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PARALLEL ANTIPORT MECHANISMS FOR Na^+ AND Cl^- TRANSPORT IN HERBIVOROUS TELEOST INTESTINE

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SUMMARY

Transport mechanisms for NaCl in intestinal brush border membrane vesicles (BBMV) of herbivorous, seawater-adapted tilapia, *Oreochromis mossambicus*, were investigated with a rapid filtration technique using $^{22}Na^+$ and $^{36}Cl^-$ as tracers. Evidence to support the existence of cotransport mechanisms (Na^+/Cl^- or $Na^+/K^+/2Cl^-$) in tilapia intestinal BBMV was not found. An inwardly directed Cl^- gradient or KCl gradient did not stimulate the uptake of Na^+ into tilapia intestinal BBMV. Furthermore, the uptake of Na^+ under these conditions was not significantly affected by furosemide. Evidence was found to indicate the existence of Na^+/H^+ and Cl^-/HCO_3^- antiport mechanisms for NaCl transport in tilapia intestinal BBMV. An outwardly directed proton gradient (pH 6·0 in, pH 7·5 out) stimulated the uptake of Na^+ above that of the control (pH 7·5 in, pH 7·5 out). Exogenous amiloride (1 mmol l^{-1}) significantly reduced Na^+ uptake in the presence of an outwardly directed proton gradient. Apparent influxes of 1 mmol l^{-1} Na^+ were (nmol mg protein^{-1} 15 s^{-1} ± 1 S.E.): control, 1·46 ± 0·09; pH gradient, 3·36 ± 0·14; pH gradient plus amiloride, 1·55 ± 0·05. The uptake of Cl^- was stimulated by outwardly directed HCO_3^- and Cl^- gradients in comparison with an outwardly directed gluconate gradient. Apparent influxes of 10 mmol l^{-1} Cl^- were (nmol mg protein^{-1} 15 s^{-1} ± 1 S.E.): gluconate, 12·90 ± 0·19; HCO_3^-, 15·83 ± 0·34; Cl^-, 14·62 ± 0·42. These results suggest that, in contrast to the cotransport mechanisms for NaCl that have been reported for other fish intestine, the herbivorous marine teleost, *Oreochromis mossambicus*, possesses dual antiport mechanisms for the transport of NaCl across its intestinal brush border membrane.

INTRODUCTION

The intestine of marine teleost fish actively absorbs NaCl and water from the lumen as a part of the osmoregulatory process that takes place in these animals (Ando, 1981; Skadhauge, 1974). The transport mechanism by which NaCl traverses the brush border membrane of intestinal epithelial cells has not been studied in a wide variety of marine teleosts. There are currently four mechanisms by which the

Key words: brush border membrane vesicles, NaCl transport, cotransport, antiport, intestinal physiology, membrane transport, fish, tilapia, *Oreochromis mossambicus*.

electroneutral transport of NaCl across epithelial cell membranes is known to occur. One mechanism involves the cotransport of Na^+, K^+ and Cl^- and has been found in the shark rectal gland (Hannafin, Kinne-Saffran, Friedman & Kinne, 1983), flounder intestine (Eveloff, Field, Kinne & Murer, 1980; Musch *et al.* 1982) and other tissues (Epstein & Silva, 1985). A second mechanism of NaCl transport is K^+-independent cotransport of Na^+ and Cl^-. This mechanism has been found in flounder urinary bladder (Stokes, 1984) and *Necturus* gallbladder (Ericson & Spring, 1982). A third mechanism of NaCl transport involves the action of dual antiporters: Na^+/H^+ antiport and Cl^-/OH^- or Cl^-/HCO_3^- antiport. This mechanism has been found in rat and rabbit small intestines (Knickelbein, Aronson, Atherton & Dobbins, 1983; Knickelbein *et al.* 1985; Liedtke & Hopfer, 1982*a*,*b*) and *Necturus* proximal tubules (Seifter & Aronson, 1984). The final mechanism for NaCl transport involves the action of Na^+/H^+ antiport and $Cl^-/formate^-$ antiport. This mechanism has been found in rabbit renal cortex (Karniski & Aronson, 1985).

The transport of NaCl across the brush border membrane of the intestinal epithelium of marine teleosts could occur by any or all of these four mechanisms. The only transport mechanism that has been documented in marine teleost fish is the $Na^+/K^+/2Cl^-$ mechanism in the winter flounder, *Pseudopleuronectes americanus* (Eveloff *et al.* 1980; Musch *et al.* 1982; Halm, Krasny & Frizzell, 1985). Whether other marine teleosts use this mechanism for NaCl transport or one of the other three has not been documented. This study was undertaken to determine the mechanism(s) of NaCl transport across the brush border membrane of intestinal epithelial cells in *Oreochromis mossambicus*, a herbivorous, euryhaline species of tilapia.

MATERIALS AND METHODS

Seawater-adapted adult tilapia (100–150 g) were captured locally and kept for 1 day prior to being used. Each animal was killed by a blow to the head, and the upper intestine removed and placed in 15 ml of buffer 1 [300 mmol l^{-1} mannitol; 60 mmol l^{-1} Hepes/Tris, 6 mmol l^{-1} EGTA, 0·1 mmol l^{-1} phenylmethylsulphonylfluoride (PMSF), pH 7·4]. After the tissue had been removed from the animals, 60 ml of ice-cold deionized water was added to the 15 ml of buffer 1. This mixture was immediately homogenized for 15 s in a polytron. Magnesium chloride was added to the homogenate to give a final concentration of 15 mmol l^{-1}, and the resulting mixture was kept on ice for 15 min. After 15 min the mixture was centrifuged at 3000 ***g*** for 15 min. The supernatant was then centrifuged at 27 000 ***g*** for 30 min. This pellet was resuspended in 30 ml of ice-cold buffer 2 (60 mmol l^{-1} mannitol, 12 mmol l^{-1} Hepes/Tris, 6 mmol l^{-1} EGTA, pH 7·4) using a Potter–Elvehjem homogenizer. The magnesium chloride precipitation and two centrifugations were repeated on the resuspended pellet. The pellet resulting from this second set of spins was resuspended in 30 ml of ice-cold transport buffer (see figure legends for composition), and this mixture was centrifuged at 27 000 ***g*** for 30 min. The final pellet was resuspended in transport buffer at a protein concentration of approximately 10 mg ml^{-1}.

Purity of the brush border membrane vesicles (BBMV) obtained from this procedure was determined by measuring the activity of membrane marker enzymes in the final pellet and in the homogenate. The activities of alkaline phosphatase, Na^+/K^+-stimulated ATPase, K^+-stimulated *p*-nitrophenylphosphatase, and cytochrome *c* oxidase were determined. Alkaline phosphatase activity was determined using Sigma chemical assay kit no. 104. Na^+/K^+-stimulated ATPase activity was determined using the coupled assay reaction of Berner, Kinne & Murer (1976). K^+-stimulated *p*-nitrophenylphosphatase activity was determined using the procedures of Arvan & Castle (1982). Cytochrome *c* oxidase activity was determined using the procedures of Cooperstein & Lazarow (1951).

The uptake of $^{22}Na^+$ and $^{36}Cl^-$ by BBMV was measured by the Millipore membrane filtration technique of Hopfer, Nelson, Perrotto & Isselbacher (1973). The compositions of the transport buffer inside the vesicles and the incubation medium outside the vesicles are indicated in each figure. Transport experiments were started by adding a volume of membrane vesicles (e.g. 20 μl) to a volume of radiolabelled incubation medium (e.g. 180 μl) to make a reaction mixture. At predetermined incubation times (i.e. 15 s, 1·5, 3, 5 and 120 min) a known volume of the reaction mixture (20 μl) was removed and injected into 2 ml of ice-cold stop solution to stop the reaction. The composition of the stop solution varied according to the experimental conditions. Its composition was usually the same as the composition of the external control medium. The stop solution and reaction mixture sample were immediately filtered through a 0·65 μm Millipore filter and washed with 8 ml of ice-cold stop solution. The filter was then placed in a scintillation vial and 5 ml of Beckman Ready Solv HP scintillation cocktail was added. All samples were counted in a Beckman LS-8100 scintillation counter. Each experiment consisted of a minimum of three replicates per time point per treatment. Each experiment was repeated at least once to verify the results. The results of only one of these repeated experiments are presented here as means ± 1 S.E. for each time point. Treatments were compared using Duncan's multiple range test.

The amount of protein present in each experiment was determined by the Coomassie blue dye binding assay (Bio-Rad) as developed by Bradford (1976). A mixture of human albumin (50 mg ml^{-1}) and human globulin (30 mg ml^{-1}) was used for the protein standard.

$^{22}NaCl$ was obtained from New England Nuclear (Boston, MA). $H^{36}Cl$ was obtained from ICN Biomedicals, Inc. (Irvine, CA). Valinomycin, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), amiloride, furosemide and the protein standard were obtained from Sigma Chemical (St Louis, MO).

RESULTS

Enzymatic purity of the vesicle preparation

Comparisons of the activities of alkaline phosphatase, Na^+/K^+-stimulated ATPase, K^+-stimulated *p*-nitrophenylphosphatase and cytochrome *c* oxidase in the final pellet and the homogenate are presented in Table 1. Alkaline phosphatase is a

Table 1. *Enzymatic characterization of upper intestinal brush border membrane vesicles from seawater-adapted tilapia* Oreochromis mossambicus

Enzyme	Activity		Purification factor	Enzyme recovery (%)	Number of animals
	Homogenate (μmol mg^{-1} h^{-1})	Vesicles (μmol mg^{-1} h^{-1})			
Alkaline phosphatase	2·71 ± 0·09	32·58 ± 0·20	12·02 ± 0·31	29·8	3
Na^+/K^+-ATPase	2·0 ± 0·13	3·2 ± 0·37	1·62 ± 0·09	4·4	4
K^+-stimulated *p*-nitrophenyl-phosphatase	0·265 ± 0·038	0·269 ± 0·003	1·02 ± 0·19	2·7	4
Cytochrome *c* oxidase	1·27 ± 0·20	1·11 ± 0·02	0·87 ± 0·13	2·4	4

Values are means ± S.E.
Enzyme activities are in μmol product released mg protein^{-1} h^{-1}.
Purification factors are means of individual vesicles activities ÷ individual homogenate activities.
Enzyme recoveries represent comparisons between homogenate and vesicle contents.
Measurements were performed on different membrane preparations.

marker for the brush border membrane, and its activity was increased 12-fold in the final pellet. Na^+/K^+-stimulated ATPase and K^+-stimulated *p*-nitrophenylphosphatase are markers for basolateral membranes, and their activities were increased 1·62- and 1·02-fold, respectively, in the final pellet. Cytochrome *c* oxidase is a marker for mitochondrial membranes, and its activity was increased 0·87-fold in the final pellet. These results indicate that the final pellet was enriched in brush border membranes and was not appreciably contaminated with either basolateral or mitochondrial membranes. It should be noted here that, since the final pellet was not 100 % brush border membranes, the transport mechanisms that were being investigated here could be resident in some other minor contaminating membrane population (basolateral, mitochondrial, etc.), as well as being in the brush border membrane population.

Osmotic reactivity of the vesicle preparation

To substantiate that BBMV were closed and osmotically reactive, the equilibrium uptake of 1 mmol l^{-1} Na^+ was measured in the presence of increasing external raffinose concentrations (Fig. 1). If the vesicles were osmotically reactive, then as the external concentration of raffinose was increased the vesicles should shrink and hold less isotope. As shown in Fig. 1, the regression of Na^+ uptake on the reciprocal of medium osmolarity was significant ($P < 0·01$), indicating that the vesicles were osmotically reactive. The estimate of the slope is 0·64 ± 0·04 nmol mg protein^{-1} mosmol^{-1}), and the estimate of the intercept is 1·04 ± 0·09 nmol mg protein^{-1}. The intercept indicates the amount of Na^+ uptake that is independent of intravesicular space (binding). A considerable amount of the Na^+ uptake in Fig. 1 is due to the

binding of Na^+ to the vesicles. For example, at 300 mosmol l^{-1}, 33 % of the Na^+ uptake is due to binding.

Test for the cotransport models

To test for the existence of the Na^+/Cl^- cotransport model or the $Na^+/K^+/2Cl^-$ cotransport model in BBMV of tilapia upper intestine, the uptake of Na^+ was measured in the presence of different ion gradients. As shown in Fig. 2, the uptake of Na^+ was not stimulated by a Cl^- gradient or by a KCl gradient into the vesicles when compared with the control (gluconate). These gradients would be expected to stimulate Na^+ uptake if the mechanism of Na^+ uptake were Na^+/Cl^- cotransport or $Na^+/K^+/2Cl^-$ cotransport. Also, the uptake of Na^+ in the presence of a KCl gradient was not significantly affected by furosemide, which is known to affect the $Na^+/K^+/2Cl^-$ cotransport mechanism (Epstein & Silva, 1985; Hannafin *et al.* 1983). These results provide strong evidence against the presence of these two cotransport mechanisms for NaCl in the apical membrane of tilapia intestinal epithelial cells.

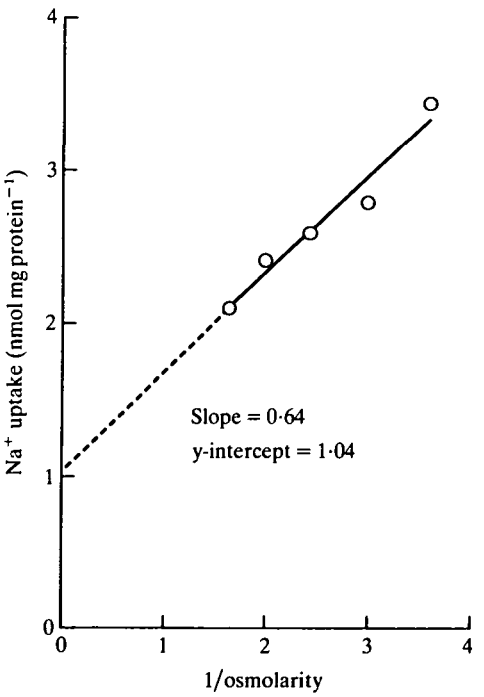

Fig. 1. The effect of increasing osmolarity on the uptake of sodium into brush border membrane vesicles from tilapia upper intestine. The vesicles contained 50 mmol l^{-1} mannitol, 50 mmol l^{-1} tetramethylammonium chloride (TMACl) and 20 mmol l^{-1} Hepes/Tris, pH 7·0. The reaction medium contained 50 mmol l^{-1} mannitol, 49 mmol l^{-1} TMACl, 1 mmol l^{-1} NaCl, 20 mmol l^{-1} Hepes/Tris, pH 7·0 and increasing concentrations of raffinose.

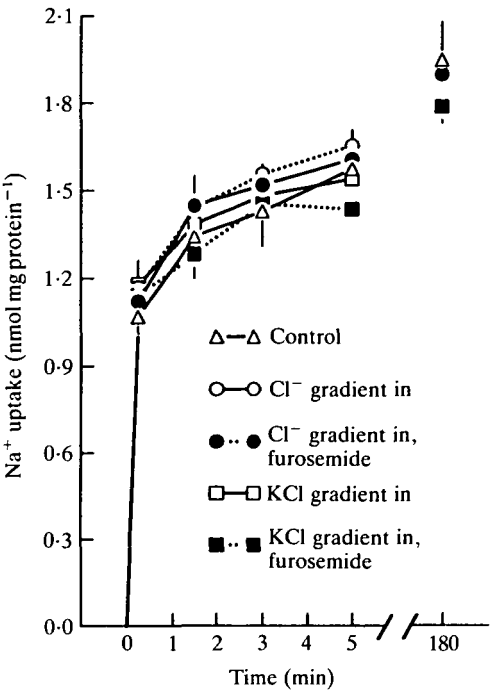

Fig. 2. The effect of chloride, potassium chloride and furosemide on the uptake of sodium into brush border membrane vesicles from tilapia upper intestine. The vesicles contained 200 mmol l^{-1} tetramethylammonium (TMA) gluconate, 100 mmol l^{-1} potassium gluconate, 12 mmol l^{-1} Hepes/Tris, pH 7·4 plus 10 μmol l^{-1} CCCP. The reaction media contained 0·5 mmol l^{-1} sodium gluconate and were the same as the vesicle medium for the control (triangles) and Cl^- treatments (open and closed circles) except that the Cl^- treatments had 100 mmol l^{-1} KCl in place of 100 mmol l^{-1} potassium gluconate. The KCl treatments had 100 mmol l^{-1} TMA gluconate, 100 mmol l^{-1} potassium gluconate, 100 mmol l^{-1} KCl and 12 mmol l^{-1} Hepes/Tris, pH 7·4 (open and closed squares). To test the effect of 0·5 mmol l^{-1} furosemide (closed symbols) the vesicles were pre-incubated with furosemide for 1 min before uptake was started.

Test for the antiport models

With evidence to suggest that the cotransport models discussed above were not the mechanisms of NaCl transport in tilapia intestinal BBMV, experiments to test for the existence of ion antiporters in tilapia intestinal BBMV were performed next. To test for the presence of a Na^+/H^+ antiporter, the uptake of Na^+ was measured in the presence and absence of a proton gradient directed out of the vesicles. The internal and external solutions for this experiment did not contain Cl^- salts. Fig. 3 shows that the uptake of Na^+ in the presence of an outwardly directed proton gradient (pH 6·0 in, pH 7·5 out) was much greater than that of the control (pH 7·5 in, pH 7·5 out). This stimulation was inhibited by 1 mmol l^{-1} amiloride which is known to inhibit Na^+/H^+ antiporters. Apparent influxes of 1 mmol l^{-1} Na^+ were (nmol mg protein^{-1} 15 s^{-1} ± 1 S.E.): control, 1·46 ± 0·09; pH gradient, 3·36 ± 0·14; pH

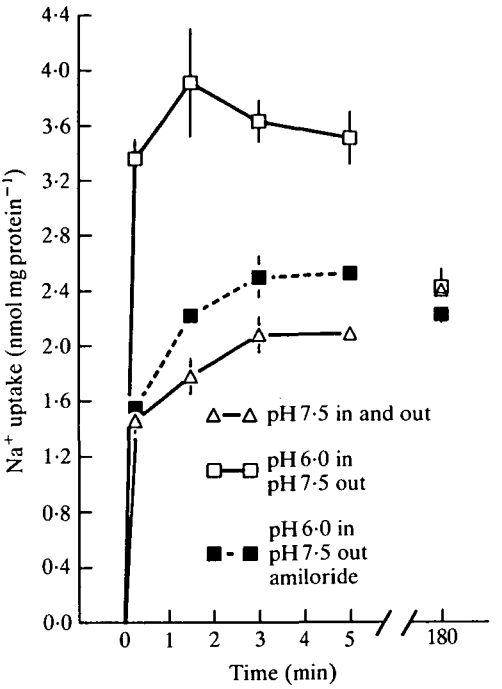

Fig. 3. The effect of a proton gradient and amiloride on the uptake of sodium into brush border membrane vesicles from tilapia upper intestine. The vesicles contained the following: 100 mmol l^{-1} tetramethylammonium (TMA) gluconate, 100 mmol l^{-1} potassium gluconate, valinomycin (15 μg mg protein^{-1}) and either 50 mmol l^{-1} Hepes/Tris, pH 7·5 (triangles) or 50 mmol l^{-1} Mes/Tris, pH 6·0 (squares). The reaction media contained 1 mmol l^{-1} sodium gluconate, 100 mmol l^{-1} TMA gluconate, 100 mmol l^{-1} potassium gluconate, 50 mmol l^{-1} Hepes/Tris, pH 7·5. The amiloride treatment (closed squares) contained 1 mmol l^{-1} amiloride in addition to the above.

gradient plus amiloride, 1·55 ± 0·05. At 15 s the pH gradient treatment was significantly different ($P < 0·05$, Duncan's multiple range test) from the control, while the pH gradient plus amiloride treatment was not. The various treatments in this experiment were short-circuited with valinomycin so that it could not be argued that the stimulation was due to diffusion potentials created by the diffusion of protons out of the vesicles, which in turn set up an electrical gradient that attracted Na^+ into the vesicles. These results support the existence of a Na^+/H^+ antiporter in tilapia intestinal BBMV.

To test for the existence of a Cl^-/anion antiporter in tilapia intestinal BBMV, the uptake of Cl^- into vesicles containing different outwardly directed anion gradients was measured. The internal and external solutions for this experiment did not contain Na^+ salts. Fig. 4 indicates that the uptake of Cl^- into these vesicles was stimulated by both an outwardly directed HCO_3^- gradient and an outwardly directed Cl^- gradient. Apparent influxes of 10 mmol l^{-1} Cl^- were (nmol mg protein^{-1} 15 s^{-1} ± 1 S.E.): gluconate, 12·90 ± 0·19; HCO_3^-, 15·83 ± 0·34;

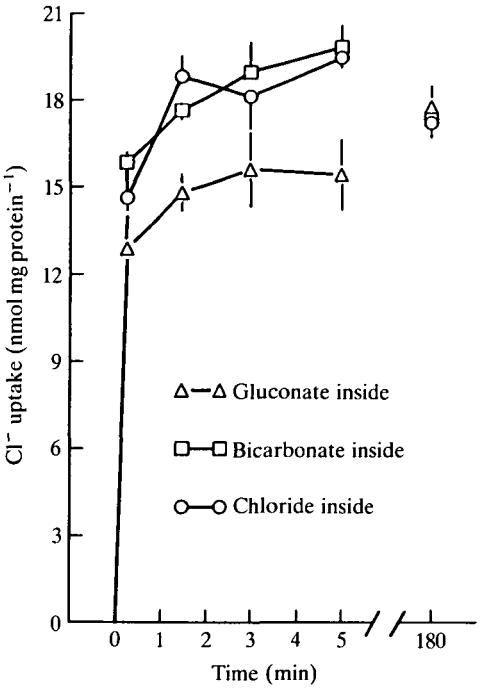

Fig. 4. The effect of internal anions on chloride uptake into brush border membrane vesicles from tilapia upper intestine. The vesicles contained 200 mmol l^{-1} tetramethylammonium (TMA) gluconate, 12 mmol l^{-1} Hepes/Tris, pH 7·7, valinomycin (15 μg mg protein^{-1}) and one of the following: 50 mmol l^{-1} potassium gluconate (triangles), 50 mmol l^{-1} $KHCO_3$ (squares) or 50 mmol l^{-1} KCl (circles). The reaction medium was the same for all treatments and consisted of 190 mmol l^{-1} TMA gluconate, 50 mmol l^{-1} potassium gluconate, 10 mmol l^{-1} TMACl and 12 mmol l^{-1} Hepes/Tris, pH 7·7.

Cl^-, 14·62 ± 0·42. At 15 s the HCO_3^- and Cl^- treatments were significantly different ($P < 0{\cdot}05$, Duncan's multiple range test) from the control. As discussed above for the Na^+/H^+ antiport experiment, the treatments were short-circuited with valinomycin so that electrical coupling can be ruled out as the reason for the stimulation of Cl^- uptake in the HCO_3^- and Cl^- treatments. These results support the existence of a Cl^-/HCO_3^- antiporter in tilapia intestinal BBMV. Overall, this supports the Na^+/H^+:Cl^-/HCO_3^- antiport model as the mechanism of NaCl transport in tilapia intestinal BBMV.

DISCUSSION

Early experiments which strongly suggested the existence of a mechanism capable of mediating electroneutral NaCl absorption in epithelia came from the work of Diamond (1962, 1964), Wheeler (1963) and Dietschy (1964). Their studies showed that Na^+ and Cl^- were both actively absorbed by fish and rabbit gallbladder at nearly

equal rates. They also found that the replacement of Na^+ by nonabsorbed cations abolished active Cl^- absorption, and that the replacement of Cl^- by nonabsorbed anions abolished active Na^+ absorption. This early work did not determine the site(s) of interaction between Na^+ and Cl^- on the gallbladder epithelial cell membrane. Studies by Frizzell, Dugas & Schultz (1975) and Cremaschi & Henin (1975) determined that the site of coupling was at the brush border membrane. These early studies were made using whole-tissue techniques (e.g. Ussing chambers). A drawback of whole-tissue techniques is that they cannot easily be used to distinguish between symport and antiport mechanisms because the investigator has little, if any, control over what is inside the cell. Later studies used membrane vesicle techniques to delineate further the mechanisms of vertebrate NaCl transport. One of the first transport mechanisms to become widely documented in vesicles from a number of different tissues was $Na^+/K^+/2Cl^-$ transport (Epstein & Silva, 1985; Eveloff *et al.* 1980; Hannafin *et al.* 1983; Musch *et al.* 1982; Halm *et al.* 1985). Since the discovery of the $Na^+/K^+/2Cl^-$ mechanism other investigators have proposed different models for NaCl transport in epithelial tissues. These models involve the action of Na^+/H^+, Cl^-/OH^-, Cl^-/HCO_3^- or Cl^-/formate$^-$ antiporters (Karniski & Aronson, 1985; Knickelbein *et al.* 1983, 1985; Liedtke & Hopfer, 1982*a*,*b*; Seifter & Aronson, 1984). Both symport and antiport models can be used to explain coupled electroneutral NaCl transport in the tissues that have been studied.

For marine teleosts the transport of NaCl across the intestinal epithelium is an important part of the osmoregulatory process that these animals perform in order to survive in the marine environment. Prior to this study, the only membrane vesicle work which had been conducted on NaCl transport in the intestine of a marine teleost had been done on the winter flounder, *Pseudopleuronectes americanus* (Eveloff *et al.* 1980). This study disclosed that Na^+ uptake into intestinal BBMV was stimulated by an inwardly directed Cl^- gradient, and this stimulation was inhibited by furosemide. It did not determine if this NaCl cotransport was K^+-dependent or K^+-independent because all the treatments had K^+ present. Later studies by Musch *et al.* (1982) and Halm *et al.* (1985) have shown that a $Na^+/K^+/2Cl^-$ cotransport mechanism is present in winter flounder intestine. To test for NaCl cotransport in tilapia intestinal BBMV, Na^+ uptake was measured in the presence of inwardly directed Cl^- and KCl gradients. The conditions used were similar to those used by Knickelbein *et al.* (1985) to test for NaCl cotransport in rabbit ileal BBMV. The results in Fig. 2 indicate that neither Na^+/Cl^- nor $Na^+/K^+/2Cl^-$ cotransport was present in tilapia intestinal BBMV, since Na^+ uptake was not stimulated by an inwardly directed Cl^- or KCl gradient. Furosemide also had no effect on Na^+ uptake (Fig. 2).

From Fig. 3 it can be seen that a Na^+/H^+ antiport mechanism exists in tilapia intestinal BBMV. This transporter has been documented in a wide variety of cells, including erythrocytes, lymphocytes, ova, sperm, skeletal muscle, cardiac muscle, neurones, capillary endothelium, fibroblasts, renal tubular cells, and intestinal and gallbladder epithelial cells (Mahnensmith & Aronson, 1985). The Na^+/H^+ antiporter has also been implicated in the following physiological processes: regulation of intracellular pH, control of cell growth and proliferation, metabolic responses to

hormones such as insulin and glucocorticoids, regulation of cell volume, and the transepithelial transport of NaCl *via* Na^+/H^+: Cl^-/HCO_3^- antiport (Mahnensmith & Aronson, 1985).

Fig. 4 indicates that a Cl^-/HCO_3^- antiport mechanism exists in tilapia intestinal BBMV. This transporter has been documented in rabbit small intestine by Knickelbein *et al.* (1985). A Cl^-/OH^- antiporter has been documented by Liedtke & Hopfer (1982*b*) in rat small intestine. Both Cl^-/HCO_3^- and Cl^-/OH^- exchange have also been documented in erythrocytes (Scarpa, Cecchetto & Azzone, 1970).

A possible mechanism for the coupling of the Na^+/H^+ antiporter and the Cl^-/HCO_3^- antiporter could be *via* the activity of carbonic anhydrase. Carbonic anhydrase facilitates the production of carbonic acid (H_2CO_3) from CO_2 and H_2O. Carbonic acid then dissociates into HCO_3^- and H^+, thus providing substrates for the antiporters. The net effect of this coupling mechanism is to move CO_2 out of the cell into the intestinal lumen and to move Na^+ and Cl^- electroneutrally from the lumen into the cell. This provides a means for the cell to get rid of metabolic CO_2 while absorbing NaCl. A model of this coupling mechanism can be found in the paper by Knickelbein *et al.* (1985).

The reason for the presence of $Na^+/K^+/2Cl^-$ symport in winter flounder and Na^+/H^+: Cl^-/HCO_3^- antiport in tilapia is not yet understood. It should be noted here that the presence of Na^+/H^+: Cl^-/HCO_3^- antiport in winter flounder has not been ruled out. In fact, the paper by Halm *et al.* (1985) suggests that there is some other mechanism for the transport of NaCl present in the brush border membrane of winter flounder intestinal epithelial cells. This mechanism could be Na^+/H^+: Cl^-/HCO_3^- antiport. Eveloff *et al.* (1980) have already shown that Na^+/H^+ antiport is present in winter flounder intestinal BBMV. All that remains to be shown is that Cl^-/HCO_3^- antiport is present too. While the exact physiological details of NaCl transport in winter flounder intestinal epithelial cells are still somewhat unclear, our present experiments suggest that NaCl cotransport mechanisms are not present in tilapia intestinal epithelial cells. The lack of cotransport mechanisms in tilapia intestinal BBMV could be related to the diet and/or salinity-tolerance of this species. Tilapia are euryhaline herbivores, whereas winter flounder are stenohaline carnivores.

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REFERENCES

ANDO, M. (1981). Effects of ouabain on chloride movements across the seawater eel intestine. *J. comp. Physiol.* **145**, 73–79.

ARVAN, P. & CASTLE, J. D. (1982). Plasma membrane of the rat parotid gland: Preparation and partial characterization of a fraction containing the secretory surface. *J. Cell Biol.* **95**, 8–19.

BERNER, W., KINNE, R. & MURER, H. (1976). Phosphate transport into brush border membrane vesicles isolated from rat small intestine. *Biochem. J.* **160**, 467–474.

BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72**, 248–254.

COOPERSTEIN, S. J. & LAZAROW, A. (1951). A microspectrophotometric method for the determination of cytochrome oxidase. *J. biol. Chem.* **189**, 665–670.

CREMASCHI, D. & HENIN, S. (1975). Na and Cl transepithelial routes in rabbit gallbladder. Tracer analysis of the transports. *Pflügers Arch. ges. Physiol.* **361**, 33–41.

DIAMOND, J. M. (1962). The mechanism of solute transport by the gallbladder. *J. Physiol., Lond.* **161**, 474–502.

DIAMOND, J. M. (1964). Transport of salt and water in rabbit and guinea pig gallbladder. *J. gen. Physiol.* **48**, 1–14.

DIETSCHY, J. M. (1964). Water and solute transport across the wall of the everted rabbit gallbladder. *Gastroenterology* **47**, 395–408.

EPSTEIN, F. H. & SILVA, P. (1985). Na–K–Cl cotransport in chloride-transporting epithelia. *Ann. N.Y. Acad. Sci.* **456**, 187–197.

ERICSON, A. & SPRING, K. R. (1982). Coupled NaCl entry into *Necturus* gallbladder epithelial cells. *Am. J. Physiol.* **243**, C140–145.

EVELOFF, J., FIELD, M., KINNE, R. & MURER, H. (1980). Sodium-cotransport systems in intestine and kidney of the winter flounder. *J. comp. Physiol.* **135**, 175–182.

FRIZZELL, R. A., DUGAS, M. & SCHULTZ, S. G. (1975). Sodium chloride transport by rabbit gallbladder: direct evidence for a coupled NaCl influx process. *J. gen. Physiol.* **65**, 769–795.

HALM, D., KRASNY, E., JR & FRIZZELL, R. (1985). Electrophysiology of flounder intestinal mucosa. II. Relation of the electrical potential profile to coupled NaCl absorption. *J. gen. Physiol.* **85**, 865–883.

HANNAFIN, J., KINNE-SAFFRAN, E., FRIEDMAN, D. & KINNE, R. (1983). Presence of a sodium–potassium chloride cotransport system in the rectal gland of *Squalus acanthias*. *J. Membr. Biol.* **75**, 73–83.

HOPFER, U., NELSON, K., PERROTTO, J. & ISSELBACHER, K. J. (1973). Glucose transport in isolated brush border membrane from rat small intestine. *J. biol. Chem.* **248**, 25–32.

KARNISKI, L. P. & ARONSON, P. S. (1985). Chloride/formate exchange with formic acid recycling: a mechanism of active chloride transport across epithelial membranes. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6362–6365.

KNICKELBEIN, R., ARONSON, P. S., ATHERTON, W. & DOBBINS, J. W. (1983). Sodium and chloride transport across rabbit ileal brush border. I. Evidence for Na–H exchange. *Am. J. Physiol.* **245**, G504–510.

KNICKELBEIN, R., ARONSON, P. S., SCHRON, C. M., SEIFTER, J. & DOBBINS, J. W. (1985). Sodium and chloride transport across rabbit ileal brush border. II. Evidence for Cl–HCO_3 exchange and mechanism of coupling. *Am. J. Physiol.* **249**, G236–245.

LIEDTKE, C. M. & HOPFER, U. (1982*a*). Mechanism of Cl^- translocation across small intestinal brush-border membrane. I. Absence of Na^+–Cl^- cotransport. *Am. J. Physiol.* **242**, G263–271.

LIEDTKE, C. M. & HOPFER, U. (1982*b*). Mechanism of Cl^- translocation across small intestinal brush-border membrane. II. Demonstration of Cl^-–OH^- exchange and Cl^- conductance. *Am. J. Physiol.* **242**, G272–280.

MAHNENSMITH, R. L. & ARONSON, P. S. (1985). The plasma membrane sodium–hydrogen exchanger and its role in physiological and pathophysiological processes. *Circulation Res.* **57**, 773–788.

MUSCH, M. W., ORELLANA, S. A., KIMBERG, L. S., FIELD, M., HALM, D. R., KRASNY, E. J., JR & FRIZZELL, R. A. (1982). Na^+–K^+–Cl^- co-transport in the intestine of a marine teleost. *Nature, Lond.* **300**, 351–353.

SCARPA, A., CECCHETTO, A. & AZZONE, G. F. (1970). The mechanism of anion translocation and pH equilibration in erythrocytes. *Biochim. biophys. Acta* **219**, 179–188.

SEIFTER, J. L. & ARONSON, P. S. (1984). Cl^- transport via anion exchange in *Necturus* renal microvillus membranes. *Am. J. Physiol.* **247**, F888–895.

SKADHAUGE, E. (1974). Coupling of transmural flows of NaCl and water in the intestine of the eel (*Anguilla anguilla*). *J. exp. Biol.* **60**, 535–546.

STOKES, J. B. (1984). Sodium chloride absorption by the urinary bladder of the winter flounder. A thiazide-sensitive electrically neutral transport system. *J. clin. Invest.* **74**, 7–16.

WHEELER, H. O. (1963). Transport of electrolytes and water across wall of rabbit gallbladder. *Am. J. Physiol.* **205**, 427–438.