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Review

Inorganic and organic anion transport by insect renal epithelia

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Abstract

Insect renal organs typically exhibit high rates of transport of inorganic and organic anions, and therefore provide useful models for the study of epithelial anion transport and its control. Isolated Malpighian tubules of some species secrete a volume of iso-osmotic fluid equal to their own volume in 10–15 s, which means that cellular Cl^- content is exchanged every 3–5 s. Anion transport can also be achieved against extreme thermodynamic gradients. The concentration of K^+ and Cl^- in the lumen of the Malpighian tubules of some desert beetles approaches or exceeds saturation. A basolateral Na^+:K^+:2Cl^- cotransporter plays an important role in vectorial ion transport in Malpighian tubules of many species, but there is also evidence for coupling of Cl^- transport to the movement of a single cationic species (Na^+ or K^+). Although an apical vacuolar H^+-ATPase plays a primary role in energizing transepithelial secretion of chloride via channels or cotransporters in the secretory segment of the Malpighian tubule, several different ATPases have been implicated in reabsorption of Cl^- by the lower Malpighian tubule or hindgut. Chloride transport is known to be controlled by several neuropeptides, amines and intracellular second messengers. Insect renal epithelia are also important in excretion of potentially toxic organic anions, and the transporters involved may play a role in resistance to insecticides of natural or anthropogenic origin.

Keywords: Malpighian tubule; Hindgut; Chloride channel; Cation-coupled cotransport; Patch clamp; Ion-selective microelectrode

1. Introduction

1.1. Insect epithelia as models for the study of epithelial anion transport

For studies of epithelial anion transport, insects provide numerous illustrations of August Krogh's principle that "For many problems there is an animal on which it can be most conveniently studied". The major excretory epithelia in insects are the Malpighian tubules and hindgut, which act in concert to form the functional kidney. The most striking features of insect Malpighian tubules are their dramatic rates of ion and water transport, particularly so in species which deal with fluid-rich diets. Fully stimulated Malpighian tubules of the blood-feeder *Rhodnius prolixus* or the fruit fly *Drosophila*, for example, can secrete a volume of iso-osmotic fluid approximately equal to their own volume every 10–15 s [1,2]. Given that intracellular Cl^- activity is typically about one third of that in the bathing media, this means that cellular Cl^- content is exchanged every 3–5 s. The primary urine is formed not by ultrafiltration, as in most vertebrate kidneys, but by a process of secretion, whereby active ion transport drives the flow of osmotically obliged water [1]. Another important contrast with vertebrate epithelia is a preeminent role of an apical vacuolar type proton ATPase in energizing Malpighian tubule ion transport and a subordinate or negligible role for the basolateral Na^+/K^+ ATPase [3]. Both chloride channels and alkali cation-coupled chloride transporters are implicated in transcellular movement of chloride.

Insects may experience differences in the degree of potassium and sodium loading through the diet, and therefore provide useful model systems for studies of cation-selectivity of cation-coupled chloride cotransport. For example, the mosquito *Aedes aegypti* and blood feeding hemipterans such as *Rhodnius* must deal primarily with the excretion of sodium and chloride in the few hours following a large blood meal and then with excretion of potassium, albeit at slower rates, as the red blood cells are broken down in the gut in the hours and days after the blood meal [4,5]. Secretion of fluid and ions by Malpighian

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tubules may be followed by reabsorption of water, ions or useful metabolites. Reabsorption may occur downstream either in a proximal segment of the Malpighian tubules [6,7] or in the hindgut [8].

Insect Malpighian tubules also provide useful model systems for studies of hormonal and intracellular second messenger systems that regulate epithelial transport. Urine formation in insects may be modulated by both diuretic and antidiuretic factors. Moreover, in *Drosophila* tubules there is clear evidence for separate control of cation and anion transport by distinct neuropeptide and second messenger systems [9]. Surprisingly, hormones which stimulate fluid secretion by isolated Malpighian tubules are found even in desert species where there is no need for a diuretic factor. In these cases, the term clearance hormone may be more appropriate than diuretic hormone, since the function of the hormone is presumably to increase the cycling of water through the excretory system, and thereby augment the clearance of waste products and toxins [10].

Antidiuretic hormones in insects can act in one of two ways. They may act by decreasing ion transport and associated fluid secretion by the Malpighian tubules, or they may stimulate reabsorption of ions and water across the hindgut [8,11]. The Malpighian tubules of *Rhodnius* provide a useful model for studies of both diuretic and antidiuretic effects. Moreover, the mechanisms and control of the secretion of Cl^- by the upper Malpighian tubule of *Rhodnius* [12] and the reabsorption of Cl^- by the lower Malpighian tubule [13,14] can be studied in a single whole isolated tubule.

Another striking feature of insect epithelia is the ability to transport ions against dramatic electrochemical gradients. In tenebrionid beetles the distal ends of the Malpighian tubules are applied to the rectal epithelia to form a cryptonephridial complex. In the lumen of the cryptonephridial Malpighian tubules of *Tenebrio molitor*, K^+, Na^+, Cl^- are accumulated to produce osmotic gradients of 6.8 osmol [15]. These osmotic gradients serve to minimize water loss by recovering water from the fecal material, and also to provide for a net gain of water through the process of atmospheric water vapor absorption. Another striking example of the extent to which insect epithelia can concentrate ions is evident in the cryptonephridial tubules of the larvae of the Namibian beetle *Onymacris* [16]. In this species, K^+ and Cl^- are accumulated in the tubule lumen to such an extent that they apparently form a supersaturated solution (i.e. >4500 mmol l^{-1}) [16].

Our understanding of hindgut function owes much to the elegant and extensive studies of the hindgut in locusts and mosquitoes by Phillips and co-workers over recent decades. Most of their studies have dealt with the migratory locust *Schistocerca gregaria*, and this material has been reviewed in detail elsewhere [8]. KCl is reabsorbed by the locust hindgut against large opposing electrochemical gradients during the dehydration of the fecal material [8]. Transepithelial chloride transport, measured as short-circuit current in Ussing chambers, is stimulated by the ion transport peptide (ITP) and chloride transport stimulating hormone (CTSH) via cAMP as second messenger. Chloride transport is not coupled to, or driven secondarily by, fluxes of Na^+, K^+, HCO_3^-, Ca^{2+}, or Mg^{2+}. An apical V-type H^+-ATPase acidifies the hindgut lumen but H^+ flux is only 10–15% of Cl^--dependent short-circuit current. It is unclear whether the resulting large apical proton gradient is used to drive Cl^- transport secondarily by an apical H^+/Cl^- symport, or if there is a primary active Cl^- pump (i.e. Cl^- ATPase) in this epithelium. Hormonal control of the *Schistocerca* hindgut is clearly separated from that of the Malpighian tubules [17]. ITP has no stimulatory action on fluid secretion by isolated Malpighian tubules of *S. gregaria*, nor does it act synergistically or antagonistically in combination with locustakinin (Lom-K) or Locusta-diuretic hormone (Locusta-DH). Conversely, stimulants of locust Malpighian tubules (Lom-K and Locusta-DH) have no effect on active Cl^- transport or the rate of fluid reabsorption across *Schistocerca* ilea and recta in vitro [17].

The excretory systems of many insects also transport organic anions at impressive rates [18–20]. This capacity is perhaps not surprising given the co-evolution of insects with flowering plants that produce secondary compounds to protect them from herbivory. The ability of insects to render harmless or to excrete an ingested xenobiotic allows them to live on such plants. The xenobiotic may, in fact, become an attractant for an insect species which adapts itself to it.

This paper examines recent advances in our understanding of inorganic and organic anion transport primarily by the functional renal system of insects consisting of the Malpighian tubules and the hindgut. Both similarities and differences with functionally analogous vertebrate epithelia will be noted. The first sections below examine mechanisms of chloride transport in Malpighian tubules composed of a single cell type (*Rhodnius*). These mechanisms will be contrasted with those in tubules containing two cell types, the primary and secondary or stellate cells (*Drosophila*, *A. aegypti*). In addition, the mechanisms and electrochemical driving forces for basolateral Na^+:K^+:2Cl^- cotransport during ion secretion by Malpighian tubules will be contrasted with cotransporters involving a single cationic species (Na^+ or K^+).

1.2. Malpighian tubule ion transport in insects faced with Na^+ loading: tertiary active chloride transport by the secretory segment of the Malpighian tubules of the blood feeding insect R. prolixus

Most insect species are phytophagous and thus deal with diets rich in K^+. By contrast, the hemipteran *R. prolixus* must deal with a Na^+ load imposed by feeding on blood which is also hypo-osmotic to its own body fluids. After a blood meal exceeding 10 times the unfed body mass, there is a dramatic postprandial diuresis which results in the elimination of a NaCl-rich urine by a two-step process. The upper Malpighian tubule first secretes a fluid containing approximately equimolar NaCl and KCl. Potassium and chloride, but not

water, are subsequently re-absorbed by the lower Malpighian tubule, resulting in elimination of a hypo-osmotic urine enriched in NaCl. Fig. 1A shows the current model of ion transport across stimulated Malpighian tubules of *R. prolixus* [12–14]. Ion transport can be stimulated 1000-fold by the diuretic factors 5-hydroxytryptamine and a peptide related to corticotropin releasing factor (CRF) [21].

Stimulation with the diuretic hormone serotonin produces a characteristic triphasic change in transepithelial potential (TEP). The TEP initial value is approximately −25 mV, lumen negative in unstimulated tubules. Upon stimulation with serotonin the TEP shifts to ∼ −33 mV in phase 1, ∼ +30 mV in phase 2 and −32 mV in phase 3 [22]. Each of the three phases of the electrical signature is attributed to the activation of a particular ion transporter (Fig. 2). There are only minor changes in basolateral membrane potential in response to serotonin, so the changes in transepithelial potential primarily reflect changes in apical membrane potential.

Ion substitution and pharmacological experiments suggest that the three phases of the response of transepithelial potential to serotonin correspond to sequential activation of an apical chloride channel, an apical vacuolar type H^+ ATPase and a basolateral Na^+:K^+:2Cl^- cotransporter (Figs. 1B and 2). The apical membrane potential reflects contributions of the V-type H^+-ATPase, tending to drive the lumen to more a positive electrical potential, and compensating move-

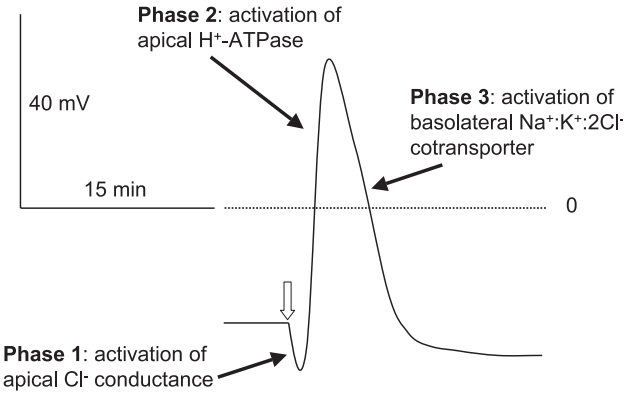

Fig. 2. Schematic diagram showing the triphasic response of transepithelial potential (TEP) to stimulation with serotonin (10^{-6} mol l^{-1}) or cAMP (10^{-3} mol l^{-1}) in the upper Malpighian tubules of *R. prolixus*. The open arrow indicates the addition of the stimulant. The pre-stimulation potential and the three different phases of the TEP response are indicated. The bath potential (0 mV) is indicated by the dotted line.

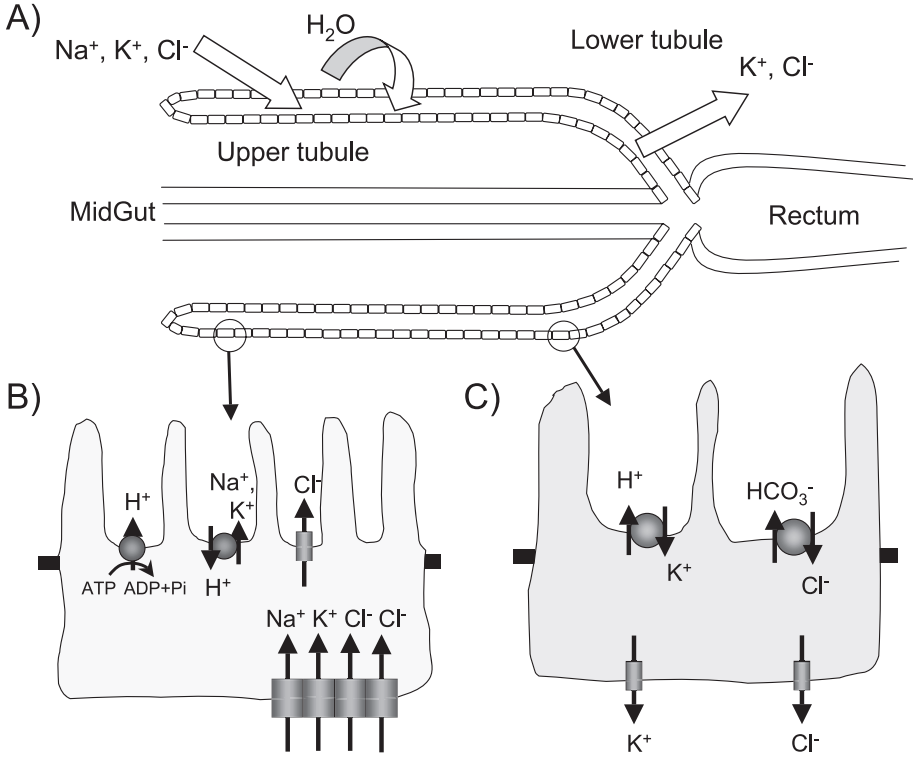

Fig. 1. Schematic diagram summarizing the current working hypotheses for ion transport by the Malpighian tubules of *R. prolixus*. (A) Diagram showing the two segments of the *Rhodnius* Malpighian tubule. The upper (distal) tubule is responsible for secretion of Na^+, K^+, Cl^- and osmotically obliged water. The lower tubule reabsorbs K^+, and Cl^- but not water. (B) Schematic diagram of the putative ion transporters involved in vectorial ion and fluid transport by Malpighian tubule cells of the secretory segment. Ion secretion is driven by an apical vacuolar-type (V-type) H^+-ATPase, which pumps protons from cell to lumen, thereby providing the driving force for secondary movement of Na^+ and/or K^+ from cell to lumen through Na^+/H^+ and K^+/H^+ exchangers. The pump also establishes a favourable electrical gradient for movement of Cl^- from cell to lumen through putative Cl^- channels. Entry of Na^+, K^+, and Cl^- into the upper tubule cell is through a bumetanide-sensitive Na^+:K^+:2Cl^- cotransporter. (C) Schematic diagram of the putative ion transporters in the lower (proximal) reabsorptive segment of the tubule. K^+ reabsorption is via an omperazole-sensitive apical H^+/K^+-ATPase and Ba^{+2}-sensitive K^+ channels. Cl^- moves from lumen to cell through a stilbene-insensitive Cl^-/HCO_3^- exchanger and exits the cell through basolateral Cl^- channels.

ment of Cl^- from cell to lumen, tending to drive the lumen to a more negative potential. Stimulation of tubules in Cl^--free bathing saline abolishes both phases 1 and 3 of the response to serotonin. Pre-incubation with bumetanide in Cl^--replete saline blocks phase 3 of the response to serotonin, but does not alter phases 1 and 2. Taken together, these results suggest that phase 1 of the triphasic response corresponds to an increase in apical membrane chloride permeability. Although the basolateral Na^+:K^+:2Cl^- cotransporter is electroneutral and has no direct effect on basolateral membrane potential and transepithelial potential, it indirectly affects apical membrane potential by increasing the availability of cellular Cl^- for movement from cell to lumen during phase 3.

The primary intracellular second messenger involved in the response to serotonin is cAMP, and isolated tubules can be stimulated to secrete at the maximal rate by addition of exogenous cAMP to the bathing saline. Moreover, addition of cyclic AMP to the saline bathing unstimulated tubules produces a triphasic change in transepithelial potential identical to that produced by serotonin (Ianowski and O'Donnell, unpublished observations). Pre-incubation of tubules in Cl^--free saline shows that phase 2, the activation of the H^+ ATPase, is in fact initiated within 7 s of the addition of serotonin [22]. This finding suggests that the production of intracellular cAMP in response to stimulation with serotonin is a relatively rapid event. In saline containing control levels of chloride (158.6 mmol l^{-1}), the change in transepithelial potential in the positive direction produced by activation of the apical H^+-ATPase is masked by the increase in chloride permeability during phase 1 of the triphasic response to serotonin.

It is worth pointing out that a paracellular pathway for chloride is not feasible for the Malpighian tubules of *Rhodnius*. Chloride concentration is 180 mmol l^{-1} in the lumen and 158.6 mmol l^{-1} in the bath and the transepithelial potential is lumen-negative. As a result, chloride movement from bath to lumen is clearly against both chemical and electrical gradients. By contrast, the lumen-positive transepithelial potential in tubules of other insect species (discussed below) means that Cl^- may move, at least in theory, by either a transcellular or paracellular route.

Simultaneous measurement of intracellular ion activity and basolateral membrane potential with double-barrelled ion-selective microelectrodes provides evidence for the thermodynamic feasibility of a Na^+:K^+:2Cl^- cotransporter in the basolateral membrane of *Rhodnius* tubules (Fig. 3). The bafilomycin-sensitive H^+ pump (Fig. 1B) creates a H^+ gradient across the apical membrane, and this gradient energizes secondary active transport of K^+ and Na^+ from the cell to the lumen through amiloride-sensitive alkali cation/proton exchangers. The reduction in intracellular Na^+ activity creates a large electrochemical gradient favouring Na^+ entry into the cell across the basolateral membrane [12]. The Na^+ gradient then drives uphill tertiary active movement of Na^+, K^+ and Cl^- into the cell through a process involving a bumetanide sensitive Na^+:K^+:2Cl^- cotransporter (Fig. 3). Application of bumetanide to stimulated Malpighian tubules decreases transepithelial fluxes of Na^+ and K^+ and Cl^-. There is an associated decline in intracellular Cl^- activity. Calculation of electrochemical potentials shows that a Na^+:Cl^- cotransporter is also thermodynamically feasible, whereas a K^+:Cl^- cotransporter would result in movement of K^+ and Cl^- from cell to bath (Fig. 3). In addition, measurements of intracellular Cl^- activity suggest that basolateral Cl^- entry is dependent upon the presence of both Na^+ and K^+ in the bathing saline. Removal of either cation results in a decrease in intracellular Cl^- activity. Taken together these results are consistent with a major role of the Na^+:K^+:2Cl^- cotransporter in vectoral ion transport in serotonin-stimulated Malpighian tubules of *Rhodnius* (Fig. 1A).

On the apical membrane Cl^- moves down a favourable electrochemical gradient from cell to lumen, presumably through channels. However, there is to date no direct patch clamp evidence for the presence of Cl^- channels in the apical membrane of *Rhodnius* tubules.

It has long been known that in K^+-free saline the Malpighian tubules of *Rhodnius* secrete at ~ 50% of the control rate [5], raising the possibility of Cl^- entry by a K^+-independent mechanism. Preliminary experiments show that fluid secretion rates in K^+-free saline are bumetanide-sensitive but thiazide-insensitive (Ianowski and O'Donell, unpublished observations). The transporter involved remains to be identified, but one possibility is that cation-coupled Cl^- transporters in *Rhodnius* Malpighian tubules may accept variable stoichiometries. For example, the basolateral cotransporter may operate either as Na^+:K^+:2Cl^- or Na^+:Na^+:2Cl^-.

Molecular biological evidence indicates the presence of a Na^+:K^+:2Cl^- cotransporter in Malpighian tubules of the tobacco hornworm *Manduca sexta* [23]. Northern blot analysis reveals that this Na^+:K^+:2Cl^- cotransporter is a Malpighian tubule specific isoform, and that the protein consists of 1060 amino acids and contains 12 putative membrane spanning regions. The *Manduca* cotransporter shares 42–46% sequence identity with Na^+:K^+:2Cl^- cotransporters that have been cloned from shark, flounder, rat, rabbit and mouse, and contains a protein kinase A and several other putative phosphorylation sites. Evidence for a Na^+:K^+:2Cl^- cotransporter in *Manduca* is somewhat surprising in view of the very low Na^+ levels in the haemolymph of Lepidoptera. Although ion transport by isolated tubules of *Manduca* has not been studied in detail, these data may again indicate that cation-coupled Cl^- cotransporters in insect epithelia can operate when exposed to low levels of Na^+, perhaps through cotransport of K^+ and Cl^-, as discussed below for tubules of *Drosophila* and the ant *Formica*.

1.3. Reabsorption of Cl^- by the lower Malpighian tubule of Rhodnius

The excretory system of *Rhodnius* must eliminate NaCl and water from the insect at an osmotic concentration lower than that of the haemolymph because the ingested vertebrate

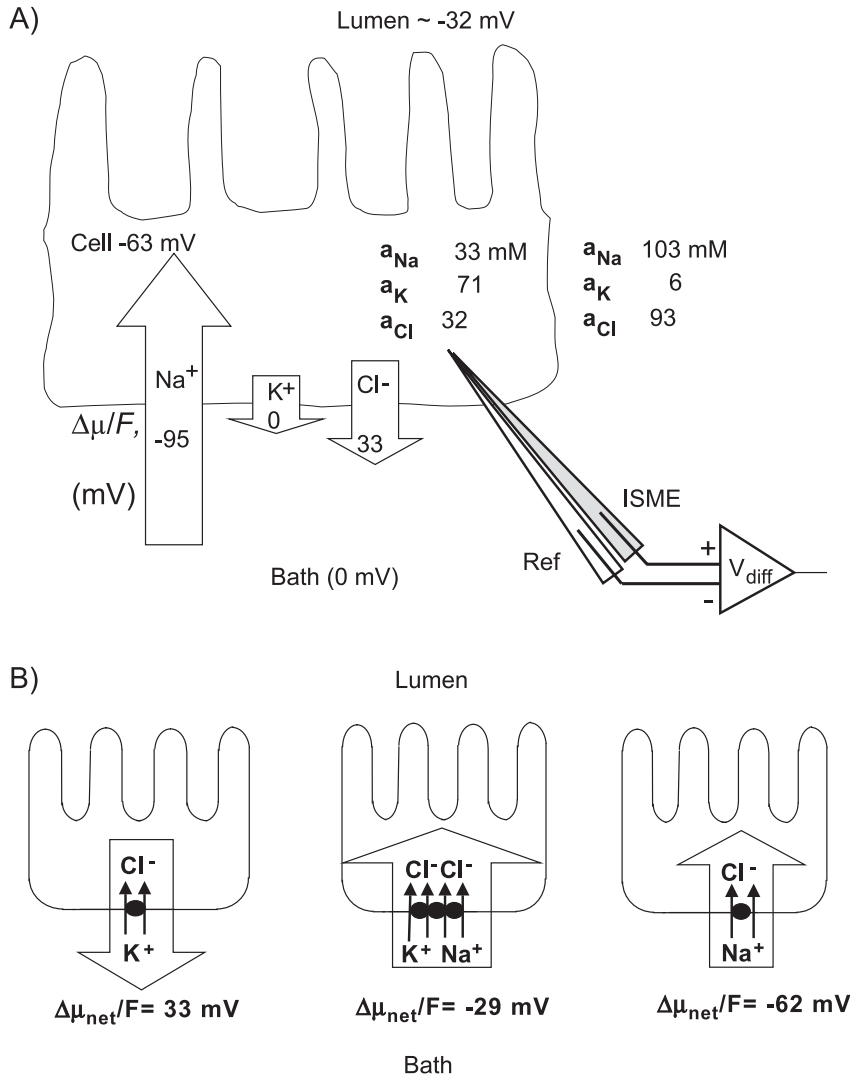

Fig. 3. (A) Schematic diagram showing intracellular Na^+, K^+ and Cl^- activities and electrical potentials in the secretory segment of the *Rhodnius* Malpighian tubule. Basolateral membrane potential and ion activities were used to calculate the electrochemical potential ($\Delta\mu/F$) across the basolateral membrane for each ion. The arrows indicate the direction of ion movement favoured by the electrochemical potential. There is a large inwardly directed electrochemical gradient for Na^+ and an outwardly directed electrochemical gradient for Cl^-. The electrochemical gradient calculated for K^+ is close to zero. However, other evidence (i.e. V_{bl} depolarization after Ba^{2+} treatment) indicates that the electrochemical gradient K^+ is small but outwardly directed, as indicated. (B) Net electrochemical potentials (calculated in Ref. [12]) for three possible cation: Cl^- cotransporters. Figure redrawn from [12].

blood is usually about 20% more dilute than the haemolymph. Secreted fluid contains 90 mmol l^{-1} KCl and 110 mmol l^{-1} NaCl and has an osmotic concentration of 345 mOsmol l^{-1}, slightly hyperosmotic to the bathing fluid. In the lower reabsorptive segment of the tubule, there is extremely rapid reabsorption of KCl with very little water, leaving a solution of 120 mmol l^{-1} NaCl and only 3 mmol l^{-1} KCl. The osmotic concentration of the final urine is 250 mOsmol l^{-1}. Reabsorption of KCl occurs in only 3–4 s as the fluid passes through the lowermost 30% of the lower tubule, so that the concentration of KCl in the lumen of the lower tubule falls at a rate of up to 20 mmol l^{-1} per second [24]. One indication of the intensity of ion reabsorption by the lower tubule is evident in the increase in K^+ concentration in the unstirred layer next to the surface of the lower tubule. In vitro the concentration of K^+ in the unstirred layer adjacent the basolateral surface of the tubule exceeds that in the bathing saline >1 mm distant by as much as 5.3-fold [25]. In saline containing 4 mmol l^{-1} K^+, the K^+ concentration within the unstirred layer may exceed 16 mmol l^{-1} [25]. Gradients in unstirred layer ion activity extend several hundred microns away from the basolateral surface of isolated tubules in vitro, and are evident, though reduced, in semi-in situ preparations as well, in spite of the mixing of the haemolymph by the contractions of the gut and the heart [25].

The membrane transporters involved in reabsorption of K^+ and Cl^- appear to be dramatically different than those involved in secretion of cations and Cl^- by the upper tubule. Reabsorption is unaffected by compounds which inhibit

vacuolar-type H^+ pump or cation:Cl^- cotransporters. A working hypothesis of ion movements during KCl reabsorption proposes that K^+ is pumped from lumen to cell by an ATP-dependent pump that is sensitive to the compounds omeprazole and SCH 28080 and may thus resemble the H^+/K^+ ATPase of the vertebrate gastric mucosa (Fig. 1C). Cellular K^+ then leaks from the cell to the bathing saline through Ba^{2+}-sensitive K^+ channels [14]. Chloride reabsorption is inhibited both by acetazolamide and by Cl^- channel blockers. It is proposed that Cl^- moves from lumen to cell through a stilbene-insensitive Cl^-/HCO_3^- exchanger and then exits the cell through basolateral Cl^- channels. Measurements of transepithelial potential and basolateral membrane potential during changes in bathing saline Cl^- concentration suggest the presence of Cl^- channels in the basolateral membrane that are blocked by diphenylamine-2-carboxylate (DPC) and 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) [13].

1.4. Separate control of anion and cation transport by multiple peptides in Drosophila Malpighian tubules

The Malpighian tubules of the fruit fly *Drosophila* consist of two cell types. The larger principal cells are the primary site of hormonally stimulated active cation transport. Fig. 4 shows that, as in the case of *Rhodnius*, it appears that the H^+ gradient created by the apical electrogenic H^+-ATPase is used to drive the exchange of K^+ for H^+ through amiloride-sensitive K^+/H^+ exchangers in the apical membrane [9]. Two peptides acting via cAMP [26,27] and one via cGMP [28] increase the activity of the apical ATPase in the principal cells. The smaller stellate cells are an important site of Cl^- transport. Chloride channels appear to be important during basal rates of secretion in tubules of both mosquitoes and fruit flies [29,30]. In response to stimulation of Cl^- transport by the leucokinin peptides, the situation is less clear. As discussed below, Cl^- transport appears to involve a transcellular pathway through stellate cells in *Drosophila*, whereas a paracellular pathway may be involved in tubules of the mosquito *A. aegypti*.

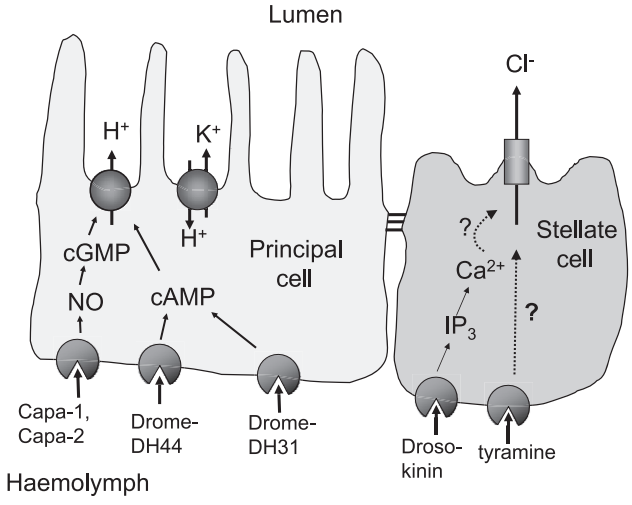

Fig. 4. Schematic diagram showing first and second messenger pathways involved in stimulation of fluid secretion in *D. melanogaster* Malpighian tubules. Three separate neuropeptides have been proposed to stimulate cation transport across principal cells; capa-1 and capa-2 (Cap2b-like peptides) activate the apical proton pump via a NO-cGMP-mediated pathway. The apical proton pump can also be activated via a cAMP-mediated pathway stimulated by either drome-DH44 (CRF-like peptide) or drome-DH31 (calcitonin-like peptide). Stimulation of transcellular Cl^- transport by drosokinin involves a Ca^{2+}/IP_3-mediated pathway in the stellate cells. Recent evidence has suggested that stellate cell Cl^- transport may also be stimulated by the amino acid tyrosine and the amine tyramine.

In tubules of several insect species, the transepithelial Cl^- conductance is increased by members of the leucokinin peptide family. Studies of *Drosophila* tubules using a combination of physiological and transgenic techniques [30] show that leucokinin-stimulated Cl^- transport involves Cl^- channels in stellate cells. Fluid secretion by both unstimulated and leucokinin stimulated *Drosophila* tubules is sensitive to the classical Cl^- channel inhibitors DPC, NPPB, anthracene-9-carboxylic acid and niflumic acid at concentrations at or below the levels effective at blocking Cl^- channels of vertebrate epithelial cells [30]. Within 2 s of application of leucokinin, there is a precipitous collapse of the lumen positive transepithelial potential. Exposure to saline with a low Cl^- concentration reversibly returns the transepithelial potential to its previous value indicating that the collapse in potential is due to an increase in transepithelial Cl^- conductance. Application of Cl^- channel blockers inhibits the effects of Cl^- substitution [30].

Several types of data indicate that the effects of leucokinin are mediated through increases in intracellular calcium levels in the stellate cells. First, an increase in fluid secretion rate and a collapse of transepithelial potential are also produced by the intracellular calcium mobilizing agent thapsigargin [9,30]. Second, application of leucokinin is associated with an increase in intracellular calcium levels [30]. This increase was demonstrated using a targeted expression system based on an aequorin transgene. This system permits measurement of calcium in genetically defined subpopulations of cells [31] and was developed, in part, because of the difficulties in measuring intracellular calcium using ion-selective microelectrodes or fluorescent Ca^{2+} indicators. The small size of stellate cells precludes direct measurement of calcium by microelectrodes, and spectrophotometric measurements of intracellular calcium levels are difficult because the tubules actively transport organic anions, including calcium chelating agents such as BAPTA [9] and fluorescent calcium indicators such as FURA2. Calcium levels in stellate cells that express the aequorin transgene rise significantly after application of leucokinin, from less than 100 nmol l^{-1} to more than 200 nmol l^{-1} within 100 ms [30,32]. No increases are seen in principal cell Ca^{2+} levels in response to *Drosophila* leucokinin [32], but the wide range of concentrations over which this kinin acts suggests the involvement of more than one receptor type in the stellate cells. Antibodies raised

against the G-protein-coupled leucokinin receptor bind to the stellate cells and also to cells in the nervous system [33].

Recent studies have provided evidence that Cl^- permeability of the *Drosophila* Malpighian tubule is controlled not only by leucokinins but also by tyramine [34]. Much of the evidence relies upon measurements of the transepithelial potential, which is not constant in *Drosophila* tubules but instead undergoes large oscillations in amplitude. These voltage oscillations reflect fluctuations in transepithelial Cl^- conductance, and are dependent upon increases in intracellular calcium levels in the stellate cells. The oscillations are eliminated by chelation of intracellular calcium. Tyrosine and several of its metabolites also produce oscillations; the most potent agonist is tyramine, which is active at concentrations of a few nanomoles per liter. The oscillations can be blocked by the tyramine antagonist yohimbine, suggesting that they are produced by activation of tyramine receptors. The oscillations in transepithelial potential produced by tyramine are similar to those which result from changes in intracellular calcium levels, suggesting that tyramine produces its effects through alteration in stellate cell calcium levels (Fig. 4). Both tyrosine [35] and tyramine [34] increase fluid secretion rates when applied at physiological levels, suggesting a physiological role for these compounds. Tyramine is a product of bacterial metabolism, and is therefore likely to be present in the rotting fruit on which *Drosophila* feeds [34]. Food containing tyramine might therefore be expected to elicit a postprandial diuresis, which may be important for clearing toxins from the haemolymph. An additional source of tyramine may be the principal cells of the tubules themselves. The presence of tyrosine decarboxylase activity in the principal cells suggests that the Malpighian tubule can synthesize tyramine from tyrosine. Release of tyramine from the principal cells might thereby alter transepithelial Cl^- permeability through effects on neighbouring stellate cells [34].

CRF-related peptides also produce an increase in transepithelial Cl^- permeability in *Aedes* Malpighian tubules [36,37]. High concentrations of CRF-related peptides increase transepithelial secretion of Na^+, Cl^- and fluid, consistent with stimulation of active transcellular Na^+ transport, probably by elevation of cAMP. By contrast, low concentrations of CRF-related peptides significantly reduce transepithelial resistance and produce depolarizing oscillations of the transepithelial potential, consistent with an increase in transepithelial Cl^- permeability. Similar effects are produced by the calcium ionophore A23187 suggesting that calcium may act as the second messenger for low concentrations of CRF-related peptides. Taken together with the discussions above, these findings indicate that Cl^- permeability may be modulated by kinins, amines such as tyramine, as well as CRF-related peptides. This raises the question of why there are so many factors, which control Cl^- permeability in dipteran Malpighian tubules. One possibility is that some of these factors affect more than one tissue. Effects on the Malpighian tubules may be coordinated with absorption across the midgut or the hindgut, for example.

1.5. Evidence for Cl^- channels in Malpighian tubules of dipterans

Several types of pharmacological and electrophysiological evidence indicate that Cl^- channels are the sites of transcellular Cl^- flux in the stellate cells of *Drosophila* Malpighian tubules. Application of the vibrating microelectrode technique reveals a small number of Cl^--dependent current density hotspots, coincident with stellate cells (Fig. 6). This technique is based on the principle that transepithelial current produces voltage gradients in the extracellular fluid above an epithelium, and these gradients are proportional in magnitude to the local current. Vibrating a voltage probe in these electrical fields yields a.c.-signals, at the same frequency as the vibration, with amplitudes proportional to the local current. A detailed description of this technique has been previously published [38]. Current density peaks associated with stellate cells in the main segment are abolished by reductions in saline Cl^- concentration or by the Cl^- channel blockers NPPB and niflumic acid [30]. Leucokinin or the calcium mobilizing agent thapsigargin significantly increases current density associated with stellate cells. No

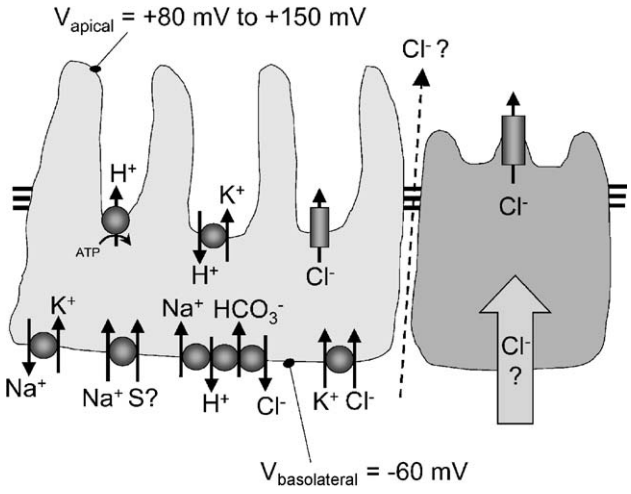

Fig. 5. Schematic diagram of putative ion transporters in Malpighian tubules of *D. melanogaster*. This model proposes that a V-type H^+ ATPase on the apical membrane of the principal cells pumps protons from cells to lumen, thereby generating a large H^+ gradient that provides the driving force for secondary movement of Na^+ and/or K^+ from cell to lumen through Na^+/H^+ and/or K^+/H^+ exchangers. The pump also generates a large apical membrane potential and lumen-positive transepithelial potential that favours the movement of Cl^- from cell to lumen through putative Cl^- channels in both stellate and principal cells. The entry of Na^+, K^+, and Cl^- across the basolateral membrane of principal cells involves a K^+:Cl^- cotransporter, an unidentified Na^+-dependent solute (S) transporter, a ouabain-sensitive Na^+/K^+ pump and a Na^+-dependent Cl^-/HCO_3^- exchanger. In addition, paracellular pathways for Cl^- conductance have been proposed for other dipterans (*A. aegypti*). The mechanism of Cl^- entry across the basolateral membrane of stellate cells is unknown.

current density hotspots are found in either the distal segment or in the lower tubule which lack stellate cells [30].

Further evidence for a role of Cl^- channels in *Drosophila* tubule Cl^- secretion has been provided by the patch clamp technique. Clusters of channels with a conductance of 256 pS are found in the apical membrane [30]. These 'maxi' channels have been identified as Cl^- channels in part because the single channel current voltage relationship in excised patches shows a reversal potential very close to the equilibrium potential for Cl^-. Multiple channels are usually seen in each patch and the channels have a high frequency of opening (P_O>0.9) and a mean open time of 13 ms. Channel activity is voltage-dependent and highest at the resting membrane potential. Open probability declines in response to the Cl^- channel blocker DIDS.

Two additional types of Cl^- channels have been found in the apical membrane of stellate cells of mosquito Malpighian tubules [29]. Type I channels have a conductance of 24 pS, an open probability of 0.82 and a mean open time of 867 ms. Type II channels have a conductance of 8 pS, an open probability of 0.07 and a mean open time of 8 ms. The high density and halide selectivity of the type I Cl^- channel are consistent with a role in transepithelial Cl^- secretion in unstimulated Malpighian tubules of *A. aegypti*. In excised patches, removal of Ca^{2+} from the cytoplasmic side did not alter channel activity. However, this does not necessarily mean that the channels are not Ca^{2+}-dependent. Other intracellular factors necessary for Ca^{2+} activation of channels (e.g. protein kinases) are not present in excised patch preparations. Future studies, perhaps using the cell-attached patch technique or perforated patch whole-cell recording, could directly assess the possibility that the apical Cl^- channels in the stellate cells of Malpighian tubules are controlled through increases in intracellular calcium in response to leucokinin. In contrast to the view that stellate channels play a cardinal role in transepithelial Cl^- flux, there has also been a suggestion of a paracellular route for Cl^- through septate junctions in *Aedes* tubules stimulated with leucokinin [39,40].

1.6. Evidence for K^+:Cl^- cotransport and Na^+-dependent Cl^-/HCO_3^- exchange activity in basolateral membranes of Malpighian tubules

It is important to point out that Cl^- is also transported not only by the stellate cells, but also by the principal cells of *Drosophila* Malpighian tubules. Several basolateral transporters in the principal cells have recently been identified (Fig. 5).

Immunohistochemical and molecular genetic evidence have revealed the presence of a Na^+-dependent Cl^-/HCO_3^- exchanger in the basolateral membrane as well [41]. Co-immunolabeling of larval tissues with an antibody to the *Drosophila* Na^+-dependent Cl^-/HCO_3^- exchanger (NDAE1) and a monoclonal antibody to the Na^+/K^+-ATPase alpha-subunit revealed that the majority of NDAE1 is located at the basolateral membranes of Malpighian tubule cells. Both NDAE1 and the Na^+/K^+-ATPase are co-localized in both the secretory main segment of the posterior Malpighian tubules, as well as in the lower (proximal) tubule and ureter. The lower tubule is involved in reabsorption of KCl and water, as well as in secretion of Ca^{2+} and acidification of the urine [42]. Neither NDAE1 nor the Na^+/K^+-ATPase is expressed in the distal non-secretory segment of the anterior Malpighian tubules. The function of the Na^+-dependent anion exchanger in Malpighian tubules is unknown, but it is of interest that it is expressed in both the secretory and reabsorptive segments. This would suggest a role in intracellular pH regulation in HCO_3^--replete bathing media. However, an earlier study suggested that the apical H^+ pump is the primary mechanism for intracellular pH regulation in Malpighian tubule cells of the related species *Drosophila hydei* in HCO_3^--free saline [43]. Future studies, perhaps using intracellular pH microelectrodes, are needed to assess the relative contributions of the Na^+-dependent Cl^-/HCO_3^- exchanger and other transporters to pH regulation in cells of the Malpighian tubule of *Drosophila*.

There is also evidence for a K^+:Cl^- cotransporter in *Drosophila melanogaster* tubules [44]. Fluid secretion is inhibited by the drug [(dihydroindenyl)oxy]alkanoic acid (DIOA) which inhibits K^+:Cl^- cotransport in some epithelial cells [45] and by 10^{-4} mol l^{-1} bumetanide, which also inhibits KCl cotransporters at high concentrations [45]. Analysis of secreted fluid ion composition indicates that when *Drosophila* tubules are treated with bumetanide, only K^+ flux declines. The latter observation suggests that K^+ flux is not Na^+-dependent, and that K^+ does not enter the tubules through a Na^+:K^+:2Cl^- cotransporter. By contrast, when *Rhodnius* tubules are treated with bumetanide, the fluxes of both Na^+ and K^+ decline, and the drug is effective at much lower concentrations ($<10^{-5}$ mol l^{-1}) [12]. The finding that *Drosophila* tubules secrete at high rates in Na^+-free saline also argues against an important functional role for Na^+:K^+:2Cl^- cotransport during vectorial ion secretion by *Drosophila* tubules [44].

The electrical potential profile across the *Drosophila* Malpighian tubule also suggests the possibility of a K^+:Cl^- cotransporter. The basolateral membrane potential is approximately -50 mV to the haemolymph, and the transepithelial potential in stimulated tubules is typically in excess of 50 mV, lumen-positive. Using these values, it can be calculated that the apical membrane potential may exceed 100 mV, lumen-positive (Fig. 6). As a result, if Cl^- approaches equilibrium across the apical membrane of *Drosophila* principal cells, it may be below equilibrium across the basolateral membrane. Therefore, a favourable gradient for Cl^- entry across the basolateral membrane in *Drosophila* tubules may drive Cl^- coupled K^+ entry. Preliminary observations suggest that the Cl^- electrochemical potential across the basolateral membrane is inwardly directed while the K^+ electrochemical potential is outwardly directed, indicating that K^+ is actively transported into the

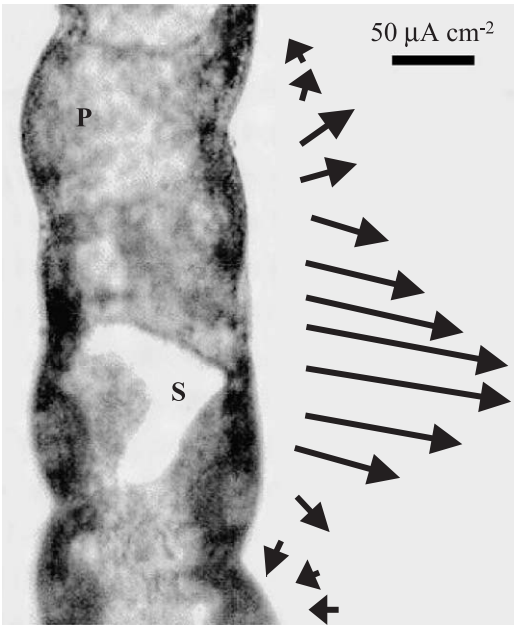

Fig. 6. Chloride-dependent current density hot spots colocalize with stellate cells in the main segment of the Malpighian tubules of *D. melanogaster*. The stellate cell is marked by an 'S' and a principal cell is marked with a 'P'. The base of each arrow indicates where the vibrating microelectrode was positioned for each current density measurement. Based on data in Ref. [30].

cell (Ianowski and O'Donnell, unpublished observations). Measurements with ion-selective microelectrodes also suggest that intracellular Cl^- activity in *Drosophila* principal cells is K^+-dependent (Ianowski and O'Donnell, unpublished observations), again suggesting that K^+ and Cl^- influx across the basolateral membrane are linked, consistent with a possible role for a Cl^--driven K^+:Cl^- cotransport in fluid secretion by Malpighian tubules of *Drosophila*. The pattern of electrochemical potentials across the basolateral membrane of *Drosophila* tubules is thus very different from that in *Rhodnius*, where the calculated electrochemical potentials indicate that active transport of Cl^- into the cell can be driven by a favourable gradient for Na^+ entry.

A small but functionally significant basolateral Cl^- conductance has been proposed for the Malpighian tubules of the ant *Formica polyctena* [46]. Calculations based on the Goldman voltage equation indicate that if the K^+:Cl^- permeability ratio varies between 10 and 1, basolateral membrane potential does not deviate very much from the K^+ equilibrium potential. In other words, significant fluxes of Cl^- into the cell through the basolateral membrane channels will not measurably alter basolateral membrane potential. Inhibition of Cl^- secretion by DIDS may be taken to support the suggestion of Cl^- channel involvement in fluid secretion. A series of experiments in which bathing saline K^+ concentration was varied and the effects of bumetanide were studied give rise to the following proposals for the mechanisms of K^+ and Cl^- transport in the Malpighian tubules of *Formica*. In Na^+-free solutions containing high levels of K^+, coupled entry of K^+ and Cl^- is of little importance. K^+ and Cl^- may enter the cells by means of channels and a Cl^-/HCO_3^- exchanger process, respectively [47]. At intermediate levels of haemolymph K^+, a substantial portion of K^+ entry appears to occur through a K^+:Cl^- cotransporter. When haemolymph K^+ concentration is reduced further, a Na^+:K^+:$2Cl^-$ cotransporter becomes important [47]. We suggest that the lumen-positive transepithelial potential in *Formica* tubules raises the possibility of a Cl^- gradient energizing basolateral K^+ uptake by K^+:Cl^- cotransport in this species as well. An important test in future studies will be to measure intracellular K^+ levels during changes in peritubular fluid Cl^-, as well as intracellular Cl^- levels during changes in peritubular fluid K^+.

Fluid secretion assays, analysis of secreted fluid Na^+/K^+ ratio and measurements of basolateral membrane potential suggest that a Na^+/K^+ ATPase contributes to fluid secretion. Fluid secretion is inhibited by the Na^+/K^+-ATPase inhibitors ouabain, dihydro-ouabain and bufalin [44]. Ouabain also produces a slight depolarization (8–10 mV) of the basolateral membrane potential, consistent with inhibition of the Na^+/K^+-ATPase. Moreoever, there is an increase in the Na^+ concentration of the secreted fluid in response to ouabain, suggesting that under these conditions Na^+ leakage into the cell across the basolateral membrane is enhanced, and that Na^+ is then more readily available for transport across the apical membrane into the lumen [44].

1.7. Transport of sulfate and bicarbonate by Malpighian tubules

Chloride is not the only anion that can be transported efficiently by insect Malpighian tubules. Active transport of sulfate ions has been demonstrated in the Malpighian tubules of mosquito larvae, which inhabit sulfate-rich lakes. In vitro preparations of the Malpighian tubules of *Aedes campestris* secrete sulfate ions actively against a threefold concentration gradient and an electrical potential difference of 20 mV. The transport is half saturated at approximately 10 mmol l^{-1} of sulfate [48]. The rate of sulfate secretion by the Malpighian tubules is sufficient to remove all sulfate ingested by larvae living in waters which contain less than 100 mmol l^{-1} of sulfate. Malpighian tubules of larvae of the related species *Aedes taeniorhyncus* soon develop an ability to transport sulfate when the larvae are reared in sulfate containing waters [49]. The rate of sulfate transport induced varies directly with the sulfate content of the water in which they are reared. The process of induction is significant within 6 h of transfer to sulfate-rich water and is about 50% complete after an additional 10 h. Kinetic analysis indicates that induction involves only a change in capacity with no change in affinity of the transporter. It is not known whether induction involves synthesis of more pump molecules, the incorporation of more such molecules into the cell membranes, the activation of in active pumps already in the membranes, or a combination of these possibilities. It is also unclear whether induction involves a hormonal mediator or

whether it is a direct response of the tubules to sulfate in the bathing saline [49].

Tubules of several species are known to transport HCO_3^- and/or carbonate into the secreted fluid. Isolated tubules of the water boatman *Cenocorixa blaisdelli* and *C. bifida* [50] secrete fluid of high pH and CO_2 content, consistent with net HCO_3^- secretion. Alkaline fluid is secreted only by a particular segment, and only in response to stimulation with cAMP. The function of HCO_3^- secretion is somewhat unclear, as it is a characteristic of species found in fresh and alkaline waters. Carbonate and HCO_3^- also appear to be transported by the lime gland, a specialized segment of the Malpighian tubules, in the alkali fly *Ephydra hians* [51]. The larvae are found in lakes containing high concentrations of HCO_3^- and carbonate, and precipitation of calcium with carbonate and/or HCO_3^- in the lumen of the lime gland may function in a regulatory capacity.

1.8. Organic anion transport: comparisons with vertebrate renal tubules

Malpighian tubules have long been known to transport both carboxylates such as *p*-aminohippurate (PAH) and benzyl penicillin as well as sulfonates such as amaranth, indigo carmine and sulforhodamine [18]. The ability of the Malpighian tubules of many insects to concentrate acidic dyes probably occurs because the dyes are similar in structure to other uncolored excreted molecules. As for mammalian renal tubules, it appears that dye molecules are concentrated by a mechanism normally used to excrete various non-metabolizable aromatic residues such as hippuric acid whose effective excretion is important. Only those molecules with strongly acidic groups and no basic groups such as -NH2 are actively transported by the tubules. For the Malpighian tubules of *Rhodnius*, dye clearance is enhanced by an increase in pH from 6.7 to 7.5. Such a change in pH has no effect on fluid secretion. This finding suggests that it is the anionic form of the dye which is transported and that at lower pH values this dissociation is suppressed. Molecules with both acidic and basic groups appear not to be able to cross the basal membranes, whereas substances with weakly acidic groups which can cross the basal membrane tend not to be concentrated in the lumen. However, they may produce vital staining of the cytoplasm. For most species, it is clear that the rate of dye secretion is not affected by the rate of fluid secretion. In other words, dye secretion and fluid secretion are separate activities of the Malpighian tubules.

Fig. 7 shows the current working hypothesis for transport of organic anions by insect Malpighian tubules. A recent study of PAH transport by *Drosophila* Malpighian tubules suggests that basolateral PAH transport is inhibited by ouabain and dependent on the Na^+ gradient. This Na^+ dependence of basolateral organic anion uptake is similar to that observed in vertebrate renal tissue [20]. However, in stark contrast to vertebrate renal studies, PAH accumulation is not affected by low concentrations of the dicarboxylic

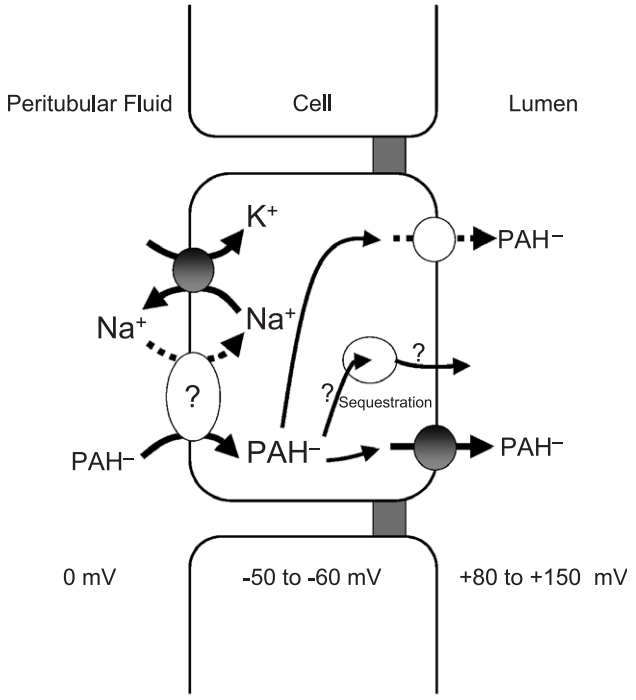

Fig. 7. Descriptive model of the steps involved in transepithelial transport of organic anions in insect Malpighian tubules, using PAH as a model substrate. Solid arrows with filled circles indicate primary active transport. Dashed arrows with open circles indicate movement down an electrochemical gradient. Solid arrows with open circles indicate movement against an electrochemical gradient by some form of secondary active transport process.

acids, α-ketoglutarate, glutarate, succinate, and citrate or the activity of protein kinase C. This suggests that the basolateral Na^+-dicarboxylate cotransporter observed in vertebrate renal uptake is not required in insects. Whether organic anion uptake in insect Malpighian tubules is a secondary transport process directly coupled to Na^+ entry or is an unknown tertiary transport mechanism remains to be elucidated. The rate of transport by isolated tubules is not increased by cAMP, cGMP, or leucokinin, suggesting that PAH transport is maximal in vivo. PAH transport is also reduced by colchicine, suggesting the involvement of microtubules in the movement of PAH containing vesicles across the cytoplasm from the basolateral to the apical surface. Studies of fluorescein transport across the cytoplasm of principal cells indicates the involvement of both diffusion and vesicular transport.

The suggestion of two distinct transporter systems in Malpighian tubules of flies (*Calliphora*, *Drosophila*) and *Rhodnius* is based primarily on the results of competition experiments [18,20]. By contrast, studies of organic anion transport by Malpighian tubules of the tropical cockroach *Blaberus giganteus* indicate that transport of fluorescein is also depressed by sulfonate, indigo carmine, and, vice versa, transport of indigo carmine is inhibited by the carboxylic acids, fluorescein and probenecid [19]. Alterations in K_m and V_{max} indicate that fluorescein transport inhibition by carbox-

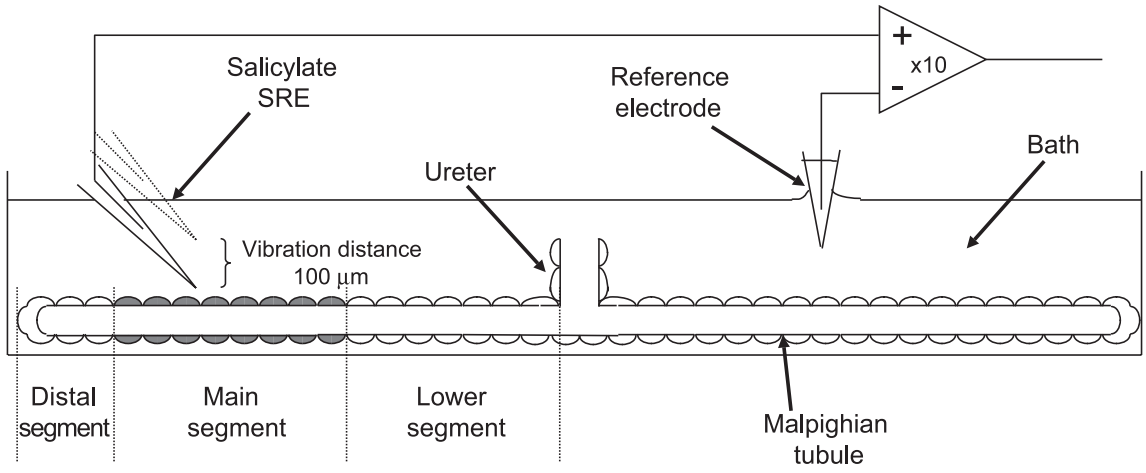

Fig. 8. Schematic diagram (not to scale) showing the self-referencing salicylate-selective (Salicylate SRE) microelectrode for the study of salicylate transport by isolated Malpighian tubules. The individual segments of the Malpighian tubule and their relationship to the ureter are shown. During measurement at each site, the salicylate SRE microelectrode was vibrated over a distance of 100 μm near the basolateral surface of the tubule.

ylic acids such as PAH and probenecid is competitive whereas its inhibition by the sulfonate Congo red is mixed [19]. The efficiency of transport is given by the ratio of V_{max} to K_m. Comparisons of this ratio for fluorescein transport by a number of insect species indicates that efficiency is highest in those species which feed on a wide variety of foods.

An additional mechanism of dye transport has been revealed by a recent study using confocal microscopy and digital image analysis to demonstrate that the Malpighian tubules of the American cockroach excrete the fluorescent organic anion sulforhodamine 101 by a mechanism sensitive to an inhibitor of multidrug resistance-associated protein 2 (MRP2) [52]. The inhibitor, chlorodinitrobenzene, does not block secretion of chlorophenol red, a substrate for the classic organic anion transporter system. These observations suggest that there may be multiple transporters involved in transepithelial movement of organic anions across Malpighian tubules.

The study of dye transport by Malpighian tubules has been greatly facilitated by the ease with which tubules can be isolated and their capacity to secrete fluids in vitro in the Ramsay preparation. Studies of organic anion transport by other tissues have been hampered by the absence of suitable in vitro preparations, particularly for small species such as *Drosophila*. We have therefore developed new methods for studying organic anion transport by small, fragile or opaque

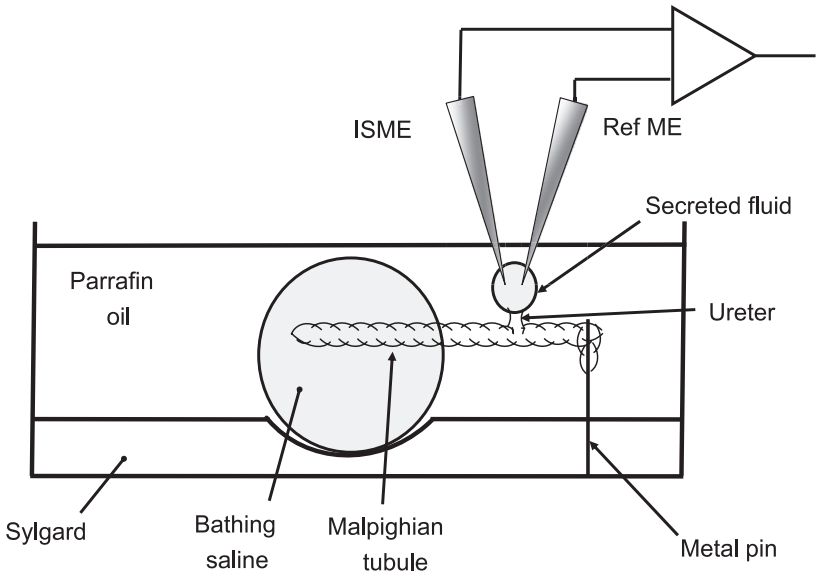

Fig. 9. Schematic representation of a modified Ramsay assay used for measurement of ion concentration in fluid secreted by an isolated Malpighian tubule. The Malpighian tubule is positioned so that the one tubule is bathed in a droplet of saline and the cut end of the ureter is in the paraffin oil. The other tubule is wrapped around a metal pin so as to secure the preparation. Secreted fluid droplets collect on the end of the ureter. An ion-selective microelectrode (ISME) and voltage-sensing reference microelectrode are positioned in the droplet when it is still attached to the ureter (as shown) or after it is removed with a fine glass rod and placed on the Sylgard-lined bottom of the Petri dish.

tissues. One method involves the use of salicylate-selective self-referencing microelectrodes for noninvasive spatial and temporal analysis of salicylate transport. The liquid membrane salicylate-selective microelectrodes are based on tetradodecylammonium dissolved in nitrophenyl octyl ether. Macroelectrodes based on a polyvinylchloride membrane impregnated with a tetraalkylammonium salicylate salts have been described previously [53]. We have previously used the self-referencing ion-selective microelectrode technique for analysis of the fluxes of inorganic cations and organic cations [54,55]. The salicylate-selective microelectrode is moved between two recording positions within the unstirred layer by computer-controlled stepper motors (Fig. 8). The voltage difference between the two positions is amplified, recorded and converted to a concentration difference. Fluxes are then calculated from the salicylate concentration difference using Fick's equation. The studies have shown that Malpighian tubules transport salicylate at high rates, and that salicylate influx is saturable and energy-dependent. The ileum and rectum also secrete salicylate, but fluxes across the midgut are negligible (Rheault and O'Donnell, unpublished observations). Salicylate-selective microelectrodes facilitate noninvasive spatial and temporal analysis of the transport of salicylate and presumably other organic anions such as benzyl penicillin. These approaches permit low-cost direct measurements of salicylate flux in real time with a spatial resolution of approximately 50 μm. This approach is also suitable for multilayered tissues such as the gut where the overlying musculature may confound optical measurements using fluorescent probes. However, there is an important caveat: ion exchanger electrodes which are sensitive to compounds such as salicylate are also sensitive to many other compounds. As a result, it is very difficult to use this technique to pharmacologically characterize the particular type of transporter for salicylate by isolated tissues. The approach is best viewed as an adjunct to the use of radiolabeled compounds or fluorescent probes for analysis of organic anion transport.

The small volume of fluid secreted by tubules of insects such as *Drosophila* hampers the measurement of organic anion fluxes using fluorescent or radiolabeled methods. We have therefore used salicylate-selective microelectrodes to measure the concentration of salicylate in nanoliter droplets of fluid secreted by isolated Malpighian tubules in the Ramsay preparation (Fig. 9). The flux of salicylate across the tubules is calculated as the product of secreted droplet salicylate concentration and secretion rate. The studies have shown that the Malpighian tubules concentrate salicylate in the lumen of the tubules approximately 50-fold relative to the concentrations in the bathing medium (Rheault and O'Donnell, unpublished observations).

An important aspect of future studies will be to determine the links between inorganic anion transport and organic anion transport. For example, PAH transport is Na^+-dependent but is not dependent upon the presence of dicarboxylic acids. It will be of interest, therefore, to determine if organic anion transport alters the concentration of intracellular Na^+, as might be expected if there is a direct coupling between the fluxes of Na^+ and organic anions. The possibility of organic anion/inorganic anion exchange processes in the lumenal membrane can be examined using perfused tubules in which the concentration of Cl^- and other anions in the perfusate is varied.

Acknowledgements

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