The Invertebrate Electrogenic $2Na^+/1H^+$ Exchanger: Polyfunctional Epithelial Workstation

Gregory A. Ahearn

Invertebrate epithelial cells from gut, kidney, and gills possess an electrogenic brush-border $2Na^+/1H^+$ antiporter protein that is analogous to the vertebrate electroneutral $1Na^+/1H^+$ exchanger but that performs an extensive array of transport functions because of its electrogenic nature and wide substrate range involving both monovalent and divalent cations.

Vertebrate epithelial and nonepithelial cells possess a Na^+/H^+ exchange protein in their plasma membranes that catalyzes the net uptake of extracellular sodium for the net extrusion of cytoplasmic protons (12). Reported biologic functions of this antiport mechanism largely stem from its relatively restricted affinity to monovalent cations and include regulation of intracellular pH, cell volume, and transepithelial ion transport. In recent years, largely as a result of the cloning efforts of J. Pouyssegur and associates, at least four structural isoforms of this transporter, termed NHE1-4, have been described and their amino acid sequences published (9).

During the last 5 years, a second class of Na^+/H^+ antiporter has been described from invertebrate epithelial cells using radioisotopic and fluorometric methods. This transporter performs the same monovalent cation exchange function as reported for the vertebrate NHE isoforms but conducts this process in an electrogenic fashion due to its $2Na^+/1H^+$ transport stoichiometry (1–3, 5, 14). In addition, as a result of a significant affinity for divalent cations, this protein also binds and transports calcium and a variety of heavy metals, thereby departing functionally from the vertebrate electroneutral $1Na^+/1H^+$ paradigm. This report summarizes our present information about this new class of Na^+/H^+ exchange proteins in invertebrate cells and discusses their potential roles in a variety of physiological activities, including transport of both monovalent and divalent cations.

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Electrogenic $2Na^+/1H^+$ and electroneutral $1Na^+/1H^+$ exchangers can occur in the same invertebrate epithelial cells

Purified brush-border and basolateral membrane preparations of gastrointestinal epithelial cells from crustaceans and echinoderms were prepared using standard centrifugation methods (8). Vesicles were loaded with high proton concentrations and were used in $^{22}Na^+$ influx measurements (as $^{22}Na^+/H^+$ exchange) at a variety of external sodium concentrations to determine the nature of the exchange process at each pole of the respective epithelium. Figure 1 shows that sigmoidal relationships between the variables were obtained for gastrointestinal brush-border membranes from freshwater and marine crustaceans and from the marine starfish, whereas hyperbolic $^{22}Na^+/H^+$ exchange by hepatopancreatic basolateral membrane from the seawater lobster resulted from the same experimental protocol (8). The sigmoidal appearance of the brush-border data for each tissue examined was shown to be due to the operation of an exchange process that responded to a transmembrane electrical potential, was inhibited by amiloride, and transferred two sodium ions into the vesicle with each outwardly flowing proton (1, 3, 5). The different positions of the curves on Fig. 1 for freshwater and marine epithelia suggest the possible accommodation of the antiport process to environmental salinity. A similar amiloride-sensitive, sigmoidal $^{22}Na^+/H^+$ antiport, resulting from electrogenic cation exchange, was also reported for lobster renal epithelial brush-border membranes, providing support for the notion of a general epithelial transport system rather than one limited to the invertebrate gastrointestinal tract (2).

". . . a second class of Na^+/H^+ antiporter has been described from invertebrate epithelial cells . . ."

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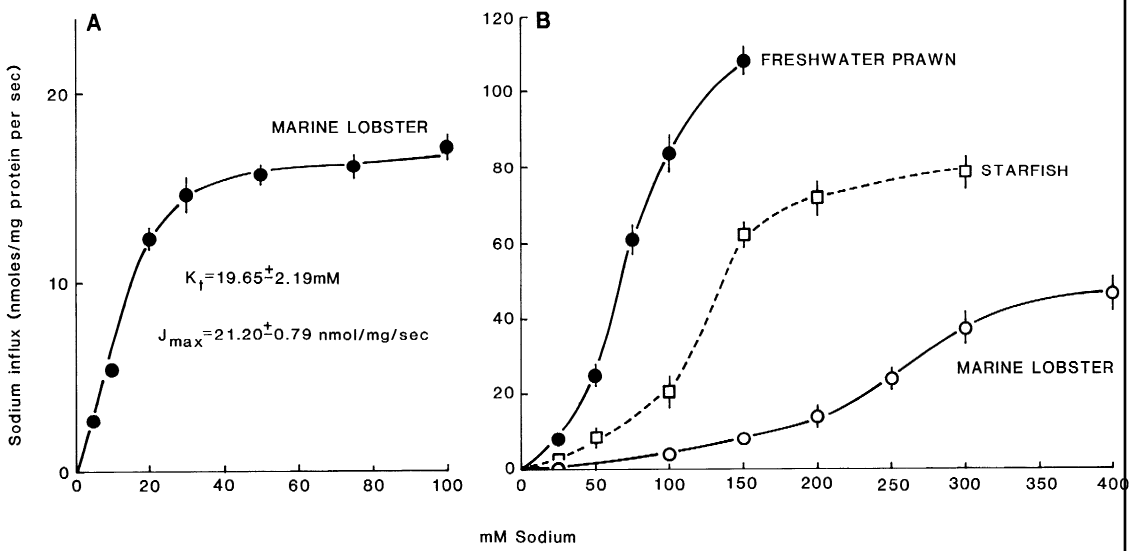

FIGURE 1. *A*: $^{22}Na^+/H^+$ exchange kinetics of lobster hepatopancreatic epithelium basolateral membrane vesicles (BLMV). Vesicles were loaded at pH 5.5 with an inside-negative membrane potential (K^+/valinomycin) and were incubated for 2.5 s in medium at pH 8.0 containing variable concentrations of $^{22}Na^+$ gluconate from 5 to 100 mM. Hyperbolic line and apparent influx kinetics were obtained from an iterative computer curve-fitting program. J_{max}, maximum flux; K_t, [Na^+] at 1/2 J_{max}. *B*: $^{22}Na^+/H^+$ exchange kinetics of invertebrate hepatopancreatic or pyloric cecal epithelial brush-border membrane vesicles (BBMV). Vesicles were loaded as above and were incubated for 2.5 s in medium at pH 8.5 containing variable concentrations of $^{22}Na^+$ gluconate from 5 to 400 mM. Sigmoidal lines were obtained from an iterative computer curve-fitting program. Values are means ± SE of 3 replicates. [From Ahearn et al. (8), with permission from Company of Biologists Ltd.]

". . . the electrogenic $2Na^+/1H^+$ antiporter may also serve as a primary means of hemolymph sodium regulation . . ."

Basolateral $^{22}Na^+/H^+$ antiport by lobster hepatopancreatic epithelium departed significantly from that described for the brush-border membrane of the same tissue. In this instance, the hyperbolic nature of the antiport process resulted from an amiloride-sensitive, electroneutral $1Na^+/1H^+$ exchange resembling that reported for all the vertebrate NHE isoforms (8). These findings suggest that invertebrate gastrointestinal and renal epithelia display two different classes of Na^+/H^+ antiporters in their plasma membranes, performing monovalent cation exchange by either electrogenic or electroneutral means, using apparently distinct types of proteins.

Role of electrogenic $2Na^+/1H^+$ exchange in gastric acidification and transcellular Na^+ transport

The asymmetrical arrangement of cation binding sites on the internal and external faces of the invertebrate $2Na^+/1H^+$ exchanger leads to transmembrane flux ratios for the cations very different from those that occur for virtually all analogous mammalian antiporters. The presence of the second sodium-binding site and resulting electrogenicity of the exchange event confer on the invertebrate transporter the thermodynamic capacity of moving protons out of the epithelium against a 10,000- to 100,000-fold concentration gradient (cell pH, 7.0; lumen pH, 2.0). In crustaceans, the contents of the stomach lumen, receiving secretions from the hepatopancreatic epithelium by way of the hepatopancreatic ducts, have been measured at pH 4.0–5.0 during digestion (10), and it is clear that the epithelial electrogenic antiporter is thermodynamically capable of providing the necessary protons for this activity. It is likely, therefore, that a primary role of this brush-border transport system in invertebrates is acid secretion.

In addition to moving hydrogen ions out of invertebrate epithelial cells against considerable concentration gradients, the electrogenic $2Na^+/1H^+$ antiporter may also serve as a primary means of hemolymph sodium regulation, particularly in freshwater species that are faced with continual ion losses to the environment. *Macrobrachium rosenbergii*, the freshwater Malaysian prawn, has a blood sodium concentration of 219 mM (6) and possesses an electrogenic $2Na^+/1H^+$ antiporter in its hepatopancreatic brush-border membrane (Fig. 1; Ref. 5), which likely works in conjunction with a basolateral Na^+-K^+-ATPase to bring about the net transfer of sodium ions from lumen to blood. The role of this brush-border antiporter in transcellular monovalent cation transport may not be as important in marine species as in freshwater animals, since the former have blood sodium concentrations quite similar to seawater. However, as the following section suggests, the electrogenic exchanger is likely equally important in freshwater and marine crustacean species for transcellular calcium transport, particularly during specific

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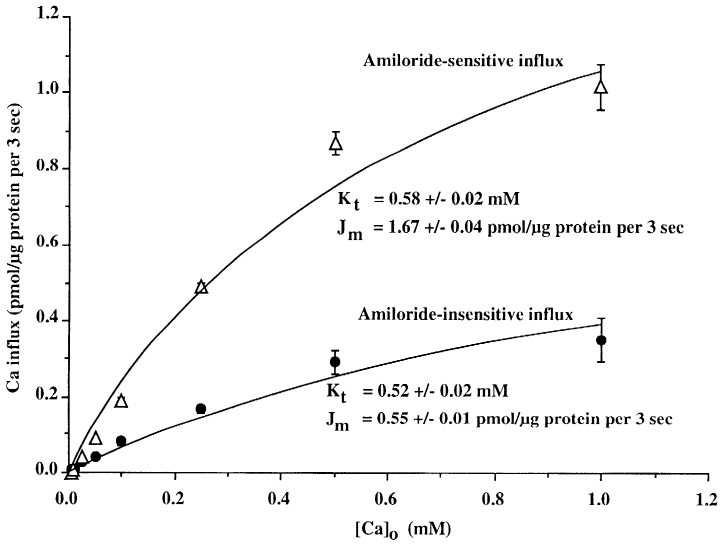

FIGURE 2. Amiloride-sensitive and amiloride-insensitive components of carrier-mediated $^{45}Ca^{2+}$ influx in short-circuited lobster hepatopancreatic brush-border membrane vesicles. Data were obtained by subtraction of amiloride-insensitive carrier influx from total carrier influx. Kinetic constants are as defined in Fig. 1 and were obtained using a computer curve-fitting program and Michaelis-Menten equation. Values are means ± SE of 3 replicates. $[Ca]_o$, extracellular Ca^{2+} concentration.

stages of the molt cycle when large fluxes of this divalent cation occur across all epithelial cell layers.

Role of the electrogenic $2Na^+/1H^+$ exchanger in calcium transport and sequestration

Recent work characterizing the electrogenic $2Na^+/1H^+$ antiporter of lobster antennal gland epithelial brush-border membranes indicated that exogenous calcium strongly inhibited $^{22}Na^+/H^+$exchange by an electrogenic, amiloride-sensitive transport system (2). This inhibition was competitive in nature, and later studies confirmed that in epithelia of both antennal glands and hepatopancreas a major calcium uptake pathway into these cells occurred by calcium displacing sodium from the $2Na^+/1H^+$ antiporter (4, 7, 8). Figure 2 illustrates a kinetic analysis of $^{45}Ca^{2+}/H^+$ antiport in lobster hepatopancreatic brush-border membrane vesicles (BBMV) and indicates that the divalent cation gains access to the epithelial cytosol by way of amiloride-sensitive and amiloride-insensitive carrier-mediated pathways (7). Once in hepatopancreatic epithelial cells, calcium appears to have at least two potential fates. First, efflux of the divalent cation can occur out the basolateral side of the epithelium to the blood by way of either a low-affinity Ca^{2+}/Na^+ exchanger of unknown stoichiometry or through a high-affinity, vanadate-inhibited Ca^{2+}-ATPase (7). A second fate of cytoplasmic calcium in lobster hepatopancreas is sequestration within membrane-bound calcium phosphate granules by a process that appears to be linked to the molt cycle of these animals. The crustacean hepatopancreatic epithelium is known to be a cyclic storage site for exoskeletal calcium during the premolt phase of ecdysis. As an animal approaches molting, much of the calcium that was originally contained in the exoskeleton is transferred to the blood and either lost to the environment, across the gills and other permeable sites, or stored as calcium phosphate granules in the hepatopancreatic epithelium and elsewhere (11). Calcium storage in these granules likely also occurs as a result of luminally derived calcium from the diet via the electrogenic $2Na^+/1H^+$ antiporter. After the animal sheds its old exoskeleton, the most immediate source of calcium for the new shell is that still located in the discarded shell. Many crustaceans eat their old exoskeleton after the molt and therefore obtain needed calcium to harden the newly formed shell by transfer across the hepatopancreatic epithleium by transcellular transport involving the transport processes described above.

"Many crustaceans eat their old exoskeleton after the molt . . ."

Role of the electrogenic $2Na^+/1H^+$ exchanger in heavy metal detoxification

Both terrestrial and aquatic invertebrate epithelial cells are known to possess a variety of endocytic vacuoles containing a variety of concretions composed of divalent and trivalent cations complexed with anions (13). The calcium phosphate granules discussed above are one type of concretion vacuole found in cells of crustaceans. In addition, other types of membrane-bound concretions are found in gut, renal, and gill epithelial cells of these animals and contain a variety of divalent and trivalent heavy

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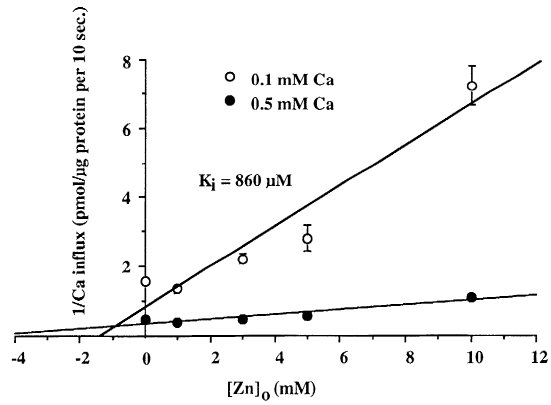

FIGURE 3. Dixon plot of an experiment to demonstrate effects of variable external Zn^{2+} concentrations on $^{45}Ca^{2+}/H^+$ exchange in short-circuited lobster hepatopancreatic brush-border membrane vesicles. Vesicles were loaded with 100 mM mannitol, 100 mM K^+ gluconate, and 50 μM valinomycin at pH 5.5 and were incubated for 10 s in medium at pH 8.5 containing either 0.1 or 0.5 mM $^{45}Ca^{2+}$ gluconate, 100 mM mannitol, 100 mM K^+ gluconate, and one of the following concentrations of $ZnCl_2$: 0, 1.0, 3.0, 5.0, and 10.0 mM. Lines were drawn by linear regression analysis. Values are means ± SE of 3 replicates. K_i, inhibitory constant; $[Zn]_O$, extracellular Zn^{2+} concentration.

metals complexed with both phosphate and sulfate. Concentrations of metals in vacuolar concretions can be considerably higher than environmental concentrations of the individual metal. Heavy metal concretion formation is a detoxification mechanism used by these animals to remove and isolate potentially lethal substances from the blood or food. Such concretions are eventually eliminated from the storage cells by exocytosis into the gastrointestinal lumen, where they are voided from the animal in the feces.

"Heavy metal concretion formation is a detoxification mechanism . . ."

Recent studies with lobster and starfish gastrointestinal epithelial brush-border membrane vesicles indicated that the heavy metals zinc and cadmium were alternative substrates for sodium or calcium on the electrogenic $2Na^+/1H^+$ antiporters in the respective animals (8, 15). Figure 3 shows that in lobster BBMV zinc was a competitive inhibitor of $^{45}Ca^{2+}/H^+$ exchange [inhibitory constant (K_i) = 860 μM]. A similar study with starfish BBMV indicated that external zinc was a mixed inhibitor of the exchange process in this animal with a K_i of 920 μM (15). The intracellular fate of cadmium or zinc in either lobster or starfish epithelia is unclear at the present time, but the presence of heavy metal concretions in these animals suggests that physiological mechanisms are present in the two animals for sequestering these potentially harmful materials.

Working model for a polyfunctional role of the invertebrate electrogenic $2Na^+/1H^+$ antiporter

Figure 4 is a working model of the polyfunctional involvement of the invertebrate electrogenic $2Na^+/1H^+$ antiporter in a wide variety of biologic activities of gut and renal epithelial cells. At least four major biologic functions are served by ion transport using this exchange protein: *1*) luminal acidification, *2*) transcellular sodium and calcium transport, *3*) calcium uptake and sequestration, and *4*) heavy metal detoxification. Because of its exchange function and transport stoichiometry, luminal acidification against a steep hydrogen ion concentration may occur in epithelial cells at the same time that

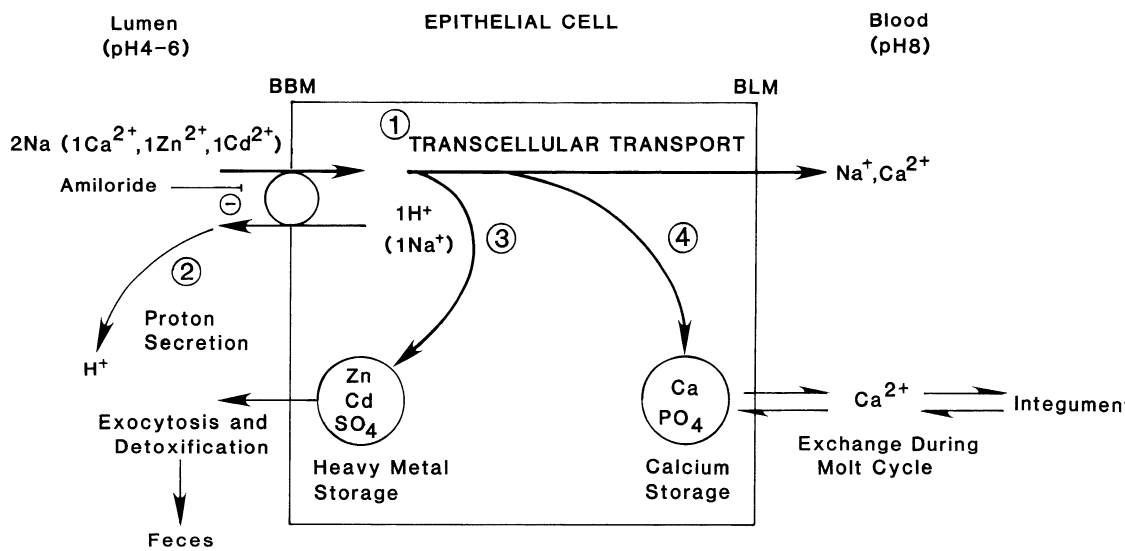

FIGURE 4. Working model of polyfunctional role of invertebrate electrogenic $2Na^+/1H^+$ antiporter for absorption, secretion, and sequestration of monovalent and divalent cations in crustacean hepatopancreatic epithelium. Involvement of brush-border exchanger with the 4 physiological activities of this tissue shown is described in text. Some or all of these processes are common to a wide variety of invertebrate epithelial cells. BBM, brush-border membrane; BLM, basolateral membrane.

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sodium and calcium absorption from lumen to blood is taking place. As a result of this simultaneous operation, digestion and organismic osmoregulation may both be strongly influenced by brush-border electrogenic monovalent and divalent cation exchange.

Calcium uptake from food or from a molted exoskeleton by hepatopancreatic cells occurs largely via the electrogenic $2Na^+/1H^+$ antiporter. This activity is not subserved by vertebrate electroneutral $1Na^+/1H^+$ antiporters likely because of differential substrate specificities between the two classes of exchangers and because of molecular structural differences in the external cation binding sites of the two proteins. The transport of calcium by the invertebrate exchangers may be a result of external cation binding sites that can either accommodate two monovalent cations or single divalent cations, whereas the vertebrate external binding site appears only suited for specific monovalent cations. On entry into the epithelial cytosol by way of the electrogenic $2Na^+/1H^+$ antiporter, calcium may either be translocated across the cell and transported to the blood across the basolateral membrane or be sequestered in calcium phosphate concretion vacuoles until needed during molting.

The invertebrate electrogenic $2Na^+/1H^+$ antiporter is a significant avenue of heavy metal uptake into animals from the environment. Certain metals, such as zinc, are required by animals in trace amounts that are needed in some instances for optimal enzyme function. Other metals (e.g., cadmium), however, have no known biologic function. Both enter invertebrate epithelial cells by this transport system and subsequently can take part in metabolic activites or become sequestered in concretion vacuoles for later exocytosis. Sequestration of potentially toxic metals in these vacuoles of the gut, or other epithelial cells, may prevent these toxic substances from entering the blood and impairing nerve and muscle function.

Whereas the invertebrate electrogenic $2Na^+/1H^+$ antiporter is analogous to the vertebrate electroneutral $1Na^+/1H^+$ antiporter and performs a transmembrane exchange of the respective monovalent cations, it is clear that because of its electrogenicity and anatomically complex external cation binding site it is able to conduct several physiological activities outside the scope of the vertebrate system. This report has focused on some of the functions of this transport system in those animals and cell types where it has been investigated. Future investigations may lead to a fuller understanding of the diversity of biologic roles played by this membrane protein and how its activities may be regulated to meet the overall demands of the organism.

"The invertebrate . . . antiporter is a significant avenue of heavy metal uptake . . ."

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