Pestycydy, 2008, (1-2), 87-94.
ISSN 0208-8703

The endocrine control of Malpighian tubule secretion: past, present and future

Geoffrey M. COAST

School of Biological and Chemical Sciences
Birkbeck (University of London),
Malet Street, London WC1E 7HX, UK
e-mail: g.coast@bbk.ac.uk

Abstract: Work by Ramsay in the 1950's established the foundations for our current understanding of primary urine production by Malpighian tubules and we now have detailed knowledge of the epithelial transport processes underlying fluid secretion. Identified neuropeptides and biogenic amines have been shown to stimulate fluid secretion and, for some, detailed information is available on the second messenger pathways and transport processes they activate. There are significant gaps in our knowledge, however, particularly concerning the role of identified diuretics *in vivo*. The hormonal status of many has yet to be established and it is also unclear whether they are used to control different types of diuresis.

Keywords: insects, primary urine formation, ion transport, diuretic hormones

INTRODUCTION

Primary urine production by insect Malpighian tubules differs from that in the mammalian kidney because it is not driven by blood pressure (ultrafiltration), but by a transepithelial osmotic gradient (osmotic filtration) generated by active transport of cations (K^+/Na^+) into the tubule lumen accompanied by a counterion (normally Cl^-). Divalent ions, amino acids and sugars enter the primary urine by passive diffusion whereas some toxic molecules are actively secreted into the lumen. This model for primary urine production was developed by Ramsay in the 1950's and has since been refined in terms of the transport processes involved (Figure 1).

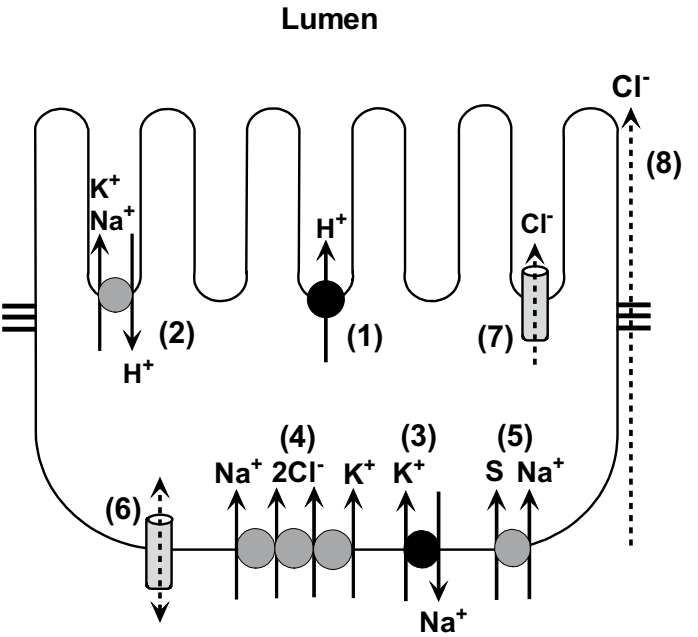

Figure 1. Major ion transport processes in Malpighian tubule principal cells. Active K^+/Na^+ secretion across the apical membrane involves a V-type ATPase (1) and cation/H^+ antiports (2). Cation uptake across the basal membrane is via an Na^+/K^+-ATPase (3), $Na^+/K^+/2Cl^-$ cotransport (4), Na^+-coupled organic solute transport (5), and ion channels (6). Chloride may reach the lumen either transcellularly, through channels in basal and apical membranes (6, 7) and $Na^+/K^+/2Cl^-$ cotransport (4), or follow a paracellular pathway (8). Solid circles indicate primary active transport processes. Dotted arrows are used to indicate passive diffusion through conductive pathways (ion channels) along electrochemical potential gradients.

Active K^+/Na^+ transport occurs through principal cells and is driven by a vacuolar-type (V-type) ATPase in the apical membrane [1]. The V-type ATPase transports H^+ into the lumen, establishing a proton gradient that drives the secondary active transport of K^+/Na^+ into the lumen via apical membrane cation/H^+ antiports. The transport of cations into the lumen establishes a favourable transepithelial gradient for passive Cl^- entry, which crosses the tubule wall through either a transcellular or paracellular pathway (see later).

Transport processes in the apical membrane are highly conserved, but there

is more variability at the basal membrane where cations and anions enter either through conductive pathways (ion channels) or via a variety of cotransporters and exchangers (Figure 1). The 'Ramsay model' for Malpighian tubule secretion emphasised the role of ion transport in generating a flow of primary urine with organic solutes entering by passive diffusion, but the transcriptome of *Drosophila* Malpighian tubules [2] has an abundance of mRNAs encoding organic solute transporters, emphasising their role in the removal of toxic compounds.

DIURETIC HORMONES

Much of Ramsay's work on primary urine production was with Malpighian tubules of the stick insect *Carausius morosus*. During these studies he developed a method for studying tubule secretion *in vitro* [3]. Tubules are severed at their connection with the gut and transferred individually to small drops of bathing fluid beneath paraffin oil. The cut end of the tubule is withdrawn from the bathing fluid into the surround paraffin oil where discrete droplets of secreted fluid form. The 'Ramsay assay' has since been used to study tubule secretion in insects as small as fruit flies and mosquitoes. Although Ramsay used a saline similar in composition to stick insect haemolymph the rate of secretion and survival of tubules in artificial bathing fluid was inferior to that in serum (haemolymph minus proteins), and he referred to an 'active principle' that was needed to support high rates of secretion [4]. Ramsay was unable to identify this factor and studied secretion by tubules bathed in a 3:1 mixture of saline and serum. Work with the blood sucking bug, *Rhodnius prolixus*, later showed the 'active principle' to be a diuretic hormone [5]. Tubules from newly fed insects secrete initially at a very high rate, but the rate falls dramatically over 150 min. High rates of secretion are restored by adding haemolymph from an insect fed a blood meal 1 h previously. The diuretic hormone is present in posterior-lateral cells of the fused mesothoracic ganglion mass and is released into the haemolymph from neurohaemal sites on the lateral abdominal nerves [5, 6]. A number of biogenic amines and neuropeptides have since been shown to stimulate tubule secretion (Table 1) and therefore are candidate diuretic hormones.

If all the diuretics listed are functional hormones the question arises as to why so many are needed to control primary urine production. One possibility is that different hormones stimulate different types of diuresis, and there is some evidence for this. A CRF-related peptide (Manse-DH) stimulates post-eclosion diuresis in lepidopterans [7], when large quantities of urine are voided so as to reduce haemolymph volume (and hence weight) prior to the first flight.

Serotonin stimulates a post-prandial diuresis in *Rhodnius* [8], during which large quantities of ions and water derived from the blood meal are voided. Terrestrial insects generally need to conserve water, however, and rarely, if ever, increase excretory water loss. Yet even desert dwelling insects have diuretics that accelerate secretion by isolated tubules. This is important because high rates of urine flow accelerate the clearance of toxic compounds from the circulation by minimising back diffusion of actively secreted solutes to the haemolymph [9]. Since virtually all of the primary urine is reabsorbed in the hindgut the diuretic here functions as a clearance hormone. An example of this is the CRF-related peptide (Locmi-DH) of the locust, *Locusta migratoria*, which is released into the haemolymph of feeding insects and accelerates the clearance of amaranth from the circulation [10, 11].

Table 1. Neuropeptides and biogenic amines that stimulate secretion by isolated Malpighian tubules

• Corticotropin-releasing factor (CRF)-related peptides
• Calcitonin (CT)-like peptides
• Kinins
• Cardioacceleratory peptides (CAP2b)
• Tachykinins
• Serotonin
• Tyramine

MODE OF ACTION OF DIURETIC HORMONES

The osmotic permeability (P_{osm}) of Malpighian tubules is high and a transepithelial osmotic gradient of a few milliosmolar supports primary urine formation [12]. Importantly, this study with *Rhodnius* tubules showed that serotonin does not increase P_{osm} although it accelerates fluid secretion 1000-fold. Diuretics therefore act by stimulating transepithelial KCl/NaCl transport and hence the movement of osmotically obliged water into the tubule lumen. They target various ion transport processes and there is considerable interspecific variation. In *Drosophila*, it is likely that three diuretics belonging to different peptide families (the CRF-related Drome-DH_{44}, the CT-like Drome DH_{31}, and the CAP_{2b} peptides CAPA-1 and 2) stimulate V-type ATPase activity [13, 14]. Drome-DH_{44} and Drome-DH_{31} act via cAMP [15, 16], and they may increase V-type ATPase activity by promoting re-assembly of the V_1V_O holoenzyme from

its constituent parts, the cytoplasmic V_1 catalytic complex and the membrane-bound V_O complex, which forms a proton channel. This has been shown in blowfly (*Calliphora vicina*) salivary glands where serotonin stimulates secretion through a cAMP-protein kinase A (PKA) signalling pathway by promoting assembly of the V_1V_O holoenzyme [17]. Recent work with *Manduca* salivary glands has shown that subunit C is the only component of the V-type ATPase to be phosphorylated by PKA, and this promotes re-assembly of the holoenzyme [18]. In contrast, CAPA-1 acts via cGMP and Ca^{2+}, and has relatively little effect on recruiting V_1 to the apical membrane [3]. It causes a rapid (<1 s) elevation in intracellular Ca^{2+} levels, followed by a slower increase. The latter is tracked by mitochondrial Ca^{2+} and causes increased mitochondrial membrane polarisation, which is associated with elevated ATP production [19]. Apical mitochondria are selectively targeted by CAPA-1 and the increased supply of ATP to the V-type ATPase elevates proton transport and hence K^+/Na^+ secretion.

The CRF-related and CT-like peptides of *Drosophila* probably have identical actions, but in the mosquitoes *Anopheles gambiae* and *Aedes aegypti* their actions differ, although both act via cAMP [13]. Stimulation of secretion by the CT-like Anoga-DH_{31} is accompanied by a marked natriuresis due to the opening of a Na^+ conductance in the principal cell basal membrane, as evidenced by depolarisation of the membrane potential (V_{basal}). In contrast, the diuretic response to the CRF-related Anoga-DH_{44} is not associated with an increase in the secreted fluid $[Na^+]:[K^+]$ ratio, which remains close to unity, and V_{basal} changes in a triphasic fashion, with a transient hyperpolarisation, before briefly depolarising and then returning to close to the initial membrane potential. The complex change in V_{basal} suggests a number of transport processes are activated and this likely involves both cAMP- and Ca^{2+}-dependent actions [13].

In contrast to the diuretics referred to above, insect kinins stimulate secretion by opening a Cl^- conductance pathway, allowing increased transport of KCl/NaCl into the lumen along with osmotically obliged water [1]. Work with two dipteran insects, *Drosophila* and *Aedes*, shows kinins cause the Ca^{2+}-dependent opening of a Cl^- conductance pathway. In *Drosophila*, kinins act on a second cell type, stellate cells, to open a transcellular conductance pathway [20], while in *Aedes* they act on principal cells and open a paracellular conductance [21].

The mechanism of kinin action on tubules from non-dipteran insects has been less well studied, and work with the house cricket, *Acheta domesticus*, is instructive because they lack stellate cells. As in dipteran insects, kinin stimulation has a non-selective effect on Na^+ and K^+ transport and is Cl^- dependent; tubules secrete slowly in low Cl^- saline and do not respond to kinin stimulation [22]. As in dipteran insects, kinin stimulation results in the lumen-positive transepithelial

potential collapsing, which is also Cl^- dependent because it is reversed in low Cl^- saline. Recent work has demonstrated a high K^+ conductance in the basal membrane, which when blocked by barium reveals a smaller Cl^- conductance (G.M. Coast, unpublished observations). Ions move through conductance pathways down their electrochemical potential gradient, which can be calculated from their activity in the cytoplasm and bathing fluid, and V_{basal}. Double-barrelled ion selective microelectrodes (ISEs) are used to measure intracellular ion activities, but have yet to be used in cricket tubules. However, voltages recorded with single barrelled ISEs give some insight into intracellular ion activities once corrected using separate measurements of V_{basal}. Moreover, kinin stimulation has no effect of V_{basal} [22] so a change in voltage recorded by the ISE represents a change in intracellular ion activity. Despite the limitations of this approach, it reveals a favourable gradient for Na^+ movement from bath to cell, whereas K^+ and Cl^- are close to electrochemical equilibrium (G. M. Coast, unpublished observations). Focussing on Cl^- movement, kinin stimulation in normal saline has little effect on $[Cl^-]_{cell}$. Addition of the epithelial Cl^- channel blocker NPPB (1 μM) produces a small drop in $[Cl^-]_{cell}$, which falls further immediately after kinin stimulation, but recovers within 1 min. The initial fall in $[Cl^-]_{cell}$ is consistent with NPPB blocking Cl^- uptake from the bath, but must be confirmed by precise measurement of the electrochemical gradient using double-barrelled ISEs. The transient fall in $[Cl^-]_{cell}$ after kinin stimulation suggests it opens an apical Cl- conductance, which is consistent with depolarisation V_{apical}; there is a large electrochemical gradient favouring Cl^- movement from cell to lumen. Recovery of $[Cl^-]_{cell}$ in the continued presence of NPPB can be explained by Cl^- uptake via a $Na^+/K^+/2Cl^-$ cotransporter in the basal membrane. The basal membrane Cl^- conductance may be constitutively open since V_{basal} is unchanged after kinin stimulation.

ROLE OF DIURETIC HORMONES *IN VIVO*

The term diuretic hormone has been applied to factors that stimulate tubule secretion in the 'Ramsay assay', but evidence for these diuretics being circulatory hormones is based largely on their immunohistochemical localisation to neurosecretory cells and neurohaemal sites. Reference has already been made to the hormonal function of Manse-DH in controlling a post-eclosion diuresis [7], for serotonin controlling the post-prandial diuresis of *Rhodnius* [8], and for Locmi-DH acting as a clearance hormone in *Locusta* [10, 11], and recent work has shown the CT-like peptide Anoga-DH_{31} is a natriuretic hormone in *Anopheles*

[22]. In this study, an acute salt and water load was delivered by injecting 1 μl of 0.9% NaCl. Approximately 73% of the injected volume load was voided by 4 h post injection and this was accompanied by a natriuresis as evidenced by the loss of almost 2.5 times more Na^+ than K^+ from the body. In contrast, insects injected with 1 μl of a sucrose solution isosmotic to 0.9% NaCl excreted just 44% of the volume load after 4 h and lost 1.3 times more K^+ than Na^+. Thus, in both instances the volume load evokes a diuresis, but only with a salt load is this accompanied by natriuresis, implying that different diuretic hormones are involved. The only diuretic shown to stimulate natriuresis by isolated tubules is Anoga-DH_{31} and it is likely that it is released in response to the injected salt and water load. To test this, mosquitoes were injected with 1 μl of 0.9% NaCl containing a 1:50 dilution of an antiserum raised against the CT-like peptide of the Pacific beetle cockroach, *Diploptera punctata*. After 4 h these the insects voided only 53% of the volume load and, importantly, they lost 1.7 times more K^+ than Na^+ [22]. Thus immunoneutralization of the CT-like peptide abolished the natriuretic response to the injection of 0.9% NaCl alone, showing Anoga-DH_{31} stimulates natriuresis *in vivo*.

CONCLUSIONS

Fifty years have elapsed since the pioneering work of Ramsay on Malpighian tubule secretion. We now understand the underlying ion transport processes and have an increased awareness of the contribution organic solute transporters make to the clearance of toxic compounds from the haemolymph. The 'active principle' that sustains high rates of secretion by isolated tubules is known to be a neurohormone and a number of candidate biogenic amines and neuropeptides have been identified as putative diuretic hormones. Antidiuretic peptides that act on Malpighian tubules have also been identified (see Orchard *et al*. in this volume). The mode of action of identified diuretics has been investigated in a small number of species, and there are interspecific differences even in insects from the same Order. Questions for the future include elucidating the role(s) of different diuretics *in vivo*, which may not be restricted to regulating primary urine production since some have been implicated in the control of ecdysis behaviour (see Zitnan *et al.* in this volume). Finally, it remains to be seen whether the hormonal control of tubule secretion is a valid target for the development of novel insecticides [24].

REFERENCES

[1] Beyenbach K.W., J. Exp. Biol., 2003, 206, 3845-3856.
[2] Wang J., Kean L., Yang J.L., Allan A.K., Davies S.A., Herzyk P., Dow J.A.T., Genome Biol., 2004, 5, R69.
[3] Ramsay J.A., J. Exp. Biol., 1954, 31, 104-113.
[4] Ramsay J.A., *ibid.,* 1956, 33, 697-708.
[5] Maddrell S.H.P., J. Exp. Biol., 1963, 40, 247-256.
[6] Maddrell S.H.P., *ibid*., 1966, 45, 499-508.
[7] Kataoka H., Troetschler R.G., Li J.P., Kramer S.J., Carney R.L., Schooley D.A., Proc. Natl. Acad. Sci. USA, 1989, 86, 2976-2980.
[8] Maddrell S.H.P., Herman W.S., Mooney R.L., Overton J.A., J. Exp. Biol., 1991, 156, 557-566.
[9] Maddrell S.H.P., *ibid*., 1981, 90, 1-15.
[10] Audsley N., Goldsworthy G.J., Coast G.M., Peptides, 1997, 18, 59-65.
[11] Patel M., Hayes T.K., Coast G.M., J. Exp. Biol., 1995, 198, 793-804.
[12] O'Donnell M.J., Aldis G.K., Maddrell S.H.P., Proc. R.. Soc. Lond. B, 1982, 216, 267-277.
[13] Coast G.M., Garside C.S., Webster S.G., Schegg K.M., Schooley, D.A., J. Exp. Biol., 2005, 208, 3281-3291.
[14] O'Donnell M.J., Dow J.A.T., Huesmann G.R., Tublitz N.J., Maddrell S. H.P., *ibid*., 1996, 199, 1163-1175.
[15] Cabrero P., Radford J.C., Broderick K.E., Costes L., Veenstra J.A., Spana E.P., Davies S.A., Dow J.A.T., *ibid*., 2002, 205, 3799-3807.
[16] Coast G.M., Webster S.G., Schegg K.M., Tobe S.S., Schooley D.A., *ibid*., 2001, 204, 1795-1804.
[17] Dames P., Zimmermann B., Schmidt R., Rein J., Voss M., Schewe B., Walz B., Baumann O., Proc. Natl. Acad. Sci. USA, 2006, 103, 3926-3931.
[18] Voss M., Vitavska O., Walz B., Wieczorek H., Baumann O., J. Biol. Chem., 2007, 282, 33735-33742.
[19] Terhzaz S., Southall T.D., Lilley K.S., Kean L., Allan A.K., Davies S.A., Dow J.A.T., *ibid*., 2006, 281, 18849-18858.
[20] O'Donnell M.J., Rheault M.R., Davies S.A., Rosay P., Harvey B.J., Maddrell S.H.P., Kaiser K., Dow J.A.T., Am. J. Physiol., 1998, 274, R1039-R1049.
[21] Yu M.-J., Beyenbach K. W., *ibid*., 2002, 283, F499-F508.
[22] Coast G.M., Nachman R.J., Schooley D.A., J. Exp. Biol., 2007, 210, 3979-3989.
[23] Coast G.M., Gen. Comp. Endocrinol., 2007, 152, 332-338.
[24] Nachman R.J., Fehrentz J.-A., Martinez J., Kaczmarek K., Zabrocki J., Coast G.M., Peptides, 2007, 28, 146-152.