]. This diuresis is under the neurohormonal control of serotonin, and at least one neuropeptide (a putative peptidergic diuretic hormone, DH). Both hormones

stimulate a rapid fluid secretion by the upper secretory segment of the Malpighian tubules that is mediated by the second messenger cAMP [2]. Depletion of K^+ from the haemolymph during this time is prevented by reabsorption of KCl from the lower tubules; a process that is also controlled by serotonin [3].

Peptides with diuretic activity have been identified in other insects and typically belong to one of at least 3 families of peptides; the calcitonin (CT)-related DHs, the corticotropin-releasing factor (CRF) – related DHs, and the kinin-like DHs [1]. In *R. prolixus,* serotonin is co-localized with a CT-related peptide, $RhoprDH_{31}$, in 5 dorsal unpaired median (DUM) cells of the mesothoracic ganglionic mass (MTGM). These DUM cells produce neurohaemal sites on the abdominal nerves, from which serotonin and $RhoprDH_{31}$ are believed to be released into the haemolymph [4, 5]. *Locusta* DH – like immunoreactivity (CRF-related) is co-localized with kinin-like immunoreactivity in lateral neurosecretory cells of the MTGM, that also release their product into the haemolymph via neurohaemal sites on the abdominal nerves [6-8].

It is unclear why there are multiple DHs in this insect and also unknown if the peptidergic DHs mimic serotonin in stimulating KCl reabsorption from the lower Malpighian tubules. This paper examines some of the questions posed by these uncertainties.

MATERIALS AND METHODS

Insects: Unfed 5^{th} instars of *R. prolixus* were used throughout. The laboratory reared colony was maintained at 25 °C under high humidity.

Malpighian tubule secretion assays: Malpighian tubules were dissected from insects under saline and the ability of serotonin or diuretic peptides to stimulate secretion tested using the Ramsay assay as previously described [9]. In addition, ion selective electrodes were used to measure the K^+ concentration of secreted fluid droplets as previously described [9].

RESULTS

DUM Cells

Five DUM cells of the MTGM co-localize serotonin and $RhoprDH_{31}$ in *R. prolixus* [2, 4]. Serotonin is capable of stimulating a thousand-fold increase in secretion rate by the upper Malpighian tubules, whereas $RhoprDH_{31}$ is weakly,

though variably, diuretic (0 to 14 fold increase in secretion) [2, 4]. The fluid secreted by the upper Malpighian tubules in response to serotonin contains approximately equal concentrations of Na^+ and K^+; however, the fluid that is ultimately secreted after passage through the lower Malpighian tubules is rich in Na^+ but not K^+ [1]. Serotonin (1 μmol l^{-1}) stimulates reabsorption of KCl from the lower Malpighian tubules (Figure 1), thereby decreasing the amount of KCl secreted [3]. $RhoprDH_{31}$, on the other hand, does not stimulate KCl reabsorption from the lower Malpighian tubules (Donini, O'Donnell and Orchard, unpublished observations).

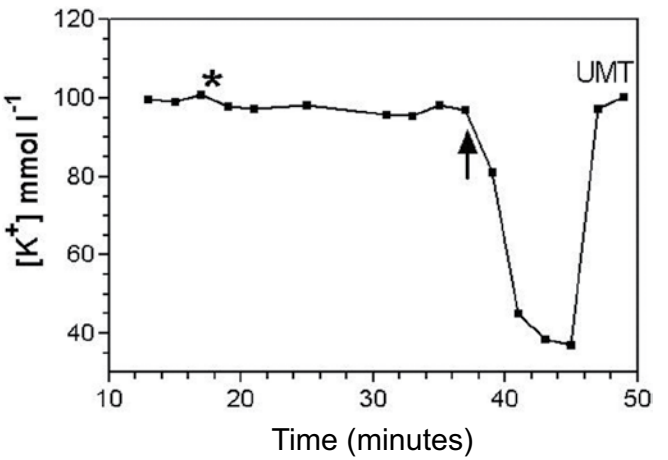

Figure 1. Malpighian tubules were removed from animals and a modified Ramsay secretion assay was utilized to collect secreted fluid. The secretory upper tubule was bathed in saline containing 24 mmol l^{-1} K^+ and stimulated with 1 μmol l^{-1} serotonin. The reabsorptive lower tubule was bathed in a separate droplet of saline containing 4 mmol l^{-1} K^+. The junction between the upper and lower tubule stretched between the two bathing droplets immersed in mineral oil. The K^+ concentration of secreted fluid collected after passage through the lower tubule is reported. The asterisk denotes the addition of 1 μmol l^{-1} ZooneDH to the lower tubule. ZooneDH does not stimulate K^+ reabsorption. The upward arrowhead denotes the addition of 1 μmol l^{-1} serotonin to the lower tubule leading to a rapid reabsorption of K^+. The junction between the upper and lower tubule was severed at approximately 45 mins and the K^+ concentration of fluid emerging from the upper Malpighian tubule (UMT) was measured.

Interestingly, although having differing effects on the secretion by Malpighian tubules, both serotonin and $RhoprDH_{31}$ are myotropic, stimulating an increase in frequency of contractions of hindgut and crop [2, 7, unpublished observations].

Lateral Neurosecretory Cells

Lateral neurosecretory cells (approx 5-6) of the MTGM co-localize CRF-related and kinin-like immunoreactivity [6, 8]. The CRF-related peptide, ZooneDH (see 1), mimics serotonin in stimulating maximum secretion by the upper Malpighian tubules [8-10]. The kinin, Leucokinin 1, has no diuretic activity on *R. prolixus* tubules. Although mimicking serotonin on secretion rate, ZooneDH (1 μmol l^{-1}) does not stimulate KCl reabsorption by the lower Malpighian tubules (Figure 1, Donini, O'Donnell and Orchard, unpublished observations), and neither does Leucokinin 1.

While having differing effects on upper Malpighian tubules, serotonin and co-localized peptides are each capable of increasing the frequency of contractions of hindgut and crop [2, 7, unpublished observations].

DISCUSSION

Why are there multiple diuretic factors?

It is possible that there are multiple diuretic factors because they each have different physiological effects and/or are released at different times. Although serotonin and ZooneDH have similar actions (probably identical) on the upper Malpighian tubules, it is important to note that ZooneDH cannot activate KCl reabsorption by the lower tubules, and neither can the co-localized peptides $RhoprDH_{31}$ or Leucokinin 1. During post-feeding diuresis there is a rapid release of serotonin from DUM cells within the first 5 minutes of gorging [10, 11] which serves to activate KCl reabsorption by the lower tubule and rapid fluid secretion by the upper tubule. The serotonin titres subsequently fall and are insufficient to maintain the high rates of fluid secretion by the upper tubules. It is possible that a native CRF-related peptide is released from the lateral neurosecretory cells at this time [6], which can maintain these high rates of secretion but which need not stimulate KCl reabsorption. This scenario also implies that the native CRF-related peptide might be used when diuresis is needed, but KCl reabsorption is not. This might occur at times of digestion of the red blood cells of the blood meal which could lead to a K^+ load that the insect needs to eliminate. Thus, this latter diuresis may be controlled by the native CRF-related peptide in the absence of serotonin.

Why co-localization?

The DUM cells co-localize the diuretic hormone serotonin and the very weakly diuretic peptide $RhoprDH_{31}$ [2]. It is possible, although yet to be demonstrated, that $RhoprDH_{31}$ might influence the actions of serotonin and thereby contribute to diuresis. In addition both of these factors have biological effects on other tissues that are associated with feeding. Thus, the frequency of contractions of the crop is increased following feeding and this increase in frequency might be mediated through serotonin and co-localized $RhoprDH_{31}$ [2, 7, unpublished observations]. Similarly, contractions of the hindgut are needed to void the urine, and the frequency of contractions of the hindgut is increased by both serotonin and $RhoprDH_{31}$. These factors, therefore, might be co-localized in order to produce complementary actions on feeding-related physiological events. It is also possible that there are other tissues that are responsive to serotonin but not to $RhoprDH_{31}$, and vice-versa.

In a similar manner the lateral neurosecretory cells co-localize a CRF-related DH, and a kinin-like peptide that has no diuretic activity. Again, both of these factors are capable of increasing the frequency of contractions of crop and hindgut and so might participate with serotonin and $RhoprDH_{31}$ in the overall control of feeding-related physiological events.

The possession of multiple diuretic hormones and co-localized factors allows for a flexibility of messaging in both timing and physiological responsiveness that might allow for a fine control of diuresis under differing osmotic challenges.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada. We are grateful to Prof. Michael O'Donnell for the use of unpublished observations.

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