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Coupled Na^+/H^+ Exchange in Rat Parotid Basolateral Membrane Vesicles

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Summary. pH gradient-dependent sodium transport in highly purified rat parotid basolateral membrane vesicles was studied under voltage-clamped conditions. In the presence of an outwardly directed H^+ gradient (pH_{in} = 6.0, pH_{out} = 8.0) ^{22}Na uptake was approximately ten times greater than uptake measured at pH equilibrium (pH_{in} = pH_{out} = 6.0). More than 90% of this sodium flux was inhibited by the potassium-sparing diuretic drug amiloride (K_I = 1.6 μM) while the transport inhibitors furosemide (1 mM), bumetanide (1 mM), SITS (0.5 mM) and DIDS (0.1 mM) were without effect. This transport activity copurified with the basolateral membrane marker K^+-stimulated *p*-nitrophenyl phosphatase. In addition, ^{22}Na uptake into the vesicles could be driven against a concentration gradient by an outwardly directed H^+ gradient. pH gradient-dependent sodium flux exhibited a simple Michaelis-Menten-type dependence on sodium concentration consistent with the existence of a single transport system with K_M = 8.0 mM at 23°C. A component of pH gradient-dependent, amiloride-sensitive sodium flux was also observed in rabbit parotid basolateral membrane vesicles. These results provide strong evidence for the existence of a Na^+/H^+ antiport in rat and rabbit parotid acinar basolateral membranes and extend earlier less direct studies which suggested that such a transporter was present in salivary acinar cells and might play a significant role in salivary fluid secretion.

Key Words exocrine gland · parotid · acinar cell · ion transport · fluid secretion · pH regulation · antiport

Introduction

The plasma membranes of a wide variety of animal cells contain a Na^+/H^+ exchanger which utilizes the extracellular to intracellular electrochemical gradient for Na^+ to mediate the uphill extrusion of protons. This transport system is thought to play a critical role in a variety of physiological functions including fertilization, the regulation of intracellular pH and cell volume, the initiation of cell growth and proliferation, the metabolic response to hormones, and the transepithelial transport of Na^+ and HCO_3^- [1, 3, 7].

Several recent publications have provided evidence for the presence of a Na^+/H^+ exchanger in salivary acinar cells [4, 12, 17]. Pirani et al. [17] observed that the intracellular pH of intact rat submandibular glands decreased in the presence of amiloride, a potent inhibitor of Na^+/H^+ exchange. In addition, Novak and Young [12] found that in rabbit submandibular glands perfused with HCO_3^--free or Cl^--free solutions, amiloride reduced acetylcholine-stimulated fluid secretion by 60 and 95%, respectively. Since the primary salivary fluid is known to be secreted in the acini, and there is little if any fluid absorption or secretion by the salivary ducts [26], these effects of amiloride on salivary secretion suggest a direct action of the drug on the acinar cells.

There is now considerable evidence indicating that salivary fluid secretion is closely linked to transepithelial chloride fluxes [10, 11, 16, 26]. The uphill step in this process is thought to take place at the acinar basolateral membrane where chloride is driven into the cell against its concentration gradient. Both in vivo and in vitro data indicate that the bulk of this chloride uptake occurs via a loop diuretic-sensitive Na/K/Cl cotransport system [10, 12, 16, 17, 22, 26]. A HCO_3^--dependent component of secretion is also known to be present since glands perfused with Cl^--free solutions secrete a reduced volume of HCO_3^--rich fluid [4, 12, 17, 26]. On the basis of a variety of observations, including those mentioned above, Young and collaborators [4, 12, 17] have proposed that, in addition to a Na/K/Cl cotransporter, Na^+/H^+ and Cl^-/HCO_3^- exchangers localized to the acinar basolateral membrane also play a significant role in salivary fluid secretion. In this regard we have recently demonstrated the existence of a potent Cl^-/HCO_3^- exchanger in a basolateral membrane vesicle preparation from the rabbit parotid [21].

Although the presence of Na^+/H^+ exchange in submandibular acinar cells is suggested by the above results, definitive studies are required to confirm its presence in other salivary glands as well as to define its role in salivary fluid secretion and other aspects of acinar cell physiology. In the present paper we extend the work of Young and collaborators by providing direct evidence for the existence of an amiloride-sensitive Na^+/H^+ exchanger in rat parotid basolateral membrane vesicles. This exchanger has properties similar to those found in other cell types. We also show that in relative terms Na^+/H^+ exchange activity in this preparation is considerably higher than in a similar preparation from the rabbit.

Abbreviations

HEPES: N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; MES: (2[N-morpholino] ethanesulfonic acid); EDTA: ethylenediaminetetraacetic acid; PMSF: phenylmethylsufonyl fluoride; NMDG: N-methyl-D-glucamine; BLMV: basolateral membrane vesicles; KpNPPase: K^+-stimulated *p*-nitrophenyl phosphatase; SITS: 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid; DIDS: 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid.

Materials and Methods

Vesicle Preparation

Basolateral membranes vesicles (BLMV) were prepared from the parotid glands of male Wistar strain rats (Harlan-Sprague-Dawley, Indianapolis, Ind.) using a modification of previously published procedures [19, 22]. The parotid glands were removed, dissected free of lymph nodes, superficial fat and connective tissue and placed in Homogenization Buffer (10 mM HEPES/Tris, pH 7.4, containing 10% sucrose [292 mM], 1 mM EDTA and 0.1 mM PMSF) at 4°C. All subsequent steps were carried out at 4°C. The glands were finely minced, diluted to a total volume of approximately 5 ml/g in Homogenization Buffer and left on ice for several minutes. Floating tissue fragments were removed by aspiration and the mince was then diluted to 10 ml/g with Homogenization Buffer. Ten-ml aliquots of the parotid mince were homogenized in a Polytron (Brinkman Instruments, Westbury, N.Y.) with one 10-sec burst at power level 5. The resulting homogenate was centrifuged at 2500 × *g* for 5 min in a Beckman J2-21 centrifuge fitted with a JA-20 rotor. The supernatant from this step was saved on ice while the pellets were resuspended in the same volume of Homogenization Buffer and rehomogenized and centrifuged as before. This latter procedure was repeated two more times for a total of four homogenization/centrifugation steps. The combined supernatant from these four steps was then filtered through a fine nylon mesh (Nitex 155u from Tetko Inc., Elmsford, N.Y.) and centrifuged at 22,000 × *g* for 20 min.

The supernatant from the above high-speed spin was discarded and the pellet (the "crude membrane fraction") was suspended in a few ml of Homogenization Buffer, passed once through a 25-gauge needle and once through a 30-gauge needle, then diluted to 7.28 ml/g starting parotid tissue with the same buffer. Following the addition of 16% Percoll® (by volume) this material was centrifuged in 13-ml aliquots (equivalent to 1.5 g of starting tissue) at 41,500 × *g* for 30 min in a Beckman JA-20.1 rotor. The upper 1.5 to 2.0 ml of the resulting Percoll gradient typically contained no measurable protein. Below this there were two bands of membranous material, a small band (1.5 to 2.0 ml) on top of a dense white band. The small band (the BLMV fraction) was harvested, diluted to 20 ml in Buffer A (10 mM HEPES plus 100 mM mannitol and sufficient Tris to obtain pH 7.4) containing 100 mM KCl and 1 mM EDTA, and centrifuged at 48,000 × *g* for 20 min. This spin resulted in a soft membranous pellet covering a firm Percoll pellet. This membranous pellet was resuspended in 10 ml of Buffer A containing 100 mM KCl and 1 mM EDTA, repelleted and taken up at a protein concentration of 1.5 mg/ml in the same buffer. Aliquots of this material were fast frozen in liquid nitrogen and stored over liquid nitrogen until use.

Rabbit parotid BLMV were prepared by a previously described procedure similar to the one given above but employing a 20% Percoll gradient in Buffer A plus 1 mM EDTA [22].

Transport Studies

The uptake of ^{22}Na into BLMV was measured at 23°C by the rapid filtration technique. On the day of the uptake experiment frozen aliquots of parotid BLMV were thawed at room temperature for 20 min, diluted 25 times with an appropriate buffer (usually Buffer K: 100 mM mannitol, 100 mM K gluconate, 25 mM HEPES, 25 mM MES, 1 mM EDTA and sufficient Tris to obtain pH 6.0) and centrifuged at 48,000 × *g* for 20 min. The resulting pellets were taken up in the same buffer at a protein concentration of 0.5 to 1.5 mg/ml, incubated at 23°C for 1 hr and stored on ice for ≥1 hr before use.

The procedure for uptake measurements was as follows. A 10-μl aliquot of vesicles was placed in a 12 × 75 test tube and at time zero at 90- or 100-μl aliquot of incubation medium containing ^{22}Na and other constituents as required was added. After an appropriate time the reaction was terminated by the addition of 1.5 ml of ice-cold stop solution (*see below*). After addition of the stop solution the vesicles were applied to a Millipore filter (HAWP 0.45 μm) under light suction. The filter, which retained the BLMV, was then washed with a further 6.0 ml of stop solution, placed in a scintillation vial with 1 ml of water and 10 ml of Filtron X (National Diagnostics) and counted for radioactivity along with samples of the incubation medium and appropriate standards.

The stop solution was Buffer A containing 150 mM K acetate and 1 mM amiloride. The entire stopping and washing procedure took less than 30 sec, during which the vesicles were in contact with the stop solution for less than 15 sec. From control experiments in which the time the vesicles were left in the stop solution was prolonged [20], we have established that no significant loss of ^{22}Na occurs during the washing and stopping procedure.

All data were corrected for nonspecific trapping of ^{22}Na by the membranes and filter by subtracting the "uptake" observed at "zero time." "Zero-time uptake" (typically 0.13 ± 0.03 nmol/mg protein at 1 mM sodium) was determined by dispensing the stop solution onto the vesicles before the addition of the incubation medium, then filtering and washing as usual (*see above*).

The detailed composition of the various media used in each experiment is given in the Figure and Table legends. Tracer ^{22}Na was used at a concentration of 10 to 25 μCi/ml. All experimental points were carried out in triplicate or quadruplicate at 23°C. The

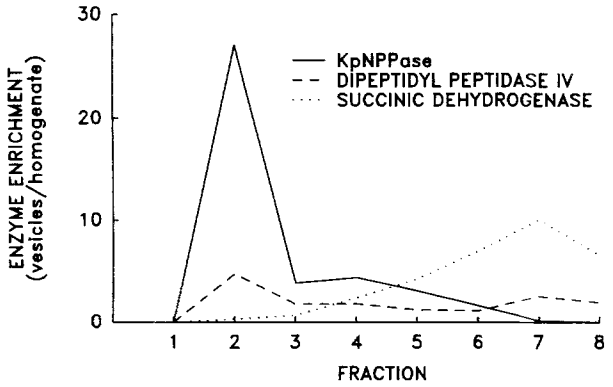

Fig. 1. Distribution of marker enzymes K^+-stimulated *p*-nitrophenyl phosphatase (KpNPPase), succinic dehydrogenase and dipeptidyl peptidase IV after centrifugation of the 22,000 × *g* "crude membrane fraction" in a self-orienting Percoll gradient (*see* Materials and Methods). The upper 1.5 to 2.0 ml of the gradient typically contained no measurable protein and was taken as fraction 1. Fraction 2 was the BLMV fraction described in Materials and Methods. The remainder of the gradient was divided into six fractions of approximately 1.5 ml each

errors shown in Tables and Figures (provided they are large enough to illustrate) are standard deviations. In control experiments (*not shown*) it was established that ^{22}Na uptake was linear with time for at least 15 sec. Accordingly initial uptake rates were measured after 10 sec of incubation. All experiments illustrated in the Tables and Figures were carried out at 100 mM K gluconate equilibrium in the presence of 10 μg/mg protein of the K^+ ionophore valinomycin (added as a 1-mg/ml stock solution in ethanol) in order to clamp membrane potentials at the K^+ diffusion potential [20]. Except where indicated the results of a single representative experiment are shown.

Calculations

In least-squares fits to the data, points were weighed according to their relative experimental errors. The errors quoted in the text on least-squares parameters are standard deviations.

Enzyme Assays

The BLMV preparation procedure was monitored by assaying for the activities of enzymes characteristic of basolateral membranes (K^+-stimulated *p*-nitrophenyl phosphatase), luminal membranes (dipeptidyl peptidase IV) and mitochondria (succinic dehydrogenase) carried out as previously described [15, 23]. Protein was determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, Calif.) with bovine gamma globulin as the standard.

Materials

^{22}Na was purchased from New England Nuclear (Boston, Mass.), amiloride was a gift from Merck Sharp and Dohme Research Laboratories (Rahway, N.J.), bumetanide was a gift from Hoffman LaRoche (Nutley, N.J.) and N-methyl-D-glucamine (NMDG) was from Aldrich (Milwaukee, Wis.). All other chemicals were from standard commercial sources and were reagent grade or the highest purity available.

Table 1. Enzymatic characterization of rat parotid basolateral membrane vesicles[a]

Enzyme	Activity in homogenate (μmol/mg protein · hr)	Enrichment (vesicles/homogenate)	% Recovery (vesicles/homogenate)
K^+-stimulated *p*-nitrophenyl phosphatase	0.75 ± 0.21	20.1 ± 5.3	17.4 ± 2.8
Dipeptidyl peptidase IV	0.15 ± 0.03	4.2 ± 1.0	3.5 ± 1.0
Succinic dehydrogenase	0.31 ± 0.13	0.28 ± 0.13	0.34 ± 0.18

[a] Activity and enrichment values are means ± SD for eight independent experiments; recoveries are means ± SD for six independent experiments. Enzyme activities are given as micromoles substrate consumed per milligram protein per hour.

Results

Enzymatic Characterization of Vesicle Preparation

Figure 1 shows the distribution of the marker enzymes KpNPPase (basolateral membranes), dipeptidyl peptidase IV (apical membranes) and succinic dehydrogenase (mitochondria) following isopycnic centrifugation of the "crude membrane fraction" in 16% Percoll (*see* Materials and Methods). KpNPPase is clearly localized to a single band on the gradient (fraction 2) far from the peak of succinic dehydrogenase. Dipeptidyl peptidase IV on the other hand is more diffusely distributed showing two peaks, one in fraction 2 and the other in fraction 7. Based on its high enrichment in KpNPPase we have chosen fraction 2 as our BLMV preparation (*see* Materials and Methods). The average activities of KpNPPase, dipeptidyl peptidase IV and succinic dehydrogenase in the initial parotid homogenate as well as the recoveries and enrichments of these enzymes in the purified BLMV are given in Table 1. The BLMV preparation is enriched 20 times over the initial gland homogenate in the basolateral marker KpNPPase. There is also a modest enrichment (4 times) in the activity of the apical membrane marker dipeptidyl peptidase IV. However, owing to the pyramidal shape of the acinar cells [26], the total area of the basolateral membrane is expected to be at least an order of magnitude

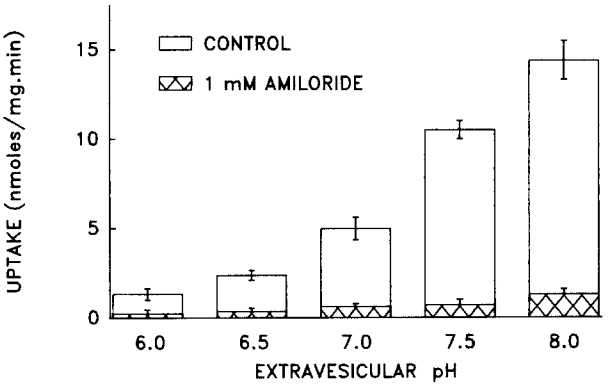

Fig. 2. Initial rate of ^{22}Na uptake into rat parotid BLMV measured as a function of increasing extravesicular pH. BLMV were preequilibrated in Buffer K (pH 6.0). The incubation media were the same buffer (without EDTA) titrated with Tris to the final pH's shown and containing (final concentrations) 1 mM ^{22}Na gluconate with or without 1 mM amiloride as indicated

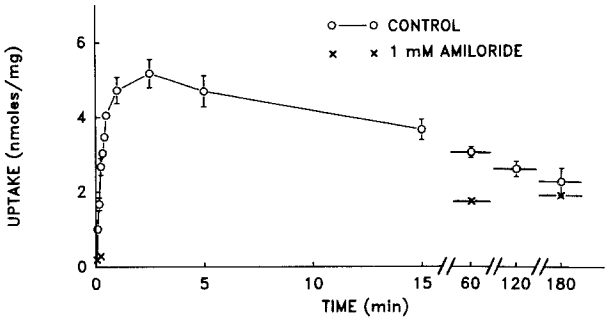

Fig. 3. Timed uptake of 1 mM ^{22}Na into rat parotid BLMV measured in the presence of an initial intravesicular to extravesicular H^+ gradient. BLMV were preequilibrated in Buffer K (pH 6.0). The incubation media were the same buffer (without EDTA) titrated with Tris to pH 8.0 and containing (final concentrations) 1 mM ^{22}Na gluconate with (×) or without (○) 1 mM amiloride

greater than that of the apical membrane. Thus the absolute contamination of the BLMV preparation by apical membranes is expected to be very small.

The present BLMV preparation represents a significant improvement in purity and yield over a similar preparation from the rat parotid reported previously from our laboratory [19], viz., a 20 times enrichment and a 17% recovery of the basolateral marker in this preparation *vs.* a 10 times enrichment and a 10% recovery obtained previously. We typically obtained approximately 2 mg vesicle protein/g rat parotid tissue with the procedure reported here.

Initial Characterization of pH Gradient-Dependent Sodium Uptake into Rat Parotid BLMV

Our initial studies of sodium uptake into the rat parotid BLMV preparation described above indicated a dramatic dependence of flux on the intravesicular to extravesicular H^+ gradient. This result is illustrated in Fig. 2. Here we compare the initial rate of uptake of 1 mM ^{22}Na into BLMV prepared with intravesicular pH 6.0 measured at a series of extravesicular pH's from 6.0 to 8.0. Compared to pH equilibrium conditions ($pH_{in} = pH_{out} = 6.0$) ^{22}Na uptake is stimulated over ten times in the presence of a pH gradient of 2 units ($pH_{in} = 6.0$, $pH_{out} = 8.0$). Ninety percent of this stimulation is inhibited by the K^+-sparing diuretic amiloride (1 mM), a specific inhibitor of mediated Na^+/H^+ exchange in many tissues [3]. Under all conditions tested in Fig. 2 over 85% of the uptake of ^{22}Na was amiloride sensitive. It should also be emphasized that the results shown in Fig. 2 cannot be accounted for by the effects of H^+ diffusion potentials acting on an amiloride-sensitive sodium-conductive pathway since this experiment, and all the other experiments reported here, were carried out under voltage-clamped conditions (*see* Materials and Methods). A small component of conductive sodium flux can in fact be demonstrated in this preparation. For example, at pH 7.4 equilibrium with $[K_{in}] = 100$ mM and $[K_{out}] = 10$ mM the uptake of 1 mM ^{22}Na is stimulated 20 to 30% by the addition of valinomycin; however, this conductive flux is not inhibited by amiloride and thus is probably due to simple diffusion (*data not shown*). Unless otherwise stated all subsequent experiments reported here were carried out in the presence of an outwardly directed H^+ gradient ($pH_{in} = 6.0$, $pH_{out} = 8.0$) in order to more easily study the pH gradient-dependent component of sodium flux.

The time course of 1 mM sodium uptake into the BLMV preparation is illustrated in Fig. 3. This sodium uptake curve exhibits a transient "overshoot" of the intravesicular ^{22}Na concentration above its equilibrium value. This indicates that the vesicle preparation is capable of catalyzing the concentrative uptake of sodium in the presence of a H^+ gradient. The observation that the equilibrium (3 hr) sodium uptakes measured in the presence or absence of 1 mM amiloride are comparable indicates that the inhibition of initial uptake rates by amiloride is not due to a nonspecific alteration of vesicle integrity.

The above results demonstrate that under the experimental conditions employed here most of the sodium flux into the BLMV's is mediated by an amiloride-sensitive, pH gradient-dependent transport pathway capable of driving sodium into the vesicles against a concentration gradient. These results provide strong evidence for the existence of

Table 2. Amiloride-sensitive sodium uptake and enzymatic enrichment of several membrane fractions[a]

Membrane fraction	Amiloride-sensitive sodium uptake (nmol/mg · min)	Enzyme enrichment (fraction/homogenate)	
		K^+-stimulated *p*-nitrophenyl phosphatase	Dipeptidyl peptidase IV
Crude membrane fraction	3.0 ± 1.3	2.6 ± 0.7	2.0 ± 0.3
Fraction 2 (BLMV)	12.4 ± 0.8	15.4 ± 5.4	4.1 ± 0.5
Fraction 7	0.8 ± 0.8	0.26 ± 0.10	2.2 ± 1.4

[a] Values are means ± SD for four independent determinations. For transport studies the membrane fractions were preequilibrated in Buffer K. The incubation media were the same buffer (without EDTA) titrated to pH 8.0 with Tris and containing (final concentrations) 1 mM ^{22}Na gluconate with or without 1 mM amiloride. The initial rate of amiloride-sensitive sodium uptake was obtained by subtracting the uptake measured in the presence of amiloride from that obtained in its absence. Fractions 2 and 7 refer to fractions of the Percoll gradient (*cf.* Fig. 1).

a Na^+/H^+ antiporter in our rat parotid basolateral membrane vesicle preparation.

In Table 2 we compare the amiloride-sensitive component of ^{22}Na uptake measured in the crude membrane fraction (i.e., the fraction loaded onto the Percoll gradient—*see* Materials and Methods and in fractions 2 (the BLMV fraction) and 7 of the Percoll gradient (*see* Fig. 1). Relative to the starting parotid homogenate fraction 7 is enriched in the apical membrane marker dipeptidyl peptidase IV and in the mitochondrial marker succinic dehydrogenase but contains little activity of the basolateral membrane marker KpNPPase (*cf.* Fig. 1 and Table 2). It is clear from Table 2 that the amiloride-sensitive component of ^{22}Na flux copurifies with the activity of the basolateral marker KpNPPase suggesting that the Na^+/H^+ exchanger is localized to the basolateral membrane.

Kinetics of pH Gradient-Dependent Sodium Uptake

Figure 4 shows the results of an experiment in which the amiloride-sensitive component of sodium flux was measured as a function of sodium concentration at $pH_{in} = 6.0$ and $pH_{out} = 8.0$. The data are presented as an Eadie-Hofstee plot. The linearity of this plot indicates that the data are fit well by the Michaelis-Menten equation and thus are consistent with the existence of a single amiloride-sensitive, pH gradient-dependent sodium transport system in this preparation with an average $K_M = 8.0 \pm 0.7$ mM ($n = 3$).

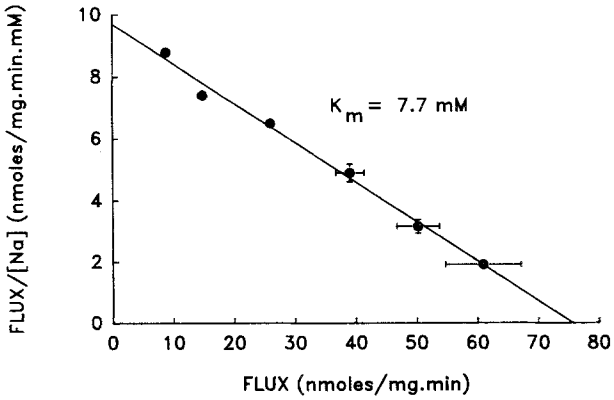

Fig. 4. Initial rate of amiloride-sensitive, pH gradient-dependent sodium uptake into rat parotid BLMV measured as a function of sodium concentration. BLMV were preequilibrated in Buffer K (pH = 6.0). The incubation media were the same buffer (without EDTA) titrated with Tris to pH 8.0 and containing (final concentrations) 1 to 32 mM ^{22}Na gluconate with or without 1 mM amiloride. Na gluconate was replaced isosmotically with NMDG gluconate to obtain the various sodium concentrations tested. Sodium uptake observed in the presence of amiloride was subtracted from all points to yield the amiloride-sensitive component of uptake illustrated. The line drawn through the data points is a least-squares fit given by $K_M = 7.7 \pm 0.4$ mM and $V_{max} = 75.1 \pm 3.1$ nmol · mg^{-1} · min^{-1} with $r = 0.993$

Table 3. Effect of inhibitors on the initial rate of pH gradient-stimulated ^{22}Na uptake into rat parotid BLMV[a]

Inhibitor	Concentration (mM)	Relative uptake
None (control)		1.00
Amiloride	1.0	0.09 ± 0.01
Furosemide	1.0	0.94 ± 0.09
Bumetanide	1.0	0.98 ± 0.07
SITS	0.5	0.97 ± 0.07
DIDS	0.1	0.97 ± 0.06

[a] BLMV were preequilibrated in Buffer K (pH 6.0). The incubation media were the same buffer (without EDTA) titrated to pH 8.0 with Tris and containing (final concentrations) 1 mM ^{22}Na gluconate and the indicated concentrations of the various inhibitors.

Effect of Various Transport Inhibitors on pH Gradient-Stimulated Sodium Uptake

Table 3 shows the results of an experiment where the effects of the transport inhibitors amiloride, furosemide, bumetanide, SITS and DIDS were measured on pH gradient-stimulated sodium uptake by rat parotid BLMV. Of these compounds only

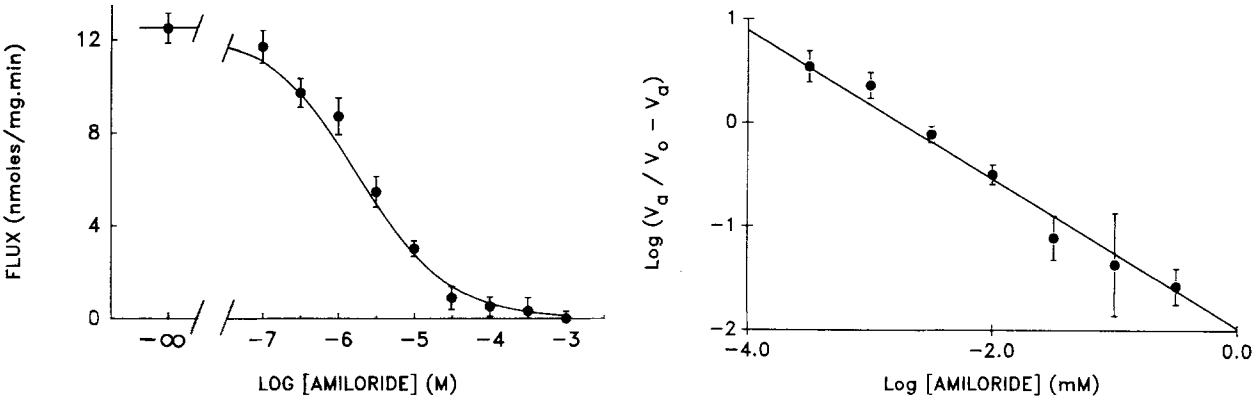

Fig. 5. Inhibition of the initial rate of pH gradient-stimulated sodium uptake by various concentrations of amiloride. BLMV were preequilibrated in Buffer K (pH = 6.0). The incubation media were the same buffer (without EDTA) titrated with Tris to pH 8.0 and containing (final concentrations) 1 mM ^{22}Na gluconate and the concentrations of amiloride indicated. The *left-hand panel* shows the flux data. The sodium uptake observed in the presence of 1 mM amiloride has been subtracted from all points. The *right-hand panel* is a Hill plot of the same results. Here V_a is the initial uptake rate in the presence of amiloride and V_o is the uptake in its absence. A least-squares fit to these points yields a Hill coefficient of 0.72 ± 0.05 and a $K_{0.5}$ for amiloride of 1.73 ± 0.14 μM. The lines drawn through the data points in both panels were calculated from this least-squares fit

Table 4. Comparison of pH gradient-dependent and Cl^- gradient-dependent sodium uptake in rat and rabbit parotid basolateral membrane vesicles[a]

Species	Sodium uptake (nmol/mg protein)	
	pH gradient-dependent	Cl^- gradient-dependent
Rat	11.9 ± 2.1 (n = 12)	1.6 ± 0.3 (n = 5)
Rabbit	2.2 ± 0.2 (n = 4)	3.5 ± 0.5 (n = 5)

[a] The initial rate of pH gradient-dependent ^{22}Na uptake was measured in rat and rabbit BLMV preequilibrated in Buffer K (pH 6.0). The incubation medium was the same buffer (without EDTA) titrated to pH 8.0 with Tris and containing (final concentrations) 1 mM ^{22}Na gluconate with or without 1 mM amiloride. The amiloride-insensitive component of uptake was subtracted in each case. The initial rate of chloride-dependent sodium uptake was measured in BLMV preequilibrated in Buffer A containing 1 mM EDTA. The incubation medium was Buffer A containing (final concentrations) 1 mM ^{22}Na gluconate and 100 mM KCl or 100 mM K methyl sulfate. ^{22}Na uptake (15 sec) was measured after mixing 10 μl of vesicle suspension with 20 μl of incubation medium. The stop solution was Buffer A containing 350 mM KNO_3 [22]. The chloride-dependent component of uptake was obtained by subtracting the uptake observed in the presence of K methyl sulfate from that observed with KCl.

amiloride produced a significant inhibition. The concentration dependence of the inhibitory effect of amiloride is illustrated in Fig. 5. Inhibition of sodium uptake is essentially complete at amiloride concentrations above 3×10^{-5} M (Fig. 5, left-hand panel) and the average K_I for amiloride is 1.6 ± 0.7 μM (n = 3). The right-hand panel of Fig. 5 shows a Hill plot of the inhibitory effect of amiloride. This plot is linear with slope 0.7 indicating that amiloride inhibits sodium uptake in an approximately 1 : 1 fashion.

Comparison of pH Gradient-Dependent and KCl-Dependent Sodium Flux in Rat and Rabbit Parotid BLMV

In a previous publication we have demonstrated the existence of a furosemide-inhibitable, KCl-dependent sodium transport pathway in rabbit parotid basolateral membrane vesicles [22]. This transporter requires the presence of both K^+ and Cl^- to express its sodium transport function. Based on these and other observations we concluded that this transport pathway is a Na/K/Cl cotransport system, presumably the Na/K/Cl cotransporter thought to be involved in parotid salivary fluid secretion [10, 12, 16, 17, 26]. The presence of a similar transport system can be demonstrated in the rat parotid BLMV preparation examined here, however, we have eliminated its contributions to sodium fluxes in the studies presented above by using chloride-free media. In Table 4 we indicate the magnitude of this KCl-dependent component of sodium flux and that of the pH gradient-sensitive, amiloride-inhibitable component of sodium flux in both rat and rabbit parotid BLMV. Bearing in mind that our rabbit and rat BLMV preparation procedures are very similar (*see* Materials and Methods, and that the rat BLMV are enriched approximately 20 times in basolateral markers *vs.* approximately 10 times enrichment in the rabbit, it is clear from Table 4 that, in relative

terms, Na^+/H^+ exchange is much more potent in the rat preparation than in the rabbit while the opposite holds true for Na/K/Cl cotransport.

Discussion

In this paper we present the results of a series of experiments which characterize the pH gradient-dependent sodium-transport properties of rat parotid basolateral membrane vesicle. The vesicle preparation used in our studies was enriched 20 times over the initial gland homogenate in the basolateral membrane marker KpNPPase (Table 1). We show that sodium uptake into this preparation is markedly dependent on the H^+ concentration in the extravesicular medium (Fig. 2). In the presence of an outwardly directed H^+ gradient (pH_{in} = 6.0, pH_{out} = 8.0) sodium uptake is more than ten times greater than the uptake measured at pH equilibrium (pH_{in} = pH_{out} = 6.0). More than 90% of this sodium flux is sensitive to inhibition by the potassium-sparing diuretic drug amiloride, while the transport inhibitors furosemide (1 mM), bumetanide (1 mM), SITS (0.5 mM) and DIDS (0.1 mM) are without effect. We also show that sodium transport can be driven into the vesicles against a concentration gradient by an outwardly directed H^+ gradient (Fig. 3). These results cannot be accounted for by the effects of H^+ diffusion potentials acting on an amiloride-sensitive sodium-conductive pathway since the above experiments were performed under voltage-clamped conditions (*see* Materials and Methods). Furthermore, the existence of such a sodium-conductive pathway could not be demonstrated (*see* Results). Thus our findings provide strong evidence for the direct coupling of sodium and proton movements via a Na^+/H^+ antiport in this preparation. In addition, the fact that this Na^+/H^+ antiport activity copurifies with the enzyme K^+-stimulated *p*-nitrophenyl phosphatase (Fig. 1 and Table 2) indicates that it is associated with the basolateral membrane.

H^+ gradient-dependent sodium flux in our parotid BLMV preparation exhibits a simple Michaelis-Menten-type dependence on sodium concentration (Fig. 4) consistent with the existence of a single transport system with K_M = 8.0 mM at 23°C. This K_M is in good agreement with values obtained for Na^+/H^+ exchange in renal and intestinal brush-border membrane vesicles in experiments carried out under similar conditions [2, 8, 25]. The sensitivity of the rat parotid Na^+/H^+ exchanger to amiloride (K_I = 1.6 μM; Fig. 5) is also in good agreement with values reported for Na^+/H^+ antiporters in other tissues [2, 6, 9, 14, 24]. This K_I value is approximately two orders of magnitude greater than that reported for sodium-conductive channels [5, 13, 18].

The results presented here provide evidence for the existence of a Na^+/H^+ exchanger in the basolateral membrane of rat and rabbit parotid acinar cells (Table 4). These observations considerably extend those of Young and collaborators who found indirect evidence for the presence of acinar Na^+/H^+ exchange in perfusion studies with intact rabbit and rat submandibular glands [4, 12, 17]. On the basis of their experiments these authors argue that basolaterally localized Na^+/H^+ exchange, along with Na/K/Cl cotransport and Cl^-/HCO_3^- exchange, plays a significant role in acinar transepithelial Cl^- and HCO_3^- movements and hence in salivary fluid secretion. The involvement of Na^+/H^+ exchange in salivary secretion is supported by the observations of Novak and Young [12] that amiloride dramatically inhibits salivary secretion in rabbit submandibular glands perfused with Cl-free or HCO_3^--free solutions (*see* Introduction). Surprisingly, however, in glands perfused with both Cl^- and HCO_3^-, these authors found that amiloride was without effect on fluid secretion [12], while in similar studies with rat submandibular glands Pirani et al. [17] observed that amiloride completely abolished the responsiveness of the gland to acetylcholine. The full significance of these differences in amiloride sensitivity in the rat and rabbit submandibular remains to be determined. However, they suggest that the involvement of Na^+/H^+ exchange in acinar fluid secretion could be a complex one and that significant species differences may exist. Our data, in fact, indicate that Na^+/H^+ exchange is much more potent in rat than rabbit BLMV (Table 4) and it is tempting to speculate that this result is indicative of a more important role for Na^+/H^+ in rat than rabbit parotid acinar physiology. A complete understanding of the role of Na^+/H^+ exchange in these cells must, however, await further experimentation.

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