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Secretion of Na^+, K^+ and fluid by the Malpighian (renal) tubule of the larval cabbage looper *Trichoplusia ni* (Lepidoptera: Noctuidae)

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ABSTRACT

The Malpighian (renal) tubules play important roles in ionic and osmotic homeostasis in insects. In Lepidoptera, the Malpighian tubules are structurally regionalized and the concentration of Na^+ and K^+ in the secreted fluid varies depending on the segment of tubule analyzed. In this work, we have characterized fluid and ion (Na^+, K^+, H^+) transport by tubules of the larval stage of the cabbage looper *Trichoplusia ni*; we have also evaluated the effects of fluid secretion inhibitors and stimulants on fluid and ion transport. Ramsay assays showed that fluid was secreted by the iliac plexus but not by the yellow and white regions of the tubule. K^+ and Na^+ were secreted by the distal iliac plexus (DIP) and K^+ was reabsorbed in downstream regions. The fluid secretion rate decreased > 50% after 25 μM bafilomycin A1, 500 μM amiloride or 50 μM bumetanide was added to the bath. The concentration of K^+ in the secreted fluid did not change, whereas the concentration of Na^+ in the secreted fluid decreased significantly when tubules were exposed to bafilomycin A1 or amiloride. Addition of 500 μM cAMP or 1 μM 5-HT to the bath stimulated fluid secretion and resulted in a decrease in K^+ concentration in the secreted fluid. An increase in Na^+ concentration in the secreted fluid was observed only in cAMP-stimulated tubules. Secreted fluid pH and the transepithelial electrical potential (TEP) did not change when tubules were stimulated. Taken together, our results show that the secretion of fluid is carried out by the upper regions (DIP) in *T. ni* Malpighian tubules. Upper regions of the tubules secrete K^+, whereas lower regions reabsorb it. Stimulation of fluid secretion is correlated with a decrease in the K^+/Na^+ ratio.

1. Introduction

The renal (Malpighian) tubules play a critical role in maintaining hemolymph homeostasis in insects (Beyenbach, 2003; O'Donnell, 2008; Coast, 2009). Ion and fluid transport mechanisms have been characterized in multiple insect species, including the dipterans *Aedes aegypti* and *Drosophila melanogaster*, the orthopteran *Acheta domesticus* (Coast, 2012) and the hemipteran *Rhodnius prolixus* (O'Donnell, 2008). In Lepidoptera, however, the mechanisms of fluid and ion transport by the Malpighian tubules have not been well characterized. Early measurements of fluid secretion rates and secreted fluid ion concentrations were carried out using tubules of *Pieris brasicae*, *Manduca sexta* and *Calpodes ethlius* (Irvine, 1969; Ramsay, 1976). Malpighian tubules of Lepidoptera are generally divided into different regions on the basis of structural difference along their length. The distal segment of the tubule is enveloped by the perinephric membrane and forms a cryptonephric complex with the rectal epithelium. A short rectal lead (RL) emerges from the cryptonephric complex. The next anterior segment of the tubule is highly convoluted and is known as the iliac plexus (IP). Farther anterior, near the junction of hindgut and midgut, the tubule undergoes a transition into the yellow region (YR). This region extends anteriorly along the midgut and the tubule is then reflected 180 degrees to form the white region (WR), which runs posteriorly to a common trunk and urinary bladder that passes fluid into the gut at the junction between the midgut and hindgut (Irvine, 1969). Studies by both Irvine (1969) and Ramsay (1976) revealed that the K^+/Na^+ ratio is higher for upper segments than for lower segments of the tubules (Irvine, 1969).

In tubules of many species, the rate of fluid secretion is greatly reduced by exposure to bafilomycin A1, an inhibitor of the apical H^+ V-ATPase, or by amiloride, an inhibitor of the apical alkali cation/proton exchangers (O'Donnell, 2008). Although these compounds reduce reabsorption by the cryptonephric complex of larval *M. sexta* (Liao et al., 2000), their effects on fluid secretion by

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the free tubules or on transepithelial transport of Na^+ and K^+ are unknown.

Fluid secretion by tubules of Lepidoptera is stimulated by multiple factors, including neuropeptides (*M. sexta* diuretic hormone, leucokinin I, cardioacceleratory peptides and tachykinin-related peptides) (Audsley et al., 1993; Skaer et al., 2002), as well as the amines 5-HT and octopamine (Skaer et al., 2002). The cyclic nucleotides cAMP or cGMP also induce an increase on fluid secretion rate (Audsley et al., 1993; Nicolson and Millar, 1983; Skaer et al., 2002). However, the effects of such factors on Na^+, K^+ and H^+ transport by the tubules are unknown.

The present work provides further information on the mechanisms and control of fluid and ion secretion by the free tubules of larvae of a lepidopteran insect, *Trichoplusia ni*. We report the effects of inhibitors and stimulants of fluid transport on transport of Na^+, K^+ and H^+ and on the transepithelial potential.

2. Materials and methods

2.1. Insects

Eggs of *T. ni* purchased from Insect Production Services of the Great Lake Forestry Centre at Sault Ste. Marie, ON, Canada were used to establish a laboratory colony at McMaster University. Larvae were maintained at room temperature (21–23 °C) and 80–90% relative humidity and were fed McMorran diet (McMorran, 1965) that contained agar, alphacel, ascorbic acid, aureomycin, casein, formaldehyde, linseed oil, methyl paraben, potassium hydroxide, sugar, vitamins, water, and wheat germ. The diet also contained Wesson's salt mixture, including 59 mM K^+ and 18 mM Na^+.

2.2. Malpighian tubule dissection

Fifth instar larvae were dissected under physiological saline (Maddrell and Gardiner, 1976) that contained (in mM): 15 NaCl, 30 KCl, 2 $MgCl_2$, 10 $KHCO_3$, 5 KH_2P0_4, 10 glucose, 10 maltose, 5 trisodium citrate, 10 glycine, 10 alanine, 10 proline, 10 glutamine, 10 valine, 5 serine, 5 histidine. Saline was adjusted to pH 7.2 with 0.5 M NaOH and 0.5 M KOH. Renal tubules were dissected using the method of Gaertner et al. (1998). Briefly, the larva's head was crushed with forceps and the larva was then pinned on its side in a 7 cm diameter petri dish lined with Sylgard. The body wall was torn lengthwise with forceps to expose the gut. After carefully removing the tracheal connections to the gut, whole Malpighian tubules were detached from the gut by transecting the rectal lead at its junction with the rectal complex and by transecting the tubule at the junction of the common trunk and urinary bladder. Isolated whole tubules were then transferred to the Ramsay assay using fire-polished glass probes. The total length of the four well-defined regions of the tubules in 5th instar larvae was ~35 mm. As a percentage of the whole tubule length, the length of each region was: rectal lead (RL) 3%, iliac plexus (IP) 57%, yellow region (YR) 20%, and white region (WR) 20%.

2.3. Measurement of fluid secretion rate

Rates of fluid secretion by the Malpighian tubules were measured using the Ramsay assay developed for tubules of the lepidopteran *C. ethlius* (Irvine, 1969), with some modifications. Briefly, whole tubules were transferred on fine glass probes from the dissecting dish to 50 µl droplets of saline in a Sylgard-lined Petri dish held under water-saturated paraffin oil. Both ends of the tubules were pulled out of the saline droplet and wrapped around steel pins stuck into the Sylgard. Pins were positioned approximately 2.5 mm and 1 mm away from the bathing droplet to hold the proximal and distal end of tubules, respectively. Droplets of secreted fluid that formed at the end of the lower segment were collected at 15–20 min intervals using a micropipette and placed on the bottom of the Petri dish. For measurement of fluid secretion by different regions of the tubule, secreted droplets were first collected from the whole tubule, then sections of the lower segments were pulled out of the bathing droplet in stages so as to collect droplets secreted by progressively more distal regions. Fluid secretion rates were then calculated for the following regions: whole tubule (WT), yellow region and iliac plexus (YR + IP), whole iliac plexus (IP) and distal iliac plexus (DIP). The rectal lead (RL), which is the most distal section, was not included in the fluid secretion assay, as this section was positioned out of the bathing droplets and wrapped around the minuten pin to anchor the distal section of the tubule. To evaluate the effects of inhibitors and stimulants of fluid secretion, the Ramsay assay was performed with lengths of tubules including YR + IP (*i.e.* excluding the white region). The white region was excluded because tubules of the larval stage used usually had luminal concretions in this section that obstructed the secretion of fluid in the Ramsay assay. Secretion rate was calculated by dividing the volume of the secreted droplets ($\pi d^3/6$), where d is the diameter measured using an ocular micrometer, by the time during which the droplets formed.

2.4. Measurement of K^+ and Na^+ concentrations and pH in secreted fluid

Ion concentrations in the secreted fluid were measured using ion-selective microelectrodes as described previously by Donini et al. (2008). Table 1 lists the ionophore cocktails, back-fill solutions and calibration solutions used. The reference microelectrodes were filled with 150 mM KCl. Ion-selective and reference microelectrodes were connected through chlorided silver wires to a high impedance ($>10^{13}\ \Omega$) electrometer (ML165 pH Amp, ADInstruments, Colorado Springs, CO, USA), which in turn was connected to a PowerLab 4/30 data acquisition system (ADInstruments).

The concentrations of Na^+ and K^+ in the secreted fluid were calculated using the following formula: $[\text{Ion}] = C \times 10^{(\Delta V/S)}$, where [Ion] is the ion concentration in the secreted fluid droplet; C is the ion concentration in one of the calibration solutions; ΔV is the voltage difference between the droplet of secreted fluid and the same calibration solution; and the slope (S) is the change in voltage in response to a ten-fold change in ion concentration.

Table 1
Characteristics of ion-selective microelectrodes.

Microelectrode	Backfilling solution	Frontfilling cocktail	Calibrations	Slope (mV)
K^+-selective	0.150 M KCl	Potassium ionophore I – cocktail B	15 mM KCl/ 135 mM NaCl and 150 mM KCl	58.2 ± 2
Na^+-selective	0.150 M NaCl	3.5% sodium ionophore X, 0.6% KtkClPB, 95.9% o-nitrophenyl ocytyl ether	15 m M NaCl/ 135 mM LiCl and 150 mM NaCl	55.1 ± 0.5
H^+-selective	0.1 M Na Citrate and 0.1 M NaCl, pH 6.0	Hydrogen ionophore I – cocktail B	Hepes ringer pH 6.5 and pH 7.5	65.1 ± 0.5