Sodium pumps in the Malpighian tubule of *Rhodnius sp.**

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ABSTRACT

Malpighian tubule of *Rhodnius sp.* express two sodium pumps: the classical ouabain-sensitive ($Na^+ + K^+$)ATPase and an ouabain-insensitive, furosemide-sensitive Na^+-ATPase. In insects, 5-hydroxitryptamine is a diuretic hormone released during meals. It inhibits the ($Na^+ + K^+$)ATPase and Na^+-ATPase activities indicating that these enzymes are involved in fluid secretion. Furthermore, in *Rhodnius neglectus*, proximal cells of Malpighian tubule exposed to hyperosmotic medium, regulate their volume through a mechanism called regulatory volume increase. This regulatory response involves inhibition of the ($Na^+ + K^+$)ATPase activity that could lead to accumulation of active osmotic solute inside the cell, influx of water and return to the normal cell volume. Adenosine, a compound produced in stress conditions, also inhibits the ($Na^+ + K^+$)ATPase activity. Taken together these data indicate that ($Na^+ + K^+$)ATPase is a target of the regulatory mechanisms of water and ions transport responsible for homeostasis in *Rhodnius sp.*

Key words: Malpighian tubule, ($Na^+ + K^+$)ATPase, Na^+-ATPase, fluid secretion, adenosine, 5-hydroxitryptamine.

FLUID SECRETION IN MALPIGHIAN TUBULE

Rhodnius sp., a bloodsucking insect, is a known vector of Chagas disease. During a meal, these animals suck about 10 times their own body weight (Beyenbach & Petzel 1987). This process is compensated by 1000 times increase in urine flux due to the synergistic action of a peptide diuretic hormone and 5-hydroxitryptamine (5-HT) (Maddrell *et al.* 1993a). The first step in the insect's urine formation is the secretion of an isosmotic fluid in the distal segment of the Malpighian tubule followed by a selective reabsorption in the proximal segment of the Malpighian tubule, hindgut and rectum. During diuresis, transcellular fluid transport across these insect epithelial cells is very fast (Phillips 1981, Nicolson 1993).

In contrast to the herbivorous insects, the hematophagous insects eliminate urine with higher sodium than potassium concentration immediately after a meal (Maddrell *et al.* 1993b). It has been proposed that fluid secretion in the Malpighian tubule cells involves two principal transporters:

1) the Na^+/H^+ or K^+/H^+ exchanger; and

2) the V-type H^+-ATPase.

The H^+-ATPase would create a proton gradient used by the Na^+/H^+ or K^+/H^+ exchanger to secrete Na^+ or K^+ into the tubular lumen (Nicolson 1993,

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Pannabecker 1995). Furthermore, Cl^- could be secreted through a transcellular or through a paracellular route. Na^+, K^+ and Cl^- could move across the basolateral membrane via the furosemide and bumetanide-sensitive $Na^+/K^+/2Cl^-$ transporter.

SODIUM PUMPS

The $(Na^+ + K^+)$ATPase is crucial for the survival of most cells (Sweadener 1989). The enzyme is an integral plasma membrane protein which actively transports three Na^+ to the outside of the cell and two K^+ to the inside, maintaining the electrochemical gradient across the cell membrane (Sweadener 1989). This ATPase is formed by two noncovalently linked subunits in an equimolar ratio: α and β (Xie & Morimoto 1995). The apparent insensitivity to ouabain of the stimulated fluid secretion in many insects tested led to the hypothesis that there was no $(Na^+ + K^+)$ATPase in the Malpighian tubule cells. However, in the Malpighian tubule of *Rhodnius*, ouabain, on the basolateral side, increased unstimulated fluid secretion (Maddrell & Overton 1988, Nicolson 1993, Pannabecker 1995). The presence of $(Na^+ + K^+)$ATPase in the Malpighian tubule was confirmed by Lebovitz *et al.* (1989) who cloned its α-subunit cDNA in the basolateral membrane of Malpighian tubule of *Drosophila melanogaster*. More recently, it was shown that a ouabain-sensitive $(Na^+ + K^+)$ATPase activity is present in the Malpighian tubule cells of *Rhodnius prolixus* (Grieco & Lopes 1997, Caruso-Neves *et al.* 1998a).

Besides the $(Na^+ + K^+)$ATPase, another sodium pump was found in Malpighian tubule of *Rhodnius prolixus* (Caruso-Neves *et al.* 1998b). This Na^+-stimulated ATPase activity has the following characteristics:

1) $K_{0.5}$ for $Na^+ = 1.49 \pm 0.18$ mM,

2) $V_{max} = 2.8 \pm 0.1$ nmol $Pi \times mg^{-1} \times min^{-1}$,

3) it is fully inhibited by 2 mM furosemide,

4) it is insensitive to ouabain concentrations up to 10^{-2} M,

5) it is sensitive to vanadate indicating it to be a P-type ATPase, and

6) it is stimulated by namolar concentrations of Ca^{2+} in the incubation medium.

This Na^+-ATPase has been described in several cell types (Proverbio *et al.* 1989, Moretti *et al.* 1991, Caruso-Neves *et al.* 1997, 1998b, 1999, Rangel *et al.* 1999). It was initially described in aged microsomal fractions from guinea-pig kidney cortex as an active Na^+ transporter not stimulated by K^+ (Proverbio *et al.* 1989). This pump has same distribution that $(Na^+ + K^+)$ATPase, and is only found in the plasma membrane (Proverbio *et al.* 1989, Caruso-Neves *et al.* 1997, 1998b, 1999, Rangel *et al.* 1999). The Na^+-ATPase of the Malpighian tubule cells from *Rhodnius prolixus* is inhibited by KCl in a dose-dependent manner with maximal effect observed at 5 mM (Figure 1). This inhibition is reversed by increasing the Na^+ concentration. These data indicate that K^+ could be a physiological modulator of the Na^+-ATPase.

PHYSIOLOGICAL ROLE OF SODIUM PUMPS

Fluid Secretion

Although the Malpighian tubules from *Rhodnius sp.* present two Na^+ pumps, their physiological role is still not clear. In general, it is postulated that the gradient created by $(Na^+ + K^+)$ATPase in epithelial cells is used for transcellular transport. The observation that ouabain did not change the stimulated fluid secretion in many insect species tested lead some authors to postulated that the $(Na^+ + K^+)$ATPase is not involved in fluid secretion (Nicolson 1993, Pannabecker 1995). However, it was observed that 5-HT, a diuretic hormone released during meals, inhibits the $(Na^+ + K^+)$ATPase activity in Malpighian tubule cells from *Rhodnius prolixus* (Grieco & Lopes 1997). Thus, the modulation of the $(Na^+ + K^+)$ATPase in the Malpighian tubule could be one of the regulatory mechanisms of fluid secretion. The inhibition of the $(Na^+ + K^+)$ATPase could lead to intracellular accumulation

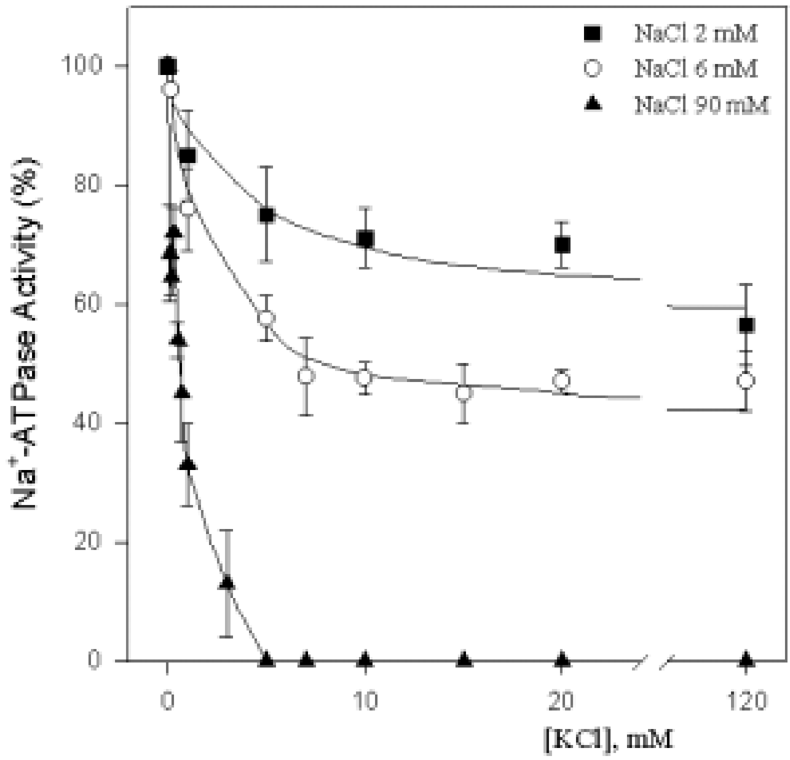

Fig. 1 – Dependence of Na^+-ATPase activity on KCl concentration. The ATPase activity was measured as described by Caruso-Neves *et al.* (1998b). The KCl concentrations range from 0.1 to 120 mM and the final osmolality was adjusted to 320 mOsm/kg. The Na^+-ATPase activity was calculated from the difference between the ATPase activity in the absence and in the presence of 2 mM furosemide, both in the presence of 1 mM ouabain. When indicated NaCl 2, 6 or 90 mM were added.

of Na^+ and, consequently, to an increase of Na^+ secretion through the luminal membrane. Since the first step in the rapid excretion phase (during meals) is the elimination of an urine enriched in NaCl and water the inhibition of Na^+ reabsorption (due to inhibition of the $(Na^+ + K^+)$ATPase activity) would be an important component in this phase. This hypothesis is supported by the observation that ouabain increases the fluid secretion in isolated and unstimulated Malpighian tubules (Lebovitz *et al.* 1989, Nicolson 1993).

The possible involvement of the ouabain-insensitive Na^+-ATPase on the fluid secretion has not been directly investigated yet. Nevertheless, it was observed that this enzyme is inhibited by 5-HT in a dose-dependent manner indicating that it is a target of regulatory mechanisms of water and ions transport responsible for homeostasis in *Rhodnius prolixus* (Grieco 1999).

Stress Conditions

The Malpighian tubule cells of *Rhodnius sp.* are exposed to different stress conditions. These cells are exposed to different osmolalities depending of the feeding state of the animal (Beyenbach & Petzel 1987, Nicolson 1993). After a blood meal the hemolymph osmolality decreases because the osmolality of the blood is lower than that of the hemolymph. On the other hand, during starvation, the osmolality of the hemolymph is increased. In this way, the presence of specific mechanisms of regulation such as cell volume regulation are necessary for the survival of the insect. In isosmotic conditions, cell volume regulation is explained by the "pump-leak" hypothesis in which the $(Na^+ + K^+)$ATPase is crucial for maintaining Na^+ and K^+ gradients (Leaf 1959, Tosteson & Hoffman 1960). Furthermore, $(Na^+ + K^+)$ATPase is also involved in cell volume regulation during anisosmotic shock (Hoffmann & Dunham 1995). During cell volume regulation there is a variation in the amount of osmotic active solute inside the cell (Hoffmann & Dunham 1995). Variation of the medium osmolality regulates several transport proteins (Yancey *et al.* 1982). Arenstein *et al.* (1995) have shown, through video-optical techniques, that proximal cells of the Malpighian tubule of *Rhodnius neglectus* exposed to hyperosmotic medium regulate their volume with a typical regulatory volume increase (RVI). On the other hand, when these cells are exposed to hyposmotic medium they are unable to regulate their volume completely. The addition of ouabain 1 mM did not change the RVI. Latter, we observed that hyperosmotic shock inhibited the $(Na^+ + K^+)$ATPase activity but did not change the Na^+-ATPase activity (Caruso-Neves *et al.* 1998a, Figure 2). So it is possible to postulate that cell volume regulation during hyperosmotic shock involves the inhibition of the $(Na^+ + K^+)$ATPase activity. This effect leads to active osmotic solute accumulation inside the cell, influx of water and to the return of the normal cell volume.

Adenosine is found in all living cells as part of the normal metabolic machinery and appears to be accumulated in different tissues in response to different stress conditions (Osswald *et al.* 1977, Olsson 1990). In addition, it has been observed that one of adenosine effects during stress is the modulation of ionic transport (Caruso-Neves *et al.* 1997). Furthermore, it was observed that adenosine increases fluid secretion in Malpighian tubule of *D. melanogaster* (Riegel *et al.* 1998). Recently, we tested the effect of adenosine on the $(Na^+ + K^+)$ATPase activity of Malpighian tubule cells from *Rhodnius prolixus* and found that adenosine inhibits the enzyme activity in a dose dependent manner (Caruso-Neves *et al.* 2000).

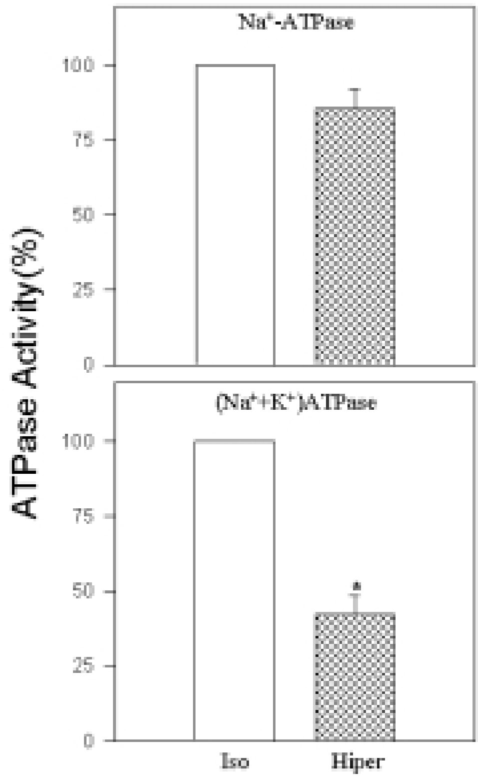

Fig. 2 – Effect of hyperosmotic shock on Na^+-ATPase and $(Na^+ + K^+)$ATPase activities. The ATPase activity was measured as described by Caruso-Neves *et al.* (1998a). The final osmolality was adjusted to 320 mOsm/kg for the isosmotic solution (open bars) or to 500 mOsm/kg for the hyperosmotic solution (dashed bars) by addition of mannitol. The Na^+-ATPase activity was calculated from the difference between the ATPase activity in the absence and in the presence of 2 mM furosemide, both in the presence of 1 mM ouabain. The $(Na^+ + K^+)$ATPase activity was calculated from the difference between the ATPase activity in the absence and in the presence of ouabain.

Taken together these data indicate that the $(Na^+ + K^+)$ATPase of the Malpighian tubule of *Rhodnius sp.* is involved in insect water and ion balance, just as it is in mammalian tissues. On the other hand, the Na^+-ATPase role in the insect physiology is still not clear.

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