The Journal of Experimental Biology 200, 2363–2367 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JEB1072

ANTI-DIURESIS IN THE BLOOD-FEEDING INSECT *RHODNIUS PROLIXUS* STÅL: THE PEPTIDE CAP_{2b} AND CYCLIC GMP INHIBIT MALPIGHIAN TUBULE FLUID SECRETION

MICHAEL C. QUINLAN[1], NATHAN J. TUBLITZ[2] AND MICHAEL J. O'DONNELL[1,*]

[1]*Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1* and [2]*Institute of Neuroscience, University of Oregon, Eugene, OR 97403, USA*

Accepted 24 June 1997

Summary

Rhodnius prolixus **eliminates NaCl-rich urine at high rates following its infrequent but massive blood meals. This diuresis involves stimulation of Malpighian tubule fluid secretion by diuretic hormones released in response to distention of the abdomen during feeding. The precipitous decline in urine flow that occurs several hours after feeding has been thought until now to result from a decline in diuretic hormone release. We suggest here that insect cardioacceleratory peptide 2b (CAP_{2b}) and cyclic GMP are part of a novel mechanism of anti-diuresis. Secretion rates of 5-hydroxytryptamine-stimulated Malpighian tubules are reduced by low doses of CAP_{2b} or cyclic GMP. Maximal secretion rates are restored by exposing tubules to 1 mmol l^{-1} cyclic AMP. Levels of cyclic GMP in isolated tubules increase in response to CAP_{2b}, consistent with a role for cyclic GMP as an intracellular second messenger. Levels of cyclic GMP in tubules also increase as urine output rates decline *in vivo*, suggesting a physiological role for this nucleotide in the termination of diuresis.**

Key words: *Rhodnius prolixus*, CAP_{2b}, cardioactive peptide, cyclic GMP, diuresis, anti-diuresis.

Introduction

The hematophagous insect *Rhodnius prolixus* (Reduviidae) is faced with a potentially serious problem every time it takes one of its infrequent but massive blood meals. Fluid gains of 10–12 times the unfed mass are typical, and replete *R. prolixus* are notably immobile and more vulnerable to predation by comparison with unfed insects. To minimize the duration of this post-prandial sluggishness, *R. prolixus* has evolved a quickly activated, high-output excretory system that unloads 50 % of the fluid mass of the meal and much of the excess NaCl within just a few hours. Indeed, the Malpighian tubules of this insect can, within a matter of minutes, increase their secretion rates by over 1000 times to levels as high as 50 nl cm^{-2} s^{-1} (Maddrell, 1991).

Tubule secretion is stimulated *in vivo* by 5-hydroxytryptamine (serotonin, 5-HT) acting synergistically with one or more neuropeptide diuretic hormones (DH) released in response to abdominal distention (Maddrell *et al.* 1993). Both DH and 5-HT are quickly inactivated or excreted *in vivo*, so maximum rates of urine formation depend on the continuous release of these molecules by the nervous system (Maddrell, 1964). Given the high rates of fluid and ion excretion, the cessation of urine production must also be tightly controlled to avoid possible dehydration and excessive loss of NaCl. No separate anti-diuretic signal has previously been identified in *R. prolixus*, and the rapid decline in urine flow that occurs 3–4 h after feeding has been assumed until now to result from a decline in DH and 5-HT release (Maddrell, 1964).

Stimulation of secretion from the tubules is mediated by cyclic AMP in *R. prolixus* and many other insects (Maddrell *et al.* 1971; Aston, 1975). The role of other second messenger systems (cyclic GMP, inositol trisphosphate/Ca^{+2}) in controlling secretion from Malpighian tubules has been largely unexamined. However, recent work by Dow and his colleagues shows that both cyclic GMP and cyclic AMP stimulate secretion in *Drosophila melanogaster* tubules (Dow *et al.* 1994; Davies *et al.* 1995). It has been suggested that both modulate a vacuolar-type H^+-ATPase (O'Donnell *et al.* 1996). Exogenous cyclic GMP is effective at doses as low as 3.5 μmol l^{-1} in *D. melanogaster*, and tubule levels of this nucleotide are elevated by both nitric oxide (NO) and a cardioactive peptide (CAP_{2b}) isolated originally from the hawkmoth *Manduca sexta* (Davies *et al.* 1995). CAP_{2b} belongs to a group of at least five peptides that collectively influence cardiac function and various stage-specific patterns of behaviour in *M. sexta* (Tublitz *et al.* 1991).

We have tested cyclic GMP and several known or potential stimulants of cyclic GMP synthesis [nitric oxide (NO), atrial natriuretic peptide (ANP), CAP_{2a}, CAP_{2b} and CAP_{2c}] on the secretory (upper) portion of Malpighian tubules from *R. prolixus*. We report here the unexpected finding that cyclic GMP

*Author for correspondence (e-mail: odonnell@mcmaster.ca).

and CAP_{2b} are powerful inhibitors of fluid secretion from these organs. CAP_{2b} depresses secretion in a dose-dependent manner and increases cyclic GMP levels in tubules. We also show with *in vivo* experiments that urine production is inversely related to tubule cyclic GMP levels. We conclude that CAP_{2b} and cyclic GMP form a novel anti-diuretic mechanism in *R. prolixus*.

Materials and methods

Secretion assays

Fluid secretion rates of isolated Malpighian tubules were determined using Ramsay tubule preparations similar to those described by Maddrell *et al.* (1988). Briefly, upper (distal) tubules from third-instar *R. prolixus* nymphs were mounted under paraffin oil in 100 µl saline droplets such that the open ends extended into the oil to allow collection of the secreted fluid. Drugs were then added to the bathing droplets, and the volume of secreted fluid was determined at fixed intervals with an ocular micrometer. Tubules were only partially stimulated with 5-HT (typically 50 % of the maximum rate; Maddrell *et al*. 1991) so that both stimulatory and inhibitory responses could be detected. Secretion rates were normalized using maximum rates obtained at the end of the experiments by exposing tubules to saturating levels of cyclic AMP.

Cyclic nucleotide determinations

Levels of cyclic GMP in tubules were determined using radioimmunoassay (RIA) kits from Amersham (RPA-525). Assays were conducted according to kit instructions except that reagent volumes were reduced by 50 %. Tubules to be analyzed were first placed in vials containing ice-cold 80 % methanol (Coast *et al.* 1991). Samples were then sonicated for 30 s, centrifuged for 10 min at 12 000 ***g***, and the supernatant dried down in a vacuum centrifuge. Dried samples were rehydrated using RIA assay buffer.

Reagent preparation and statistics

All drugs and peptides except the CAPs were obtained from Sigma, Inc. Synthetic CAP_{2a} and CAP_{2b} were obtained from Research Genetics (Huntsville, AL, USA). CAP_{2c} was obtained from HPLC-purified fractions of homogenates of the central nervous system (CNS) from the tobacco hawkmoth *Manduca sexta* using the protocol of Huesmann *et al.* (1995). In brief, abdominal portions of the ventral nerve cord were removed from pharate adult *M. sexta* and frozen at −80 °C for further processing. After a brief heat treatment (80 °C for 5 min), nerve cords were homogenized in 0.5 mol l^{-1} acetic acid and microfuged for 5 min. The supernatant was applied to a C-18 Sep-pak cartridge and eluted with step-wise increases in acetonitrile concentrations. CAP_{2c} elutes in the 60 % acetonitrile fraction and was subjected to further purification using a six-step HPLC protocol described in Huesmann *et al.* (1995). Secondary ion mass spectroscopic analysis of the CAP_{2c} fraction revealed a single resolvable peak.

Unless otherwise stated, all chemicals were dissolved in distilled water or saline, sampled and frozen until time for use. Peptide samples were lyophilized before storage at −80 °C. The NO donor, sodium nitroprusside, was prepared immediately before each experiment and stored in darkness.

All values are reported as means ± standard errors of the mean. Statistical comparisons were performed using Student's *t*-test; probabilities less than 0.05 were considered significant. When comparing normalized data, values were arcsine-transformed before applying *t*-tests.

Results

Both cyclic GMP and CAP_{2b} significantly inhibited secretion from *R. prolixus* tubules (Table 1). CAP_{2b} is clearly the more effective of the two (Table 1), and the threshold concentration for this substance is in the low nanomolar range (Fig. 1). The

Table 1. *Summary of experiments testing cyclic GMP and several agonists of cyclic GMP synthesis on Malpighian tubules from third-instar* Rhodnius prolixus *nymphs*

		Normalized secretion rates		
Compound	Concentration	Controls	Treated	Probability
Cyclic GMP	1 µmol l^{-1}	0.45±0.039 (11)	0.33±0.031 (11)	$P<0.05$
Cyclic GMP	10 µmol l^{-1}	0.45±0.039 (11)	0.16±0.020 (11)	$P<0.001$
SNP	10 µmol l^{-1}	0.70±0.058 (8)	0.66±0.055 (8)	NS
ANP	0.1 µmol l^{-1}	0.64±0.040 (8)	0.67±0.053 (8)	NS
CAP_{2a}	0.1 µmol l^{-1}	0.41±0.049 (7)	0.46±0.055 (7)	NS
CAP_{2b}	0.1 µmol l^{-1}	0.64±0.070 (8)	0.13±0.025 (8)	$P<0.001$
CAP_{2c}	0.5 NCE/V_B	0.45±0.065 (7)	0.55±0.066 (7)	NS

Tubules were partially stimulated with 50–60 nmol l^{-1} 5-HT (Lange *et al*. 1989; Maddrell *et al*. 1991) and given saline only (Controls) or test compound (Treated) at the start; 30 min later, all tubules were administered a saturating dose of cyclic AMP (2 mmol l^{-1}) so that stimulation of secretion was maximal. Values were then normalized using these maximal secretion rates.

Concentrations are either µmol l^{-1} or nerve cord equivalents per 100 µl bathing droplet (NCE/V_B) for CAP_{2c}, whose exact molecular mass is unknown.

Values are means ± S.E.M. for the number of tubules given in parentheses; NS, not significant.

SNP, sodium nitroprusside; ANP, atrial natriuretic peptide; CAP, cardioactive peptide.

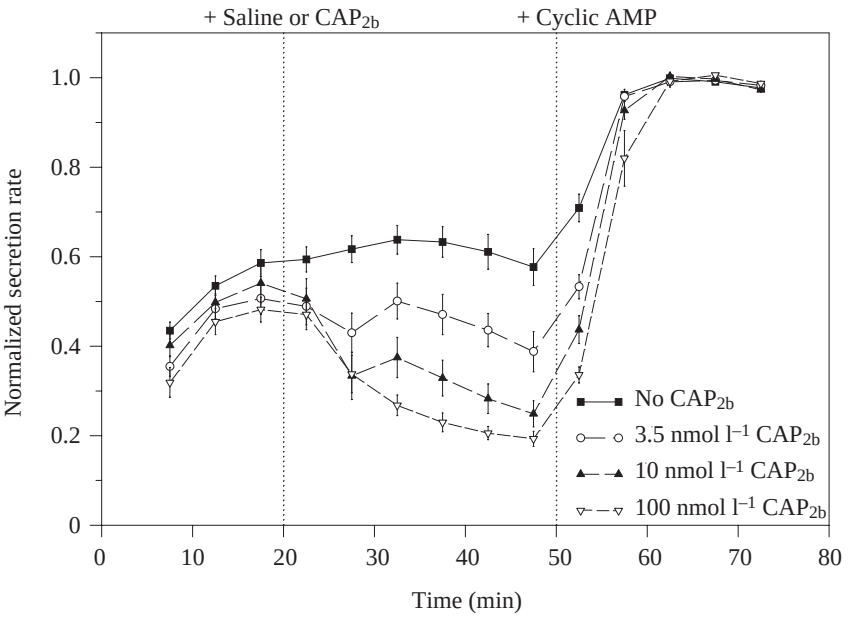

Fig. 1. Dose–response curves for CAP_{2b} action on tubule secretion rates. Tubules were partially stimulated with 60 nmol l^{-1} 5-HT and secretion rates allowed to stabilize for 20 min. Saline or CAP_{2b} was added at 20 min, and a saturating dose of cyclic AMP (2 $mmol^{-1}$) at 50 min to obtain maximum rates for normalizing the preceding values. Values are means ± S.E.M. for 8–12 tubules.

mechanism by which exogenous cyclic GMP inhibits secretion is considered in detail elsewhere (M. C. Quinlan and M. J. O'Donnell, in preparation). Note also in Fig. 1 that cyclic AMP completely reverses the inhibition (maximum rates for all groups were statistically indistinguishable). Neither sodium nitroprusside (10 µmol l^{-1}) nor human atrial natriuretic peptide (ANP; 0.1 µmol l^{-1}) affected secretion despite the comparatively high doses used in our experiments (Table 1). *D. melanogaster* tubules, for example, respond vigorously to 2 µmol l^{-1} sodium nitroprusside (Dow *et al.* 1994). With respect to ANP, 0.1 µmol l^{-1} is considered saturating for most vertebrate systems (Hamet *et al.* 1986).

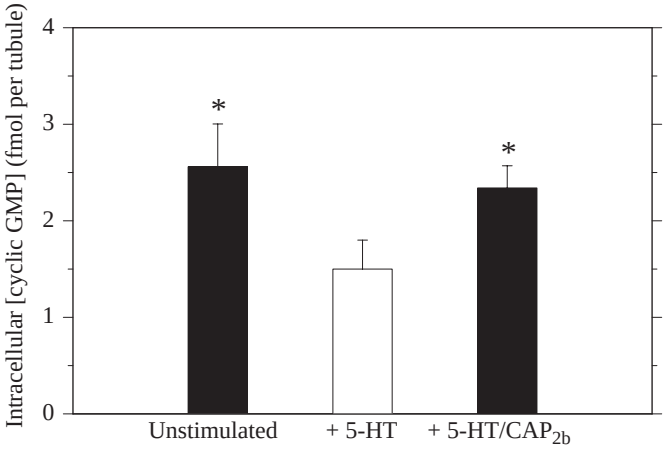

Fig. 2. Effects of CAP_{2b} on intracellular cyclic GMP levels in tubules. Treatment groups were unstimulated controls, stimulated (5-HT) controls, and stimulated tubules + 0.1 µmol l^{-1} CAP_{2b}. Neither CAP_{2b} nor 5-HT was added to unstimulated tubules. Stimulated tubules received 70 nmol l^{-1} 5-HT. All four upper tubules from each animal were incubated together in 100 µl of saline with appropriate treatments for 10 min. Reactions were terminated by adding ice-cold methanol. Values are means + S.E.M. for 12 or more tubules. Bars marked with an asterisk differ significantly (P<0.05) from the stimulated controls; cyclic GMP levels in 5-HT-stimulated tubules were significantly lower than in unstimulated tubules or in tubules stimulated with 5-HT and CAB_{2b}.

These experiments suggested that cyclic GMP may act as an intracellular second messenger for CAP_{2b}. This hypothesis was supported by the finding that exposure to CAP_{2b} *in vitro* significantly increased intracellular levels of cyclic GMP in isolated tubules (Fig. 2). Intracellular levels of cyclic GMP in isolated tubules also increased in response to an extract of the metathoracic ganglion (Fig. 3).

The foregoing experiments demonstrated that an increase in intracellular cyclic GMP concentration was associated with an inhibition of secretion from the upper region of the tubules *in vitro*. It was therefore of interest to determine whether cyclic GMP levels *in vivo* correlate with rates of urine formation, so we measured the cyclic GMP content of tubules at various points in the diuresis cycle (Fig. 4). Urine formation followed a time course similar to that described by Maddrell (1964). Rates peaked quickly and remained high for approximately 2 h, after which they fell steadily for 3–4 h. At peak output, these insects divested themselves of 16.4 % of the mass of their blood meal per hour (8.6 µl h^{-1}, or 1.35 times the unfed mass of the animals). The decline in urine output was correlated with a 56 % increase in tubule cyclic GMP content (Fig. 4).

Discussion

Our results demonstrate that low doses of cyclic GMP and CAP_{2b} suppress fluid secretion by Malpighian tubules stimulated with physiological doses of 5-HT. The inhibition can be reversed by application of sufficient concentrations of exogenous cyclic AMP. Tubule cyclic GMP content increases in response to CAP_{2b} treatment, indicating that cyclic GMP

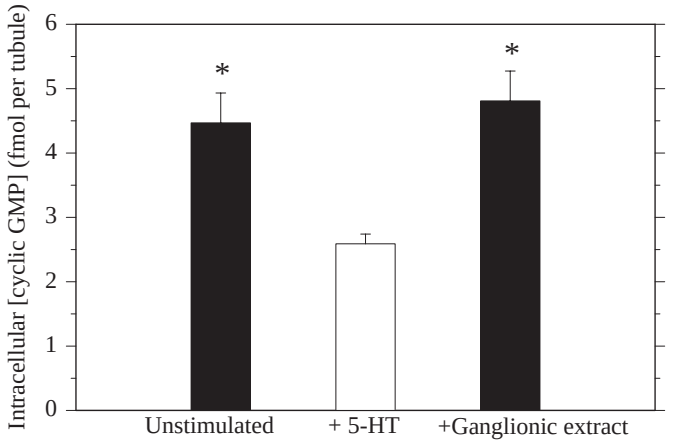

Fig. 3. Effects of ganglionic extract on intracellular cyclic GMP levels in tubules. Treatment groups were unstimulated controls, stimulated controls (5-HT) and stimulated tubules + ganglionic extract. Stimulated tubules received 70 nmol l^{-1} 5-HT. Ganglionic extract was prepared by sonication of isolated metathoracic ganglia in saline. All four upper tubules from each animal were incubated together in 100 µl of saline with appropriate treatments for 10 min. The ganglionic extract dosage was 0.5 ganglia per 100 µl of saline. Reactions were terminated by adding ice-cold methanol. Values are means + S.E.M. for 12 or more tubules. Bars marked with an asterisk differ significantly from the stimulated controls.

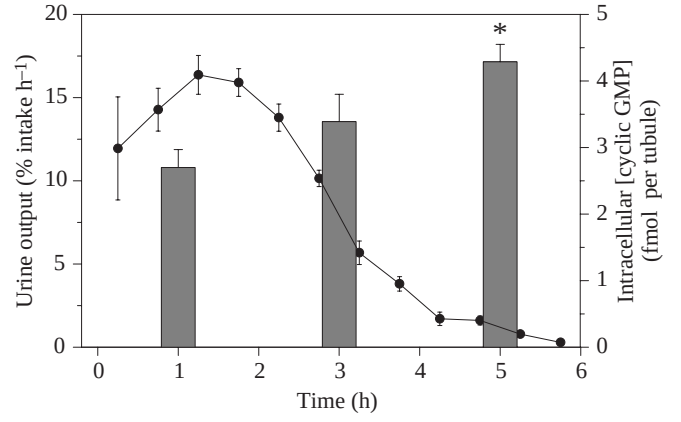

Fig. 4. Composite figure showing rates of urine formation (circles, means ± S.E.M.) and cyclic GMP levels (bars) of tubules taken from third-instar animals at various points in the diuresis cycle. Rates of urine formation were determined by weighing animals at 30 min intervals for 6 h immediately following feeding (N=8). Time 0 represents the termination of the 15–20 min feeding period. Urination rates are expressed as a percentage of the blood meal mass lost per hour. Tubules used for cyclic GMP determinations were removed quickly (<2 min) from donors under saline and immediately analyzed using radioimmunoassay (N=8). Histogram bars are positioned at the approximate time of tubule removal. The bar marked with an asterisk differs significantly from the stimulated controls (left-hand bar), i.e. from tubules removed from animals at the peak of diuresis.

functions as an intracellular second messenger for CAP_{2b}. Moreover, tubule cyclic GMP content increases as urine output from fed *R. prolixus* declines, consistent with a physiological role for cyclic GMP in the termination of diuresis.

A factor present in an extract of the metathoracic ganglionic mass also elevates tubule cyclic GMP content. The net effect of adding an extract will be a maintenance of or an increase in fluid secretion rate (cf. Maddrell, 1963), presumably because the large quantities of diuretic hormones present will mask the effects of factors leading to an increase in tubule cyclic GMP content. Nonetheless, our data indicate that a factor present within the CNS can elevate tubule cyclic GMP content, and that appropriate release of this factor into the haemolymph could counteract the effects of physiological levels of diuretic hormones and terminate diuresis.

The failure of NO to affect secretion by *R. prolixus* tubules is in contrast to the clear effect of NO on secretion rates of *D. melanogaster* tubules (Dow *et al.* 1994). Cyclic GMP synthesis is controlled by two classes of guanylate cyclase: a group of soluble enzymes stimulated by the highly diffusible NO (Schmidt *et al.* 1993), and a family of membrane-bound enzymes which are stimulated by peptide agonists such as ANP (Yuan and Garbers, 1992) and crustacean hyperglycaemic hormone (Goy, 1990). However, NO synthesis itself is frequently controlled, at least in vertebrates, by peptides (Schmidt *et al.* 1993). Hence CAP_{2b} might elevate cyclic GMP levels in *D. melanogaster* tubules by way of NO and in *R. prolixus* directly *via* a membrane-associated enzyme.

Our inclusion of ANP in the present study was motivated in part by its well-documented action *via* cyclic GMP in vertebrate systems and also because of early reports that plasma taken from dogs undergoing atrial distention caused inhibition of secretion from *R. prolixus* tubules (Kappagoda *et al.* 1979). In subsequent papers, Knapp *et al.* (1981*a*,*b*) were able to eliminate angiotensin II, bradykinin and vasopressin as potential mediators of this activity. At the time, ANP, a hormone responsible for hypervolaemic diuresis and natriuresis in mammals (Rosenzweig and Seidman, 1991) had not yet been identified and so escaped their attention. Pither *et al.* (1985) ultimately determined that the active factor had a low molecular mass (<1800 Da *versus* 3000 Da for ANP) and was lipophilic. This factor is unlikely to be ANP given these characteristics, and we have confirmed that ANP is without effect on secretion from upper Malpighian tubules. The inhibitory factor present in canine plasma remains unidentified.

The evidence presented here suggests that CAP_{2b}, or a related peptide, and cyclic GMP are part of an anti-diuretic mechanism in *R. prolixus*. Hormones with anti-diuretic effects on Malpighian tubules are rare in insects, however (Spring, 1990). Inhibition of Malpighian tubule fluid secretion by an anti-diuretic hormone has been demonstrated in crickets (Spring *et al.* 1988), but the identities of the hormone and its second messenger are unknown. Why does *R. prolixus* need a separate control system for reducing tubule secretion rates? In other words, why can termination of diuresis not be controlled simply through a reversal of the process that initiates diuresis? One reason is that haemolymph volume declines as diuresis progresses, and this will tend to elevate the concentration of

circulating diuretic hormones, thereby tending to stimulate the tubules further. In addition, the release of diuretic hormones is triggered by gross distention of the abdomen during feeding. However, plasticization of the cuticle during feeding will change the mechanical properties and dimensions of the cuticle. It is difficult to understand, therefore, how abdominal stretch receptors could be pre-set to permit subsequent detection of the appropriate reduced level of distention at which to signal the termination of diuretic hormone release. Even small errors in such an system could have deleterious consequences, since a 3% change in linear dimension corresponds to a 9% change in volume.

Our results raise several questions for further research on anti-diuresis. First, is the anti-diuretic response in *R. prolixus* mediated *in vivo* by CAP_{2b} or a similar compound? Antibodies against CAP_{2b} are already available (Tublitz *et al.* 1991), so it should be straightforward to test for the presence of this peptide within the nervous system of *R. prolixus*. Second, what could trigger the release of anti-diuretic hormone *in vivo*? One possibility is the haemolymph $[Na^+]$:$[K^+]$ ratio, which declines as the blood meal is processed (S. Maddrell, personal communication). Finally, are anti-diuretic factors present in other blood-feeders? We hope to address these problems in future studies.

References

Aston, R. J. (1975). The role of adenosine 3′,5′-cyclic monophosphate in relation to the diuretic hormone of *Rhodnius prolixus*. *J. Insect Physiol.* **21**, 1873–1877.

Coast, G. M., Cusinato O., Kay, I. and Goldsworthy, G. J. (1991). An evaluation of the role of cyclic AMP as an intracellular second messenger in Malpighian tubules of the house cricket, *Acheta domesticus*. *J. Insect Physiol.* **37**, 563–573.

Davies, S. A., Huesmann, G. R., Maddrell, S. H. P., O'Donnell, M. J., Skaer, N. J. V., Dow, J. A. T. and Tublitz, N. J. (1995). CAP_{2b}, a cardioacceleratory peptide, is present in *Drosophila* and stimulates tubule fluid secretion *via* cGMP. *Am. J. Physiol.* **269**, R1321–R1326.

Dow, J. A. T., Maddrell, S. H. P., Davies, S. A., Skaer, N. J. V. and Kaiser, K. (1994). A novel role for the nitric oxide–cGMP signaling pathway: the control of epithelial function in *Drosophila*. *Am. J. Physiol.* **266**, R1716–R1719.

Goy, M. F. (1990). Activation of membrane guanylate cyclase by an invertebrate peptide hormone. *J. biol. Chem.* **265**, 20220–20227.

Hamet, P., Tremblay, J., Pang, S. C., Skuherska, R., Schiffrin, E. L., Garcia, R., Cantin, M., Genest, J., Palmour, R., Ervin, F. R., Martin, S. and Goldwater, R. (1986). Cyclic GMP as mediator and biological marker of atrial natriuretic factor. *J. Hypertension* **4**, S49–S56.

Huesmann, G. R., Cheung, C. C., Loi, P. K., Lee, T. D., Swiderek, K. M. and Tublitz, N. J. (1995). Amino acid sequence of CAP_{2b}, an insect cardioacceleratory peptide from the tobacco hawkmoth *Manduca sexta*. *FEBS Lett.* **371**, 311–314.

Kappagoda, C. T., Knapp, M. F., Linden, R. J., Pearson, M. J. and Whitaker, E. M. (1979). Diuresis from left atrial receptors: effect of plasma on the secretion of the Malpighian tubules of *Rhodnius prolixus*. *J. Physiol., Lond.* **291**, 381–391.

Knapp, M. F., Linden, R. J. and Pearson, M. J. (1981*a*). Diuresis from stimulation of left atrial receptors: ADH and the Malpighian tubules of *Rhodnius prolixus*. *Q. J. exp. Physiol.* **66**, 333–338.

Knapp, M. F., Linden, R. J., Pearson, M. J., Pither, J. M. and Whitaker, E. M. (1981*b*). Diuresis from stimulation of left atrial receptors: initial purification steps from plasma of the causative agent. *Q. J. exp. Physiol.* **66**, 439–445.

Lange, A. B., Orchard, I. and Barrett, F. M. (1989). Changes in haemolymph serotonin levels associated with feeding in the blood-sucking bug, *Rhodnius prolixus*. *J. Insect Physiol.* **35**, 393–399.

Maddrell, S. H. P. (1963). Excretion in the blood-sucking bug, *Rhodnius prolixus* Stål. I. The control of diuresis. *J. exp. Biol.* **40**, 247–256.

Maddrell, S. H. P. (1964). Excretion in the blood-sucking bug, *Rhodnius prolixus* Stål. II. The normal course of diuresis and the effect of temperature. *J. exp. Biol.* **41**, 163–176.

Maddrell, S. H. P. (1991). The fastest fluid-secreting cell known: the upper Malpighian tubule cell of *Rhodnius*. *BioEssays* **13**, 357–362.

Maddrell, S. H. P., Herman, W. S., Farndale, R. W. and Riegel, J. A. (1993). Synergism of hormones controlling epithelial fluid transport in an insect. *J. exp. Biol.* **174**, 65–80.

Maddrell, S. H. P., Herman, W. S., Mooney, R. L. and Overton, J. A. (1991). 5-Hydroxytryptamine: A second diuretic hormone in *Rhodnius prolixus*. *J. exp. Biol.* **156**, 557–566.

Maddrell, S. H. P., Lane, N. J., Harrison, J. B., Overton, J. A. and Moreton, R. B. (1988). The initial stages in the action of an insecticidal δ-endotoxin of *Bacillus thuringiensis* var. *israelensis* on the epithelial cells of the Malpighian tubules of the insect *Rhodnius prolixus*. *J. Cell Sci.* **90**, 131–144.

Maddrell, S. H. P., Pilcher, D. E. M. and Gardiner, B. O. C. (1971). Pharmacology of the Malpighian tubules of *Rhodnius* and *Carausius*: the structure–activity relationship of tryptamine analogues and the role of cyclic AMP. *J. exp. Biol.* **54**, 779–804.

O'Donnell, M. J., Dow, J. A. T., Huesmann, G. R., Tublitz, N. J. and Maddrell, S. H. P. (1996). Separate control of anion and cation transport in Malpighian tubules of *Drosophila melanogaster*. *J. exp. Biol.* **199**, 1163–1175.

Pither, J. M., Knapp, M. F., Gregory, H. and Linden, R. J. (1985). The causative agent in atrial receptor diuresis in the dog is a low molecular weight, lipophilic and weakly acidic substance. *Clin. Sci.* **68**, 243–246.

Rosenzweig, A. and Seidman, C. E. (1991). Atrial natriuretic factor and related peptide hormones. *A. Rev. Biochem.* **60**, 229–255.

Schmidt, H. H. H., Lohmann, S. M. and Walter, U. (1993). The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. *Biochem. biophys. Acta* **1178**, 153–175.

Spring, J. H. (1990). Endocrine regulation of diuresis in insects. *J. Insect Physiol.* **36**, 13–22.

Spring, J. H., Morgan, A. M. and Hazelton, S. R. (1988). A novel target for anti-diuretic hormone in insects. *Science* **241**, 1096–1098.

Tublitz, N., Brink, D., Broadie, K. S., Loi, P. K. and Sylwester, A. W. (1991). From behavior to molecules: an integrated approach to the study of neuropeptides. *Trends Neurosci.* **14**, 254–259.

Yuan, P. S. T. and Garbers, D. L. (1992). Guanylyl cyclase-linked receptors. *A. Rev. Neurosci.* **15**, 193–225.