J. exp. Biol. 159, 359–369 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

CHARACTERIZATION OF AN ENDOGENOUS Na^+/H^+ ANTIPORTER IN *XENOPUS LAEVIS* OOCYTES

BY DAVID W. TOWLE, ADRIENNE BAKSINSKI, NATHALIE E. RICHARD AND MAREK KORDYLEWSKI

Department of Biology, Lake Forest College, Lake Forest, IL 60045, USA and *Mount Desert Island Biological Laboratory, Salsbury Cove, ME 04672, USA*

Accepted 22 April 1991

Summary

The amiloride-sensitive Na^+/H^+ antiporter in defolliculated oocytes of *Xenopus laevis* was characterized by measurements of $^{22}Na^+$ influx and apparent H^+ efflux. Uptake of $^{22}Na^+$ was linear over a 90-min incubation period and was inhibited approximately 80 % with 5×10^{-4} mol l^{-1} amiloride. Amiloride-sensitive sodium uptake was reduced following collagenase treatment or oocyte aging. $K_{0.5}$ for amiloride inhibition was $4.13\times10^{-6}\pm1.33\times10^{-6}$ mol l^{-1} and the K_m for Na^+ was 4.25×10^{-3} mol l^{-1}. Hill analysis of the kinetic data for Na^+ revealed an n_H value of 1.14, indicating an absence of interacting binding sites for Na^+. Parallel measurements of amiloride-sensitive Na^+ uptake and H^+ efflux indicated a Na^+/H^+ exchange ratio of 0.88:1. Our conclusion is that the Na^+/H^+ antiporter of *Xenopus* oocytes exhibits a nominal 1:1 Na^+/H^+ exchange stoichiometry and is similar in its properties to the antiporter of other vertebrate cells.

Introduction

The oocyte of the African clawed frog *Xenopus laevis* has proved useful in the characterization of membrane proteins translated from injected mRNA. Many genetic and physiological studies have taken advantage of the ability of the oocyte to synthesize, process and insert membrane transport proteins produced from exogenous mRNA. These studies include an early description of the acetylcholine receptor (Barnard *et al.* 1982), the sequencing of cDNA coding for the sodium/glucose cotransporter (Hediger *et al.* 1987) and numerous electrophysiological and radioisotopic analyses of sodium channel function (George *et al.* 1989; Hinton and Eaton, 1989; Kroll *et al.* 1989; reviewed in Sigel, 1990). No detailed studies of endogenous or exogenous Na^+/H^+ exchange by the *Xenopus* oocyte have been reported, however. Indeed, studies have indicated the absence of expressed Na^+/H^+ antiporter in collagenase-treated immature oocytes (George *et al.* 1989) and in fertilized oocytes (Webb and Nuccitelli, 1982).

Following our report of an electrogenic $2Na^+/1H^+$ antiporter in membrane vesicle preparations from crustacean gill (Shetlar *et al.* 1987; Towle *et al.* 1988;

Key words: *Xenopus laevis*, oocyte, Na^+/H^+ antiporter, sodium, proton.

Shetlar and Towle, 1989) and its confirmation by another laboratory (Ahearn and Clay, 1989), it became apparent that the *Xenopus* oocyte expression system would be useful in molecular studies of this apparently unique transport protein. To implement such an approach requires knowledge of endogenous Na^+/H^+ exchange in the oocyte, to provide a background against which the crustacean mRNA-derived $2Na^+/1H^+$ exchange can be studied. The objectives of the experiments reported here were to determine whether the immature *Xenopus* oocyte displays Na^+/H^+ exchange and, if so, to describe its properties in some detail. A preliminary account of some of the data has appeared in abstract form (Towle and Baksinski, 1990).

Materials and methods

Oocyte-positive frogs (*Xenopus laevis* Daudin) obtained from NASCO (Fort Atkinson, WI, USA) were maintained under a 12 h:12 h light:dark cycle in shallow tanks of dechlorinated tap water at 18–20 °C. They were fed NASCO frog 'brittle' twice a week, followed by a change of water 1 h after feeding. Prior to surgery, frogs were anesthetized by immersion in crushed ice for 30 min (Marcus-Sekura and Hitchkock, 1987). Using aseptic procedures, small incisions were made through the skin and muscle in the lower ventral abdominal area. One or two ovarian lobes were removed and the incisions were sutured. During recovery from hypothermia, the frog was placed in water supplemented with 0.5 % NaCl for 2–3 h.

The lobes of the ovary were placed in sterile modified Barth's saline containing 88 mmol l^{-1} NaCl, 1 mmol l^{-1} KCl, 2.4 mmol l^{-1} $NaHCO_3$, 0.3 mmol l^{-1} $Ca(NO_3)_2$, 0.41 mmol l^{-1} $CaCl_2$, 0.82 mmol l^{-1} $MgSO_4$, 15 mmol l^{-1} Hepes–NaOH (pH 7.6), 10 i.u. ml^{-1} penicillin and 10 μg ml^{-1} streptomycin (Colman, 1984). The ovarian material was separated into individual oocytes with watchmaker's forceps. Oocytes were then defolliculated by treatment for 1 h in Ca^{2+}- and Mg^{2+}-free Barth's saline (Shetlar *et al.* 1990), followed by return to normal Barth's medium at 18 °C overnight. Oocytes at Dumont stages V and VI (Dumont, 1972) were used in transport experiments.

Sodium uptake measurements were performed in 96-well microtiter plates by incubating a group of 8–12 oocytes in 100 μl of uptake medium containing 100 mmol l^{-1} choline chloride, 0.33 mmol l^{-1} $Ca(NO_3)_2$, 0.41 mmol l^{-1} $CaCl_2$, 0.82 mmol l^{-1} $MgSO_4$ and 5 mmol l^{-1} Hepes–NaOH, pH 7.4 (final Na^+ concentration = 4 mmol l^{-1}) (George *et al.* 1989). Following preincubation on ice for 30 min with or without amiloride (5×10^{-4} mol l^{-1}), uptake measurement was initiated by adding 2 μCi of carrier-free $^{22}NaCl$ (Dupont/NEN, specific activity = 1.00 Ci mg^{-1}). After incubation at 19–21 °C for up to 90 min, oocytes were washed four times in 3 ml of ice-cold 100 mmol l^{-1} NaCl, 5 mmol l^{-1} Hepes–NaOH, pH 7.4. Individual oocytes were then placed into scintillation vials and lysed with 200 μl of 1 % sodium dodecyl sulfate. Following addition of 6 ml of Ecolume (ICN, Irvine, CA, USA), radioactivity was determined in a liquid scintillation counter. Each experiment was performed at least twice on different batches

of oocytes from separate individuals. Representative or pooled data are presented as mean±S.E.

Apparent efflux of H^+ was measured in weakly buffered uptake medium containing 0.5 mmol l^{-1} Hepes–Tris (pH 7.4), 0 or 20 mmol l^{-1} NaCl plus choline chloride to total 104 mmol l^{-1} Cl^-, and other components as described for sodium uptake. A buffer curve was obtained by titrating the medium with dilute nitric acid, using a miniature combination pH electrode (Microelectrodes, Inc., Londonderry, NH, USA) and pH meter sensitive to 0.001 pH unit. During titration or incubation, the medium was covered with 100 μl of light mineral oil to reduce CO_2 exchange with the atmosphere. The buffering capacity of the incubation medium at about pH 7.4 was 0.78 mequiv l^{-1} pH unit^{-1}. Ten oocytes were incubated in 100 μl of medium in the absence or presence of amiloride (5×10^{-4} mol l^{-1}) at 19–20°C. Recordings of pH changes were converted to changes in $[H^+]$ using the equation for the linear region of the empirically determined buffer curve.

Results

Experiments examining the effect of length of incubation on $^{22}Na^+$ uptake by defolliculated oocytes revealed a linear rate of uptake over the 90-min incubation period (Fig. 1). Addition of 5×10^{-4} mol l^{-1} amiloride produced an 80 % decline in the rate of $^{22}Na^+$ absorption, signifying the participation of either sodium channels or Na^+/H^+ exchange in the majority of the measured sodium uptake.

Defolliculating oocytes with collagenase (0.12 % Sigma type II for 1 h at 18–19°C), rather than Ca^{2+}- and Mg^{2+}-free Barth's medium, led to a marked decline in the amiloride-sensitive portion of sodium uptake (Fig. 2). This effect of

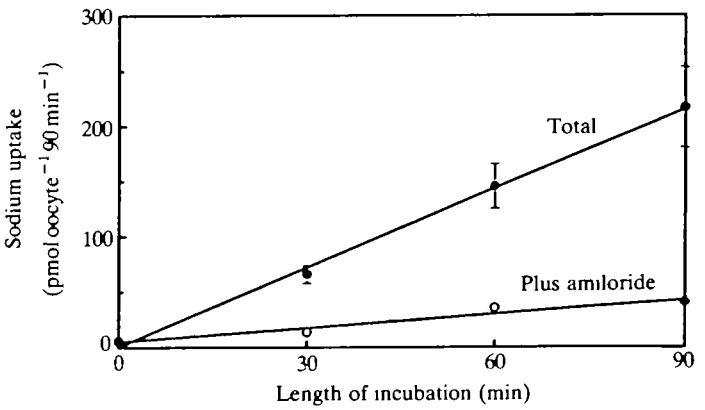

Fig. 1. Defolliculated *Xenopus laevis* oocytes were incubated in uptake medium containing 4 mmol l^{-1} $^{22}Na^+$ in the absence (●) or presence (○) of 5×10^{-4} mol l^{-1} amiloride. At each of the indicated intervals, eight oocytes were removed, washed and counted in liquid scintillation medium. Representative of three individual experiments; mean±S.E. (except where obscured by symbol).

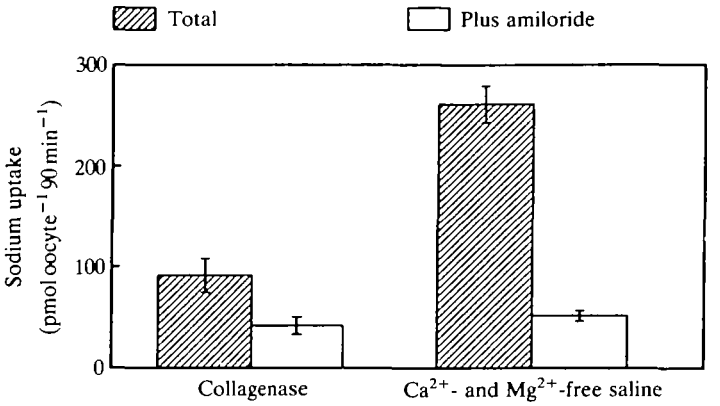

Fig. 2. Oocytes were treated for 1 h with either collagenase (0.12 % Sigma type II) or Ca^{2+}- and Mg^{2+}-free Barth's saline and returned to normal Barth's saline overnight. Uptake of $^{22}Na^{+}$ was determined the following day, in the absence or presence of 5×10^{-4} mol l^{-1} amiloride. Representative of three individual experiments; mean±S.E., eight oocytes per sample.

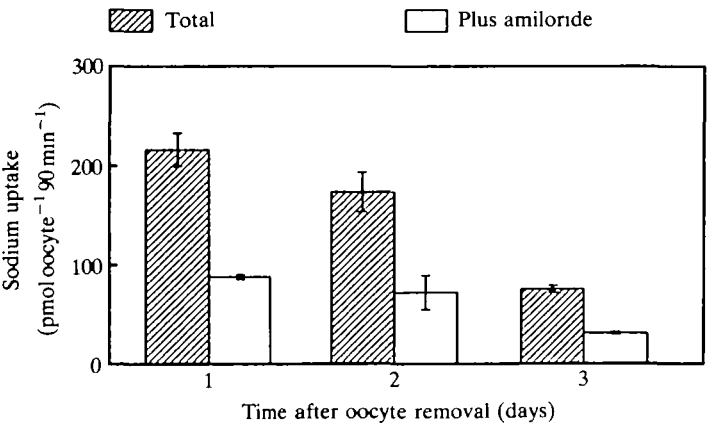

Fig. 3. Ca^{2+}- and Mg^{2+}-free defolliculated *Xenopus laevis* oocytes were incubated at 19 °C in Barth's saline which was changed daily. At 24-h intervals, samples of oocytes were tested for total and amiloride-insensitive uptake of $^{22}Na^{+}$ ($[Na^{+}]=4$ mmol l^{-1}). Representative of two individual experiments; mean±S.E., eight oocytes per sample.

collagenase could result from a particular sensitivity of the sodium transport protein(s) to non-collagenase proteases in the crude collagenase preparation. In the ensuing experiments, oocytes were treated with Ca^{2+}- and Mg^{2+}-free Barth's medium only.

Uptake assays performed on Ca^{2+}- and Mg^{2+}-free-defolliculated oocytes 48 and 72 h after removal from the animal revealed a steadily declining $^{22}Na^{+}$ uptake rate, compared with oocytes monitored 24 h after removal (Fig. 3). Uptake measurements were therefore made using oocytes obtained no more than 24 h previously.

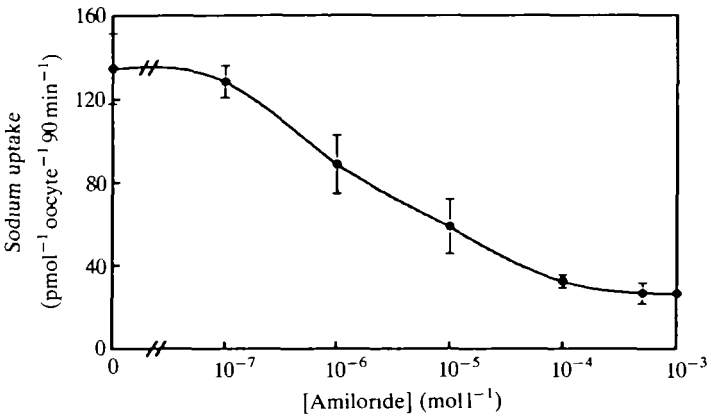

Fig. 4. The effect of amiloride concentration on $^{22}Na^+$ uptake by defolliculated *Xenopus* oocytes ($[Na^+]$=4 mmol l^{-1}). Representative of four individual experiments; mean±S.E., except where obscured by symbol, eight oocytes per sample. The calculated $K_{0.5}$ for amiloride was $4.13\times10^{-6}\pm1.33\times10^{-6}$ mol l^{-1} (mean±S.E., N=4).

Despite a uniformity of treatment conditions, oocytes from different individuals displayed non-uniform amiloride-sensitive $^{22}Na^+$ uptake rates, ranging from 100 to 250 pmol oocyte^{-1} 90 min^{-1}.

Amiloride at a concentration of 10^{-7} mol l^{-1} had no effect on $^{22}Na^+$ uptake by oocytes (Fig. 4), indicating the absence of measurable Na^+ channel activity. However, amiloride at concentrations of 10^{-6} mol l^{-1} and higher inhibited Na^+ uptake in a dose-dependent fashion, a behavior characteristic of Na^+/H^+ exchange (Kleyman and Cragoe, 1988). The $K_{0.5}$ for amiloride inhibition was calculated by taking the second derivative of the third-order polynomial equation which best fitted the data, determined with Slidewrite Plus (Advanced Graphics Software, Sunnyvale, CA, USA). The $K_{0.5}$ determined in this way for amiloride inhibition of Na^+ uptake was $4.13\times10^{-6}\pm1.33\times10^{-6}$ mol l^{-1} (mean±S.E., N=4), a value that lies at the low end of comparable $K_{0.5}$ values for Na^+/H^+ antiporters from other vertebrate sources (Mahnensmith and Aronson, 1985; Aronson and Igarashi, 1986; Kleyman and Cragoe, 1988).

The response of amiloride-sensitive Na^+ uptake to extracellular $[Na^+]$ was clearly hyperbolic, with typical Michaelis–Menten kinetics (Fig. 5). In these experiments, the total concentration of choline plus Na^+ was kept constant at 104 mmol l^{-1}. Pooling data from four experiments and calculating K_m according to the Lineweaver–Burke method revealed a K_m value for Na^+ of 4.25×10^{-3} mol l^{-1} (Fig. 5). Hill analysis of the kinetic data for Na^+ revealed an n_H value of 1.14 (Fig. 6), indicating the number of interacting binding sites and thus a likely Na^+/H^+ exchange stoichiometry of 1:1.

To investigate the exchange stoichiometry more directly, apparent efflux of H^+ from oocytes was measured in weakly buffered medium. In the presence of 20 mmol l^{-1} Na^+ in the external medium, apparent H^+ efflux demonstrated marked amiloride sensitivity (Fig. 7). Measured H^+ efflux was completely

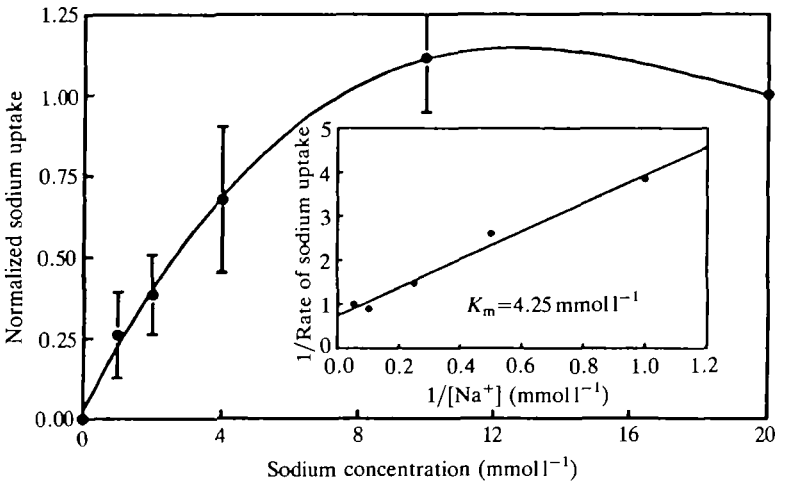

Fig. 5. The effect of sodium concentration on amiloride-sensitive $^{22}Na^+$ uptake by defolliculated *Xenopus laevis* oocytes. Data from four experiments were normalized to a value of 1.0 at 20 mmol l^{-1} Na^+, and plotted as mean±S.E. of four values. Each of the individual values was obtained by subtracting amiloride-insensitive Na^+ uptake of eight oocytes from total Na^+ uptake of eight additional oocytes, measured in the presence or absence of 5×10^{-4} mol l^{-1} amiloride. Inset: a double reciprocal plot of the kinetic data in Fig. 5, yielding a K_m value of 4.25×10^{-3} mol l^{-1} Na^+.

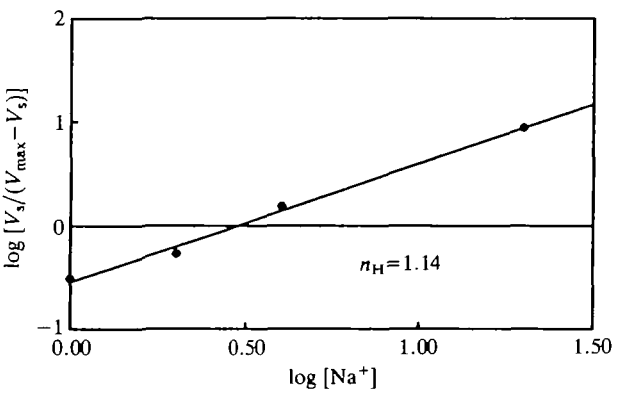

Fig. 6. Hill plot of kinetic data for Na^+ pooled from four experiments. The slope of the line was calculated by linear regression as the Hill coefficient, n_H, and was 1.14. V_s, normalized sodium uptake; V_{max}, maximum normalized sodium uptake. Sodium concentration was measured in mmol l^{-1}.

dependent upon external Na^+ under the conditions employed (Fig. 8). In experiments simultaneously measuring H^+ efflux and Na^+ uptake on oocytes prepared from the same animal, the ratio of Na^+ uptake (182.8±30.7 pmol oocyte^{-1} 90 min^{-1}) to H^+ efflux (208.5 pmol oocyte^{-1} 90 min^{-1}) was 0.88, not substantially different from the theoretical ratio of 1.0 for electroneutral Na^+/H^+

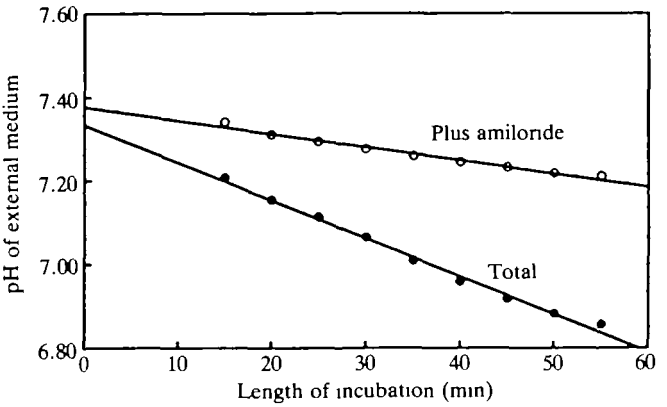

Fig. 7. Apparent efflux of H^+ from defolliculated *Xenopus laevis* oocytes into weakly buffered uptake medium containing 20 mmol l^{-1} Na^+, in the absence (●) or presence (○) of 5×10^{-4} mol l^{-1} amiloride. Values were obtained using 10 oocytes in 100 μl of uptake medium covered with mineral oil. One of three similar experiments.

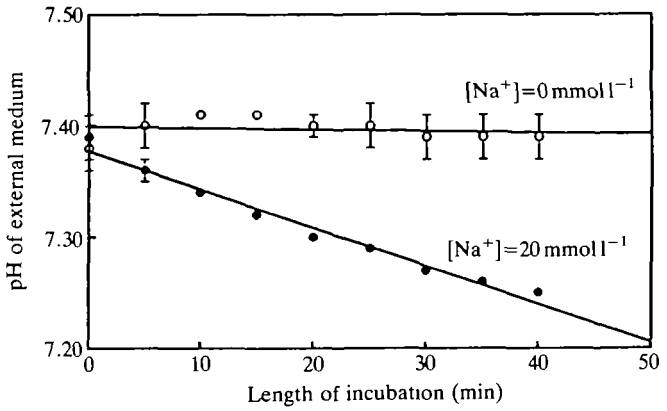

Fig. 8. Dependence of apparent H^+ efflux from *Xenopus laevis* oocytes on external Na^+ concentration, in the presence (●) or absence (○) of 20 mmol l^{-1} NaCl. Choline chloride was added to the weakly buffered medium to give a total of 104 mmol l^{-1} Cl^-. Mean±S.E. of three experiments.

exchange. Such 1:1 stoichiometry is found universally in Na^+/H^+ antiporters of vertebrate cells (Aronson and Igarashi, 1986).

Discussion

Despite some reports to the contrary (George *et al.* 1989), the immature oocyte of *Xenopus laevis* clearly exhibits amiloride-sensitive Na^+/H^+ antiporter activity. The previously reported absence of the antiporter may have arisen as a result of

treatment of oocytes with collagenase, to which the antiporter is apparently quite sensitive. The length of time after oocyte removal from the animal also has a clear effect on the activity of the Na^+/H^+ antiporter. Whether endocytosis of antiporter protein is occurring during maintenance in Barth's medium, as observed for *Xenopus* oocyte Na^+/K^+-ATPase (Schmalzing *et al.* 1990), is not known.

The function of the Na^+/H^+ antiporter in *Xenopus* oocytes is not clear. As in many vertebrate cells, it undoubtedly plays an important role in intracellular pH regulation and in volume regulation (reviewed in Mahnensmith and Aronson, 1985). Shrinkage of erythrocytes of urodele amphibians leads to rapid activation of Na^+/H^+ exchange (Cala, 1980); whether such a response also occurs in *Xenopus* oocytes is not known.

The kinetic properties of the *Xenopus* oocyte Na^+/H^+ antiporter are clearly similar to those of other vertebrate cells. The $K_{0.5}$ values with respect to amiloride inhibition have been reported to range from 7×10^{-6} to 33×10^{-6} mol l^{-1} among vertebrate Na^+/H^+ antiporters (Nord *et al.* 1984; Mahnensmith and Aronson, 1985). The $K_{0.5}$ value of 4.13×10^{-6} mol l^{-1} amiloride reported here extends this range slightly but does not argue for a unique property of the *Xenopus* antiporter. The inhibitor constant is similar to the value of 7×10^{-6} mol l^{-1} reported for the Na^+/H^+ antiporter in basolateral membrane of frog skin epithelium (Ehrenfeld *et al.* 1987). Inhibitor constants for sodium channels, in contrast, are typically in the range of 1×10^{-7} to 3×10^{-7} mol l^{-1} amiloride (Kleyman and Cragoe, 1988). For example, the $K_{0.5}$ of the sodium channel in the apical membrane of frog skin epithelium is 3×10^{-7} mol l^{-1} (Benos *et al.* 1979). Our results thus suggest that amiloride inhibition of sodium uptake into oocytes is a consequence of inhibiting Na^+/H^+ exchange rather than Na^+ channels.

The hyperbolic nature of the dependence of Na^+ uptake on external Na^+ concentration further underlines the similarity of the Na^+ uptake process of oocytes to Na^+/H^+ exchange systems of other vertebrates. The K_m value calculated for Na^+ (4.25×10^{-3} mol l^{-1}) is close to the range (6×10^{-3} to 87×10^{-3} mol l^{-1}) reported for Na^+/H^+ antiporters of cells and membrane vesicles derived from other vertebrate tissues (Aronson, 1985; Bidet *et al.* 1987). However, the apparent K_m value of the sodium channel in frog skin apical membranes lies within the same range (Sariban-Sohraby and Benos, 1986). Thus, this parameter cannot be used to distinguish between channel and antiporter mechanisms.

Hill analysis of the kinetic data indicated first-order kinetics with respect to Na^+ concentration. Deviation of n_H from unity would suggest cooperativity or multiple binding sites for sodium. Because the Hill coefficient in the present case was determined to be 1.14, the existence of multiple binding sites is not supported. Furthermore, this value of n_H is consistent with a 1:1 Na^+/H^+ exchange stoichiometry.

Measurements of apparent H^+ efflux from oocytes demonstrated its sensitivity to amiloride and its dependence on external sodium, leading to the conclusion that H^+ efflux is mediated by a Na^+/H^+ antiporter in *Xenopus* oocytes. Calculation of the Na^+/H^+ exchange stoichiometry revealed a ratio of $0.88Na^+:H^+$, approxi-

mating the ratio of 1:1 expected for electroneutral Na^+/H^+ exchange. Similar levels of inhibition of Na^+ uptake and H^+ efflux by amiloride further suggest that the two transport processes are linked. The possibility of a sodium channel acting in conjunction with an amiloride-sensitive conductive H^+ pathway cannot be totally discounted. However, the simplest explanation of our observations lies in invoking a Na^+/H^+ antiporter inhibited by concentrations of amiloride that block Na^+/H^+ antiporters of other vertebrate systems.

The intracellular pH of immature *Xenopus* oocytes is approximately 7.14 (Houle and Wasserman, 1983), thus generating an outwardly directed H^+ gradient in an incubation medium of pH 7.4. This H^+ gradient appears to be sufficient to drive Na^+ uptake *via* Na^+/H^+ exchange from experimental solutions containing as little as $1\,mmol\,l^{-1}$ Na^+.

The *Xenopus* oocyte may be unsuitable for expression of Na^+/H^+ antiporter mRNA from other vertebrate sources because of the presence of endogenous Na^+/H^+ exchange activity with properties similar to those of the exchangers of other vertebrates. However, the oocyte may be quite suited to studies of Na^+/H^+ antiporters that have distinctly different kinetic properties. For example, a comparison of the kinetic properties of the *Xenopus* oocyte Na^+/H^+ antiporter with those of the recently described crustacean gill Na^+/H^+ antiporter reveals major differences. First, the $K_{0.5}$ for amiloride, measured with membrane vesicles from gills of the green crab *Carcinus maenas*, is $2.8\times10^{-4}\,mol\,l^{-1}$ (Shetlar and Towle, 1989), compared to $4.1\times10^{-6}\,mol\,l^{-1}$ reported here for the *Xenopus* oocyte. Second, the K_m for Na^+ in *Carcinus* vesicles is $3.4\times10^{-2}\,mol\,l^{-1}$, compared to $4.25\times10^{-3}\,mol\,l^{-1}$ for the *Xenopus* oocyte. Finally, the stoichiometry of Na^+/H^+ exchange in *Carcinus* vesicles appears to be $2Na^+:1H^+$, compared with the 1:1 stoichiometry for the *Xenopus* oocyte. It thus becomes possible to design an expression system in which Na^+/H^+ antiporter activity resulting from translation of crustacean mRNA can be kinetically distinguished from the endogenous *Xenopus* antiporter.

This research was supported by the National Science Foundation (DCB-8996137 and REU Supplement). Special appreciation is expressed to Robert Shetlar and Alison Morrison of the Max-Planck-Institut für Systemphysiologie for helpful discussions.

References

Ahearn, G. A. and Clay, L. P. (1989). Kinetic analysis of electrogenic $2\,Na^+-1\,H^+$ antiport in crustacean hepatopancreas. *Am. J. Physiol.* **257**, R484–R493.

Aronson, P. S. (1985). Properties of the renal Na^+-H^+ exchanger. In *Membrane Transport Driven by Ion Gradients* (ed. G. Semeza and R. Kinne), pp. 220–228. New York: The New York Academy of Sciences.

Aronson, P. S. and Igarashi, P. (1986). Molecular properties and physiological roles of the renal Na^+-H^+ exchanger. *Curr. Topics membr. Transport* **26**, 57–78.

Barnard, E. A., Miledi, R. and Sumikawa, K. (1982). Translation of exogenous messenger

RNA coding for nicotinic acetylcholine receptors produces functional receptors in *Xenopus* oocytes. *Proc. R. Soc.* B **215**, 241–246.

BENOS, D. J., MANDEL, L. J. AND BALABAN, R. S. (1979). On the mechanism of the amiloride sodium entry site interactions in anuran skin epithelia. *J. gen. Physiol.* **73**, 307–326.

BIDET, M., TAUC, M., MEROT, J., VANDEWALLE, A. AND POUJEOL, P. (1987). Sodium–hydrogen exchanger in proximal cells isolated from rabbit kidney. I. Functional characteristics. *Am. J. Physiol.* **253**, F935–F944.

CALA, P. M. (1980). Volume regulation by *Amphiuma* red blood cells. The membrane potential and its implications regarding the nature of ion flux pathways. *J. gen. Physiol.* **76**, 683–708.

COLMAN, A. (1984). Translation of eukaryotic messenger RNA in *Xenopus* oocytes. In *Transcription and Translation: A Practical Approach* (ed. B. D. Hames and S. J. Higgins), pp. 271–302. Oxford: IRL Press Ltd.

DUMONT, J. N. (1972). Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory-maintained animals. *J. Morph.* **136**, 153–180.

EHRENFELD, J., CRAGOE, E. J. AND HARVEY, B. J. (1987). Evidence for a Na^+/H^+ exchanger at the basolateral membranes of the isolated frog skin epithelium: effect of amiloride analogues. *Pflügers Arch.* **409**, 200–209.

GEORGE, A. L., JR, STAUB, O., GEERING, K., ROSSIER, B. C., KLEYMAN, T. R. AND KRAEHENBUHL, J.-P. (1989). Functional expression of the amiloride-sensitive sodium channel in *Xenopus* oocytes. *Proc. natn. Acad. Sci. U.S.A.* **86**, 7295–7298.

HEDIGER, M. A., COADY, M. J., IKEDA, T. S. AND WRIGHT, E. M. (1987). Expression cloning and cDNA sequencing of the Na^+/glucose co-transporter. *Nature* **330**, 379–381.

HINTON, C. F. AND EATON, D. C. (1989). Expression of amiloride-blockable sodium channels in *Xenopus* oocytes. *Am. J. Physiol.* **257**, C825–C829.

HOULE, J. G. AND WASSERMAN, W. J. (1983). Intracellular pH plays a role in regulating protein synthesis in *Xenopus* oocytes. *Devl Biol.* **97**, 302–312.

KLEYMAN, T. R. AND CRAGOE, E. J., JR (1988). Amiloride and its analogs as tools in the study of ion transport. *J. Membr. Biol.* **105**, 1–21.

KROLL, B., BAUTSCH, W., BREMER, S., WILKE, M., TÜMMLER, B. AND FRÖMTER, E. (1989). Selective expression of an amiloride-inhibitable Na^+ conductance from mRNA of respiratory epithelium in *Xenopus laevis* oocytes. *Am. J. Physiol.* **257**, L284–L288.

MAHNENSMITH, R. L. AND ARONSON, P. S. (1985). The plasma membrane sodium–hydrogen exchanger and its role in physiological and pathophysiological processes. *Circulation Res.* **56**, 773–788.

MARCUS-SEKURA, C. J. AND HITCHKOCK, M. J. M. (1987). Preparation of oocytes for microinjection of RNA and DNA. *Meth. Enzymol.* **152**, 284–288.

NORD, E. P., HAFEZI, A., WRIGHT, E. M. AND FINE, L. G. (1984). Mechanisms of Na^+ uptake into renal brush border membrane vesicles. *Am. J. Physiol.* **247**, F548–F554.

SARIBAN-SOHRABY, S. AND BENOS, D. J. (1986). The amiloride-sensitive sodium channel (editorial review). *Am. J. Physiol.* **250**, C175–C190.

SCHMALZING, G., ECKARD, P., KRÖNER, S. AND PASSOW, H. (1990). Downregulation of surface sodium pumps by endocytosis during meiotic maturation of *Xenopus laevis* oocytes. *Am. J. Physiol.* **258**, C179–C184.

SHETLAR, R. E., ALEXANDER, K. E. AND TOWLE, D. W. (1987). Electrogenic Na^+/H^+ exchange in membrane vesicles from crab (*Carcinus maenas*) gill. *Bull. Mt Des. Isl. biol. Lab.* **27**, 59–61.

SHETLAR, R. E., SCHÖLERMANN, B., MORRISON, A. I. AND KINNE, R. K. H. (1990). Characterization of a Na^+–K^+–$2Cl^-$ cotransport system in oocytes from *Xenopus laevis*. *Biochim. biophys. Acta* **1023**, 184–190.

SHETLAR, R. E. AND TOWLE, D. W. (1989). Electrogenic sodium–proton exchange in membrane vesicles from crab (*Carcinus maenas*) gill. *Am. J. Physiol.* **257**, R924–R931.

SIGEL, E. (1990). Use of *Xenopus* oocytes for the functional expression of plasma membrane proteins. *J. Membr. Biol.* **117**, 201–221.

TOWLE, D. W. AND BAKSINSKI, A. (1990). Endogenous amiloride-sensitive sodium uptake by *Xenopus* oocytes. *Physiologist* **33**, A105 (Abstract).

TOWLE, D. W., HUNTER, K. C., MAIOLO, JR., N. J., WRESTLER, J. C., SHETLAR, R. E. AND

HØLLELAND, T. (1988). Stoichiometry of sodium/proton exchange in membrane vesicles from gills of osmoregulating and osmoconforming crabs. *Am. Zool.* **28**, 18A (Abstract).

WEBB, D. J. AND NUCCITELLI, R. (1982). Intracellular pH changes accompanying the activation of development in frog eggs: Comparison of pH microelectrodes and ^{31}P NMR measurements. In *Intracellular pH: Its Measurement, Regulation and Utilization in Cellular Function* (ed. R. Nuccitelli and D. W. Deamer), pp. 293–324. New York: Alan R. Liss, Inc.