Na/H Exchange in Cultured Chick Heart Cells

pH_i Regulation

DAVID PIWNICA-WORMS, RON JACOB, C. RUSSELL HORRES, and MELVYN LIEBERMAN

From the Department of Physiology, Duke University Medical Center, Durham, North Carolina 27710

ABSTRACT The purpose of this study was to establish the existence of Na/H exchange in cardiac muscle and to evaluate the contribution of Na/H exchange to pH_i regulation. The kinetics of pH_i changes in cultured chick heart cells were monitored microfluorometrically with 6-carboxyfluorescein and correlated with Na_i content changes analyzed by atomic absorption spectrophotometry; transmembrane H^+ movements were evaluated under pH stat conditions. After induction of an intracellular acid load by pretreatment with NH_4Cl, a regulatory cytoplasmic alkalinization occurred with a $t_{1/2}$ of 2.9 min. pH_i regulation required external Na^+ and was concomitant with transmembrane H^+ extrusion as well as a rapid rise in Na_i content in an Na/H ratio of 1:1. Microelectrode recordings of membrane potential demonstrated directly the electroneutral character of pH_i regulation. Acid-induced net Na^+ uptake could be either stimulated by further decreasing pH_i or inhibited by decreasing pH_o; Na^+ uptake was unaffected by tetrodotoxin (10 μg/ml), quinidine (10^{-5} M), DIDS (10^{-4} M), Cl_o-free solution, or HCO_3-free solution. Amiloride (10^{-3} M) maximally inhibited both pH_i regulation and Na^+ uptake; the ID_{50} for amiloride inhibition of Na^+ uptake was 3 μM. Na_o-dependent H^+ extrusion showed half-maximal activation at 15 mM Na_o; Li^+, but not K^+ or choline$^+$, could substitute for Na^+ to support H^+ extrusion. Ca_o-free solution also stimulated acid-induced Na^+ uptake. We conclude that pH_i regulation following an acid load in cardiac muscle cells is by an amiloride-sensitive, electroneutral Na/H exchange. Stimulation of Na/H exchange up to 54 pmol/cm^2·s indicates the rapidity of this exchange across cardiac cell membranes. Na/H exchange may also participate in steady state maintenance of pH_i.

INTRODUCTION

The intracellular pH (pH_i) of cardiac muscle in steady state is ~7.1 (Ellis and Thomas, 1976; Roos and Boron, 1981), 1 pH unit more alkaline than the pH_i expected for electrochemical equilibrium of H^+. This gradient implies the existence of an H^+ extrusion system; however, mechanisms of pH_i maintenance in heart cells in steady state have not yet been resolved. Recent evidence in non-

Address reprint requests to Dr. M. Lieberman, Dept. of Physiology, Box 3709, Duke University Medical Center, Durham, NC 27710. Dr. Piwnica-Worms' present address is Dept. of Radiology, Brigham and Women's Hospital, Boston, MA 02115.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/85/01/0043/22$1.00

Volume 85 January 1985 43-64

steady state indicates that Cl/HCO_3 exchange may regulate pH_i following cytosolic alkalinization (Vaughan-Jones, 1982), but does not appear to participate significantly in pH_i regulation following cytosolic acidification (Gonzalez and Clancy, 1981; Vaughan-Jones, 1982; Piwnica-Worms, 1983; Vanheel et al., 1984). We recently reported changes in pH_i induced by lowering external Na^+ consistent with an Na/H exchange mechanism in cultured heart cells (Piwnica-Worms and Lieberman, 1983); evidence suggestive of Na/H exchange has also been reported for the sheep cardiac Purkinje fiber (Deitmer and Ellis, 1980; Ellis and MacLeod, 1983). This mechanism participates in pH_i regulation following an acid load in several different tissues: mouse soleus muscle (Aickin and Thomas, 1977), crayfish neuron (Moody, 1981), salamander renal proximal tubule (Boron and Boulpaep, 1983), and rat lymphocytes (Grinstein et al., 1984). Thus, the purpose of this study was to firmly establish the existence of Na/H exchange in the cell membrane of a cardiac preparation and to evaluate the contribution of Na/H exchange to pH_i regulation. Monitoring pH_i with the fluorescent dye 6-carboxyfluorescein in combination with measurements of transmembrane H^+ movements, Na_i content, and membrane potential demonstrates that electroneutral Na/H exchange regulates pH_i following an acid load in heart cells and may participate in steady state pH_i maintenance.

METHODS

Tissue Culture

The techniques for producing polystrands composed chiefly of muscle (contractile) or fibroblasts (noncontractile) from 10–11-d-old chick hearts disaggregated with trypsin have been previously described (Horres et al., 1977; Piwnica-Worms et al., 1983). The contractile preparation consists of 70 strands of muscle cells formed as a sheath around a nylon core, with each strand up to 5 mm in length and an outside diameter typically <100 μm (tissue dry weight ~0.25 mg).

Experimental Solutions

The compositions of the modified Earle's solutions are given in Table I. NH_4 solution was made by equimolar substitution of 20 mM NH_4Cl for NaCl; low Na_o solution was produced by equimolar substitution of tetramethylammonium-Cl (TMA-Cl) for NaCl; Cl_o-free solution was produced by equimolar substitution of methanesulfonate for Cl (see Kenyon and Gibbons, 1977). Bicarbonate-containing solutions were maintained at pH 7.4 by gassing with 96% air/4% CO_2, while solutions buffered with various ratios of HEPES/Tris were titrated in air with HCl or NaOH to the indicated pH. Ouabain (Sigma Chemical Co., St. Louis, MO), quinidine HCl (Sigma Chemical Co.), 4,4-diisothiocyano-2,2′-disulfonic acid stilbene (DIDS; Pierce Chemical Co., Rockford, IL), and amiloride (a gift from Dr. E. Cragoe, Merck, Sharp & Dohme Research Laboratories, West Point, PA) were added directly as solid powder to perfusates. Tetrodotoxin (TTX; Sankyo Co., Tokyo, Japan) was dissolved in distilled H_2O before addition to solutions.

Intracellular Ion Content

Experiments were carried out at 37°C in an incubator containing either 96% air/4% CO_2 or 100% air at ~95% humidity (Horres et al., 1977). To follow rapid Na_i content changes, preparations were rinsed continuously in 250 ml of solution for 30–180 s. To follow Na_i

content changes over longer periods (minutes), preparations were rinsed for 30–45 s, followed by incubation in the same solution. Clearance of extracellular Na^+ in polystrands occurs with a half-time ($t_{1/2}$) of 4 s (Wheeler et al., 1982).

Each experiment was ended by rinsing polystrands in ice-cold Na-free, K-free solution (Table I) for 30 s to clear extracellular cations. Cold solution inhibits >98% of transmembrane Na efflux (Wheeler et al., 1982). Preparations were then rapidly air-dried, removed from the silver support, dried overnight in a vacuum oven at 110°C (National Appliance

TABLE I

Solutions

Component*	1 Control	2 Low Na	3 NH_4^+	4 0-Cl	5 0-HCO_3	6 pH stat	7 0-Na, 0-K rinse
Na^+	144.8	0.8	124.8	144.8	123.8	144.0	—
TMA^+	—	118.0	—	—	—	—	—
K^+	5.4	5.4	5.4	5.4	5.4	5.4	—
Mg^{2+}	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Ca^{2+}	2.7	2.7	2.7	2.7	2.7	2.7	2.7
$Choline^+$	—	26.0	—	—	—	—	145.0
NH_4^+	—	—	20.0	—	—	—	—
Cl^-	128.8	128.8	128.8	—	133.8	156.4	150.4
X^-	—	—	—	128.0	—	—	—
HCO_3^-	26.0	26.0	26.0	26.0	—	—	—
$H_2PO_4^-$	0.8	0.8	0.8	0.8	0.8	—	—
SO_4^{2-}	0.8	0.8	0.8	0.8	0.8	—	0.8
Glucose	5.6	5.6	5.6	5.6	5.6	5.6	5.6
HEPES	—	—	—	—	40–5	0.05	5.4
Tris	—	—	—	—	0–35	—	4.1
Albumin (g/liter)	1.4	1.4	1.4	1.4	1.4	—	—
pH_o	7.4	7.4	7.4	7.4	5.5–8.5	7.4	7.4

* Compositions are given in millimolar unless otherwise noted. X^- is methanesulfonate (Eastman Kodak Corp., Rochester, NY). TMA^+ (tetramethylammonium) was obtained from ICN Pharmaceuticals, Plainview, NY; HEPES (Ultrol Brand) from Calbiochem-Behring, La Jolla, CA; and Tris, bovine serum albumin (fraction V), and choline-HCO_3 from Sigma Chemical Co., St. Louis, MO. HCO_3-free, HPO_4-free solution was made from solution 5 omitting NaH_2PO_4; some Na-free solutions were made from solution 2 by substituting KH_2PO_4 for NaH_2PO_4 while adjusting KCl; Ca-free rinse was made from solution 7 by omitting $CaCl_2$.

Co., Portland, OR), and weighed on an electrobalance (Cahn G-2, Ventron Instruments, Paramount, CA). The contribution of monofilament without tissue was subtracted from the total weight of the preparations to obtain tissue dry weight (Piwnica-Worms et al., 1983). Individual preparations were then extracted for 2 h in 0.5 ml of 1 N HNO_3 (reagent grade). For Na^+ analysis, 1.5 ml of Na/K blank solution containing 0.685 g/liter CsCl and 0.125 ml/liter Acationox (Scientific Products, McGraw Park, IL) was added to the extract. (For K^+ analysis, an aliquot of the resulting solution was further diluted to 1 part to 4 parts of blank solution.) The Na^+ and K^+ concentration of each diluted extract was determined with an atomic absorption spectrophotometer (model 460, Perkin-Elmer, Norwalk, CT) along with appropriate Na/K standards using parallel reagent and substrate

controls to correct for possible cation contamination. Intracellular ionic concentrations were calculated using steady state cell water determinations for the polystrand (4.29 μl/mg dry weight; Horres et al., 1977).

The total cell Ca^{2+} content (Ca_i) was also determined by atomic absorption spectrophotometry after rinsing polystrands in ice-cold Ca-free solution for 30 s. After dry weight determination, two polystrands were pooled to increase material for analysis and extracted for 2 h in 200 μl of 1 N HNO_3. 250 μl of Ca blank solution containing 0.75 N HNO_3 and 13 mM $LaCl_3$ was then added to each extract and the Ca^{2+} concentration was measured together with appropriate reagent blanks and calibration solutions.

Ion content determinations normalized to tissue dry weight, unlike ionic activities, are not complicated by water movement under non-steady state conditions and therefore accurately reflect net transmembrane ionic movements (McManus and Schmidt, 1978). In addition, ^{42}K and ^{24}Na tracer experiments do not indicate the existence of hidden pericellular compartments in the polystrand, which, if present, would confound calculations of transmembrane ion flux from measurement of ionic content (Horres and Lieberman, 1977; Wheeler et al., 1982).

Intracellular pH Monitoring

The fluorescence intensity of 6-carboxyfluorescein (6CF) indicated relative changes in intracellular pH (Piwnica-Worms and Lieberman, 1983). Briefly, preparations were exposed to the nonfluorescent precursor dye 6CF diacetate (Molecular Probes, Inc., Junction City, OR), which diffused through the cell membrane to be cleaved by native intracellular esterases to form 6CF. Preparations were then transferred to a perfusion chamber and fluorescence intensity (excitation, 488 nm; emission, 523 nm) was monitored with a filter microfluorometer. Because the dye concentration and the pathlength of light were constant, the direction and rate of fluorescence intensity changes indicated the direction and response time of changes in pH_i. Single-wavelength fluorescence is nonlinear for pH >7.3 (Piwnica-Worms and Lieberman, 1983); therefore, response times are reported as apparent $t_{1/2}$. For pH <7.3 (acid-loaded conditions), apparent $t_{1/2}$ values are not expected to be greatly distorted by this nonlinearity.

pH Stat

To measure the transmembrane movement of H^+ equivalents, polystrands were suspended in plastic cuvettes containing 3.0 ml of test solution weakly buffered with 0.05 mM HEPES (pH 7.40; Table I). Solutions were continuously mixed with a magnetic stirbar and maintained at 37°C. Acid release by the cells was monitored using a microcombination pH electrode (MI-710, Microelectrodes, Inc., Londonderry, NH) coupled to a pH meter (model 26, Radiometer, Copenhagen, Denmark) and a pH stat titrator (model 11 with an ABU 12 Autoburette, Radiometer). Solution pH was maintained at 7.40 ± 0.01 using 0.5 mM KOH as a titrant and both the rate of titrant addition and solution pH were continuously recorded (model A5223-5, Omniscribe 2 channel, Houston Instrument, Austin, TX). Correction for background CO_2 absorption (typically 1–2 nmol H^+ equivalents/min) was made in all experiments. At the end of each experiment, preparations were removed for dry weight determination and solution buffer capacity was measured by addition of KOH. If net alkali, rather than acid, was transiently extruded by preparations, pH stat titration automatically stopped while alkaline drifts in pH_o continued to be recorded. This ΔpH_o, multiplied by the solution buffer capacity, was used to measure net extrusion of alkali equivalents.

Electrical Recordings

Transmembrane potentials were recorded using standard microelectrode techniques on spontaneously beating polystrands in a superfusion chamber (Horres et al., 1979; Piwnica-Worms et al., 1983); switching between solutions occurred with a $t_{1/2}$ of 4 s at a flow rate of 10 ml/min. Records were retained with a chart recorder (7702B, Hewlett-Packard Co., Waltham, MA).

Statistics

Values are presented as means ± SEM. Statistical significance was evaluated by the two-tailed Student's *t* test unless otherwise indicated (Remington and Schork, 1970).

RESULTS

pH_i Regulation

pH_i regulation in heart cells was examined following an acid load produced by the NH_4^+ prepulse technique (Boron and De Weer, 1976; Roos and Boron, 1981). NH_4^+-loaded cells rapidly lose NH_3 on exposure to NH_4^+-free solution; H^+ released in the cells results in cytoplasmic acidification. Fig. 1 *A* illustrates the pH_i response induced by a 15-min pretreatment with solution containing 20 mM NH_4^+. Removal of external NH_4^+ caused a rapid cytosolic acidification, followed by pH_i recovery with a $t_{1/2}$ of ~2.9 ± 0.5 min (n = 6). Regulation of pH_i was inhibited by Na_o-free solution (Fig. 1 *B*); pH_i rapidly recovered on subsequent re-establishment of an inward Na gradient. The cause of the transient acidification observed during the initial return to control Na_o is unknown; a similar transient, under these conditions, has also been recorded in renal proximal tubules with pH-selective microelectrodes (Boron and Boulpaep, 1983). The effect of amiloride (10^{-3} M), a rapid and reversible inhibitor of Na/H exchange in several cell types (for review, Benos, 1982), is illustrated in Fig. 1 *C*. The initial rapid decrease in fluorescence on addition of amiloride reflected, in part, quenching of the 6CF signal by amiloride[1] since the neutral form of the drug can permeate cells (Benos et al., 1983). Amiloride did not block pH_i changes caused by passive NH_3 movements, but did block the pH_i-regulatory response following the NH_4^+-induced acid load (n = 3). In sum, these findings suggest an Na/H exchange mechanism involved in pH_i regulation in heart cells following an acid perturbation.

pH_i-induced Changes in Na_i

Figs. 2 and 3 show changes in Na_i content caused by an NH_4^+ prepulse. In control

[1] Despite preliminary results to the contrary (Piwnica-Worms and Lieberman, 1983), a control series of experiments confirmed that amiloride can affect 6CF fluorescence as predicted by equations for either static or dynamic quenching (Lakowicz, 1983): at a fixed 6CF concentration, quenching was proportional to the amiloride concentration such that the fluorescence intensity of 1 μM 6CF in control solution was reduced 24% by 10^{-3} M amiloride, 4% by 10^{-4} M amiloride, and ~1% by 10^{-5} M amiloride. On the other hand, at a fixed amiloride concentration, quenching was a constant proportion of the fluorescence intensity at various 6CF concentrations. Quenching was identical with or without protein (albumin) and in HCO_3- or HEPES-buffered solutions.

polystrands, steady state Na_i was 68.0 ± 3.6 nmol/mg dry weight (n = 9) or 15.8 ± 0.8 mM, assuming a steady state water content of 4.29 μl/mg dry weight. This value correlated well with previously published measurements of Na_i in polystrands (Wheeler et al., 1982). After 15 min in NH_4^+ solution, Na_i was 48.7 ± 4.2 nmol/mg dry weight (n = 5). Acidifying the cytosol by shifting NH_4^+-loaded preparations to NH_4^+-free (control) solution for 1 min caused a rapid increase in Na_i to almost twice the steady state value. The difference of 75.6 ± 6.9 nmol/

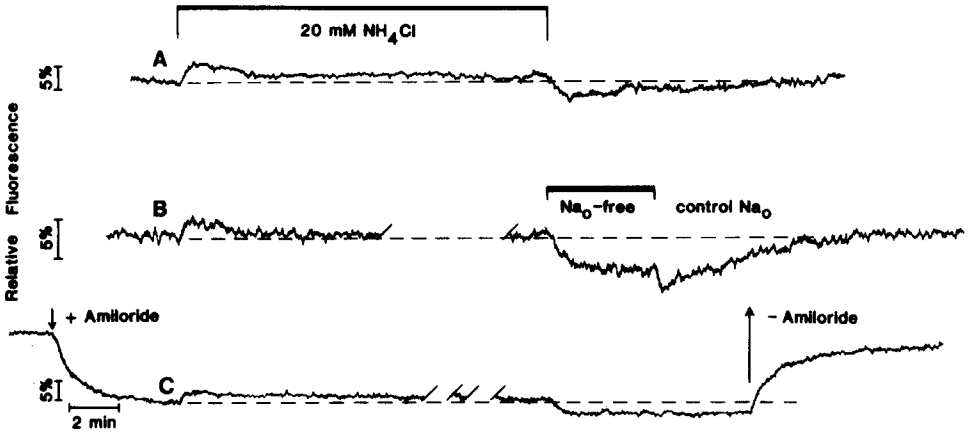

FIGURE 1. Effects of Na-free solution and amiloride on pH_i recovery after acid load as monitored microfluorometrically with the intracellular pH-sensitive dye 6CF (excitation, 488 nm; emission, 523 nm). Tracings show relative fluorescence intensity. Top bar shows time of exposure to 20 mM NH_4^+ for all traces. (*A*) pH_i recovery in control solution. Six experiments on five preparations showed similar results. (*B*) Effect of Na-free solution. Horizontal dashed lines indicate fluorescence intensity prior to NH_4Cl exposure; breaks in the records indicate where light was scattered by bubbles passing through the chamber. (*C*) Effect of exposure to amiloride (10^{-3} M). Each tracing represents a different preparation. Relative fluorescence is expressed as a percentage of total fluorescence due to the dye. Downward deflection of relative fluorescence trace indicates decreasing pH_i.

mg dry weight between the latter two values of Na_i content (Fig. 3, $b - a$) represented an acid-induced net Na uptake. The 1-min uptake proved to be the most useful measure of acid-induced net Na movements for technical reasons associated with obtaining consistent results at shorter times and because Na_i content peaked at this time. The decline in Na_i content over longer times (Fig. 2) was ouabain inhibitable (10^{-4} M; data not shown), which suggests that the Na/K pump secondarily responded to the acid-induced increase in Na_i.

pH_i regulation was previously found to be amiloride sensitive, and therefore Na_i changes during pH_i regulation were similarly examined. Amiloride (10^{-3} M) did not significantly affect the fall in Na_i during NH_4^+ exposure; however,

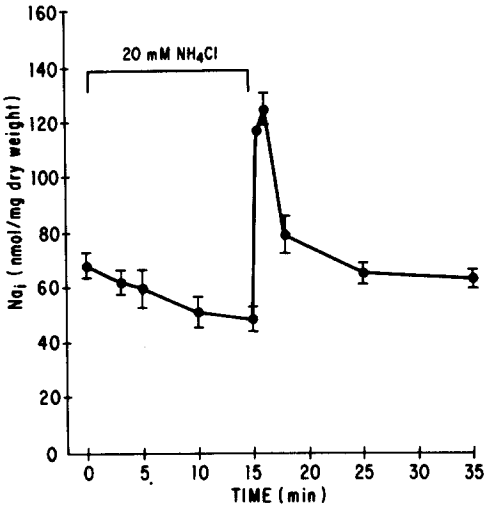

FIGURE 2. Changes in Na_i content during NH_4^+-induced acid load. Bars represent means ± SEM of three to six determinations; individual points are means of two experiments. Na_i content at time zero is the value in control solution; no significant changes occurred in preparations maintained in control solution for times similar to these experiments.

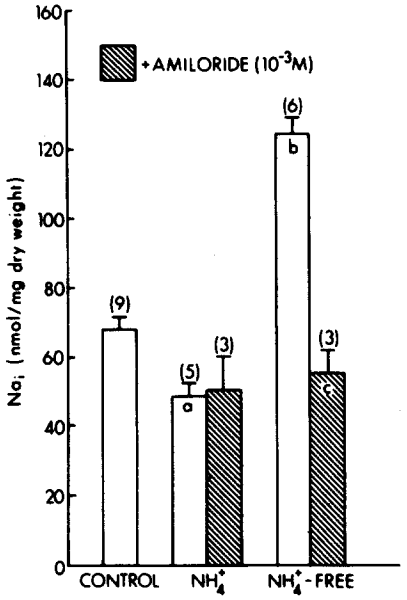

FIGURE 3. Changes in Na_i content of cultured heart cells produced by NH_4^+-induced changes in pH_i. Na_i content was measured in control solution after a 15-min exposure to 20 mM NH_4^+ (solution 3, Table I) and after a subsequent 1-min return to NH_4^+-free (control) solution. Bars represent means ± SEM; *n* is the number of determinations.

exposing polystrands to NH_4^+-free solution containing amiloride blocked net Na uptake. The difference of 68.5 ± 8.7 nmol/mg dry weight (Fig. 3, *b* − *c*) represented the amiloride-sensitive net Na uptake, which was 91% of the acid-induced net Na uptake. Similar results were obtained whether amiloride was applied at the beginning of NH_4^+ exposure and present throughout the return to NH_4^+-free solution or applied simultaneously with a return to NH_4^+-free solution (amiloride-sensitive Na uptake: 68.5 ± 8.7 vs. 67.2 ± 7.4 nmol/mg dry weight; degrees of freedom = 7, $P > 0.50$), which suggests that amiloride inhibited Na uptake within seconds.

Spontaneously contractile polystrands contain a nonmuscle component (fibroblast-like cells) that may account for up to 25% of the ionic content (Horres, 1975). No significant difference was found in Na_i content between contractile (C) polystrands and noncontractile (NC) polystrands under control conditions (C: 68.0 ± 3.6 [$n = 9$] vs. NC: 82.1 ± 11.1 [$n = 3$] nmol/mg dry weight; $P > 0.30$). Similarly, no differences were found after 15 min in NH_4^+ (C: 48.7 ± 4.2 [$n = 5$] vs. NC: 36.7 ± 3.8 [$n = 3$] nmol/mg dry weight; $P > 0.05$). However, Na_i content in noncontractile polystrands only approached control values 1 min after return to NH_4^+-free solution (75.3 ± 10.6 nmol/mg dry weight [$n = 3$]), and was significantly less than Na_i content in contractile polystrands (124.3 ± 5.5 nmol/mg dry weight [$n = 6$]; $P < 0.02$). Thus, nonmuscle cells in contractile polystrands could not account for the rapid increase in Na_i content beyond control values. In fact, their presence would cause an underestimation of net Na uptake into cardiac muscle cells.

The effects of pharmacological agents and changes in external ion content on Na uptake induced by an acid load are shown in Table II. Polystrands were exposed for 15 min to 20 mM NH_4^+ solution (except as noted) and then switched for 1 min to NH_4^+-free solutions altered as indicated. Net Na uptake is presented as a percent of Na uptake induced by a return to NH_4^+-free (control) solution. Amiloride (10^{-3} M) significantly inhibited net Na uptake. Ouabain (10^{-4} M), at a dose known to rapidly and completely inhibit the Na/K pump in polystrands (Lieberman et al., 1982), significantly increased net Na uptake to 138% of control or, accounting for the 1-min uptake period, to a rate of 103.8 ± 9.6 nmol Na/mg dry weight·1 min ($n = 7$). In the absence of an acid load, ouabain (10^{-4} M) causes Na_i content in cultured heart cells to increase at a rate of only 10–14 nmol/mg dry weight·1 min (recalculated from Horres et al., 1979, and Wheeler, 1981). Therefore, Na/K pump inhibition alone cannot account for the rapid acid-induced net Na uptake. Simultaneous addition of amiloride and ouabain produced a small net Na uptake. Either ouabain competed for amiloride binding sites, rendering amiloride ineffective, or, more likely, the simultaneous addition of these two agents induced Na uptake by an amiloride- and ouabain-insensitive pathway. For this reason, the acid-induced net Na uptake, unattenuated by Na/K pump activity, was taken to be the difference in Na uptake between each agent acting alone. Tetrodotoxin (10 μg/ml) applied throughout the maneuver did not significantly affect acid-induced net Na uptake, which suggests that Na^+ movements were not mediated by fast Na channels. Quinidine (10^{-3} M), a putative blocker of Na/Ca exchange (Parker, 1978), applied 1 min

before the return to control solution, caused Na_i content changes similar to ouabain (data not shown), which indicates that quinidine also does not inhibit acid-induced net Na uptake.

In control solution, 2 min after application of amiloride (10^{-3} M), Na_i declined to 57.8 ± 3.6 nmol/mg dry weight (n = 3); after 5 min, Na_i was 56.8 ± 3.5 nmol/mg dry weight (n = 4), a value significantly different from control (68.0 ± 3.6; $P < 0.05$). Membrane potential recordings during addition of amiloride (10^{-3} M) to control solution for 5 min showed maximum diastolic potentials to depolarize by 4–5 mV with no changes in overshoot potentials (data not shown). These findings were inconsistent with a decrease in Na^+ conductance and

TABLE II

Effects of Various Drugs and External Ions on Acid-induced Na Uptake

Condition	ΔNa_i (% control)	n	P
NH_4^+-free control	100±9	6	—
+ Amiloride (10^{-3} M)	10±4	10	<0.001
+ Ouabain (10^{-4} M)	138±13	7	0.05
+ Amiloride (10^{-3} M) + ouabain (10^{-4} M)	36±15	4	<0.02
+ TTX (10 μg/ml)	86±6	3	>0.20
+ DIDS (10^{-4} M)	82±14	4	>0.30
HCO_3^--free	96±20	3	>0.50
Cl^--free	109±22	4	>0.50

Polystrands were exposed to 20 mM NH_4Cl solution for 15 min and then incubated for 1 min in NH_4^+-free (control) solution altered as indicated (see text for details). Net Na uptake in NH_4^+-free solution was normalized to the control NH_4^+-free maneuver as seen in Fig. 3. Results are presented as means ± SEM; n is the number of determinations; P values are a two-tailed comparison with control.

suggested that part of the steady state Na^+ "leak" may actually be mediated by Na/H exchange.

Na/H Exchange vs. Na + HCO_3/Cl + H Exchange

The results shown thus far are consistent with the presence of an Na/H exchange mechanism. However, the Na uptake induced by an acid load does not distinguish between Na/H exchange and a coupled Na + HCO_3/Cl + H exchange. The latter occurs in squid axons (Boron and Russell, 1983), snail neuron (Thomas, 1977), and barnacle muscle (Boron et al., 1981), and would also produce net Na uptake in response to an acid load. This exchange is affected by the direction of the Cl^- gradient and is inhibited by removal of HCO_3^- as well as by the application of stilbene derivatives (Russell et al., 1983). Therefore, to rule out the possibility of Na + HCO_3/Cl + H exchange, Na uptake following NH_4Cl preincubation was measured under the following conditions (Table II): (*a*) in nominally HCO_3^--free solution throughout, (*b*) with a return to Cl^--free solution (which might stimulate Na uptake), and (*c*) in the presence of DIDS (10^{-4} M), at a dose 10 times that necessary to inhibit 90% of pH_i regulation mediated by Na + HCO_3/Cl + H

exchange in snail neuron (Thomas, 1976). None of these treatments influenced the acid-induced net Na uptake in cultured heart cells.

Amiloride Dose Response

The dose-response curve for amiloride inhibition of Na uptake induced by an acid load is shown in Fig. 4. At an Na_o concentration of 144 mM, half-maximal inhibition of amiloride-sensitive net Na uptake occurred at 3 μM.

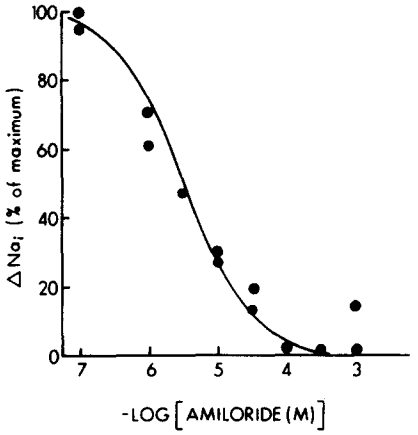

FIGURE 4. Dose-response curve for amiloride inhibition of acid-induced net Na^+ uptake by cultured heart cells. Na^+ uptake was defined as the difference between Na_i content after a 15-min incubation in 20 mM NH_4^+ solution and after a subsequent 1-min switch to NH_4^+-free (control) solution containing various concentrations of amiloride. pH_o was 7.4; Na_o was 144 mM. The maximum net Na^+ uptake in this experiment was 57 nmol/mg dry weight·1 min. The solid line represents a simple titration curve with an apparent K_I of 3 μM.

pH_o Effects on Acid-induced Na Uptake

The response of Na uptake to changes in pH_o (maintaining pH_i approximately constant) is summarized in Table III. Preparations were exposed to HCO_3^--free, HPO_4^{2-}-free NH_4^+ solution (pH_o 7.4) for 15 min before switching to HCO_3^--free, HPO_4^{2-}-free, NH_4^+-free solutions of various pH_o's. The absence of HCO_3^- and HPO_4^{2-} in the solutions allowed pH_o to be varied widely without precipitating calcium salts; ouabain (10^{-4} M) was added to NH_4^+-free solutions to prevent Na/K pump attenuation of net Na uptake. Increasing pH_o from 7.4 to 8.5 in NH_4^+-free solution did not significantly change amiloride-sensitive net Na uptake. However, exposing preparations to solutions at pH_o 6.5 decreased amiloride-sensitive net Na uptake; solutions at pH_o 5.5 further decreased net Na uptake. Although the decrease in net Na uptake upon decreasing pH_o was thermodynamically consistent with Na/H exchange, these data are also compatible with a kinetic effect due to competition between external H^+ and Na^+ for a binding site on the exchanger.

pH_i Effects on Acid-induced Na Uptake

The dependence of Na uptake on pH_i (at constant pH_o) was examined by exposing polystrands to NH_4^+ solution (pH_o 7.4) for various times before returning to NH_4^+-free solution (pH_o 7.4). Prolonging the duration of NH_4^+ exposure (but less than the time required for NH_4^+ to reach electrochemical equilibrium) increases the magnitude of the acidification produced on switching to NH_4^+-free solution (Roos and Boron, 1981). Therefore, as shown in Fig. 5, increasing the internal acidification increased amiloride-sensitive net Na uptake. Because ouabain (10^{-4} M) was applied during NH_4^+-free incubation, changes in net Na uptake could not be explained by pH_i sensitivity of the Na/K pump-mediated Na efflux (Breitwieser and Russell, 1983).

TABLE III

The Influence of pH_o on Acid-induced Na Uptake in Cultured Heart Cells

pH_o	Amiloride	ΔNa_i	Amiloride-sensitive ΔNa_i
	10^{-3} M	*nmol/mg dry wt · 1 min*	*nmol/mg dry wt · 1 min*
8.5	O*	87.2±7.0 (6)	80.3±7.6‡
	+	6.9±2.9 (4)	
7.4	O	93.9±10.7 (6)	86.4±10.9
	+	7.5±1.9 (3)	
6.5	O	65.7±3.5 (3)	56.6±5.0§
	+	9.1±3.6 (3)	
5.5	O	37.9±15.2 (3)	29.9±15.5§‖
	+	8.0±3.4 (3)	

Values are means ± SEM of n determinations. Net Na uptake was defined as the difference in Na_i after incubating in HCO_3^--free, HPO_4^{2-}-free, 20 mM NH_4^+ solution for 15 min, and Na_i after a subsequent incubation for 1 min in HCO_3^--free, PO_4^{3-}-free, NH_4^+-free solution containing either ouabain* (10^{-4} M) or amiloride (10^{-3} M).

‡ Comparison with pH_o 7.4, $P > 0.50$.

§ Comparison with pH_o 7.4, $P < 0.05$ (one-tailed).

‖ Comparison with pH_o 6.5, $P = 0.07$ (one-tailed).

Na_o-induced pH_i Changes

To examine the relationship between Na_o and pH_i, Na gradients were systematically varied while rates of pH_i changes were monitored with 6CF (Fig. 6). From a stable pH_i in control solution, lowering Na_o resulted in increased rates of intracellular acidification up to a minimum $t_{1/2}$ of ~20 s in 0.8 mM Na_o solution. Responses occurred only when Na_o was reduced below 72 mM and, within the accuracy of this technique, a half-maximal response occurred between 20 and 30 mM Na_o. On switching to progressively lower Na_o, both the rate and relative magnitude of the cytosolic acidification increased. However, this qualitative change in magnitude could not be analyzed further because the observations in this series involved different polystrands and hence the final dye concentration may have varied (see Piwnica-Worms and Lieberman, 1983). In contrast, heart cells Na_i-depleted by preincubation in low Na_o alkalinized on return to normal

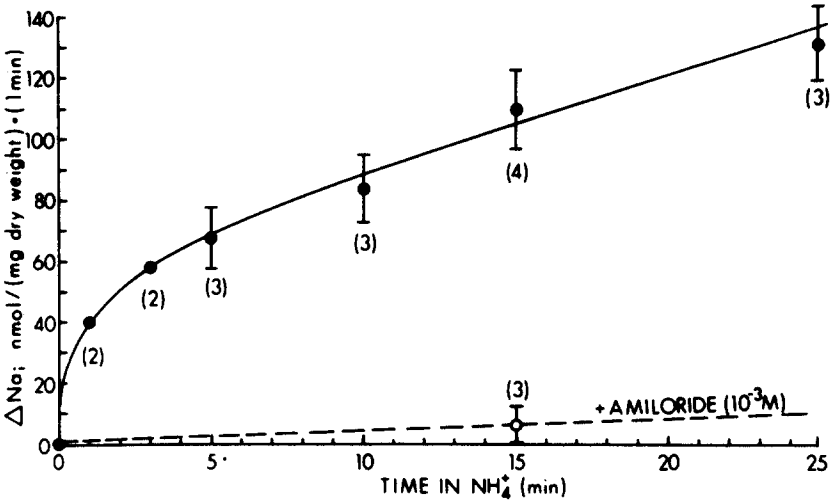

FIGURE 5. Effect of decreasing pH_i on acid-induced net Na^+ uptake in cultured heart cells. After incubating cells for various times in 20 mM NH_4^+ solution, the net change in Na_i content was determined after 1 min in NH_4^+-free (control) solution containing ouabain (10^{-4} M). Increasing the time in NH_4^+ solution decreased pH_i during the subsequent exposure to NH_4^+-free solution.

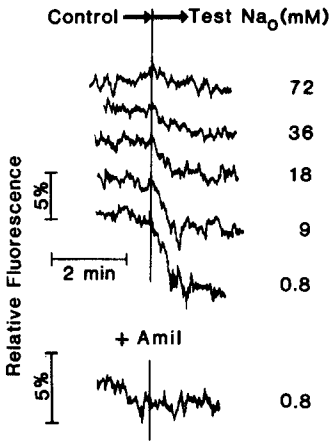

FIGURE 6. Rate of cytosolic acidification in cultured heart cells as a function of Na_o. Kinetics of pH_i changes were monitored with 6CF (excitation, 488 nm; emission, 523 nm). Relative fluorescence is expressed as a percentage of total fluorescence due to the dye. Downward deflection of relative fluorescence trace indicates decreasing pH_i. Following the stable recording of pH_i in control solution, six preparations were switched to test solutions of different Na_o (TMA substitution) produced by mixing various ratios of solutions 1 and 2 (Table I). In this series, the diameter of each monitored strand was 100 μm. In the last tracing, amiloride (10^{-3} M) was added 3 min before the switch to low Na_o solution.

Na_o. Amiloride (10^{-3} M) completely and reversibly inhibited both low Na_o-induced acidification (Fig. 6; $n = 3$) and, in Na_i-depleted cells, normal Na_o-induced alkalinization. Ouabain (10^{-4} M) did not inhibit Na_o-induced pH_i changes (data not shown).

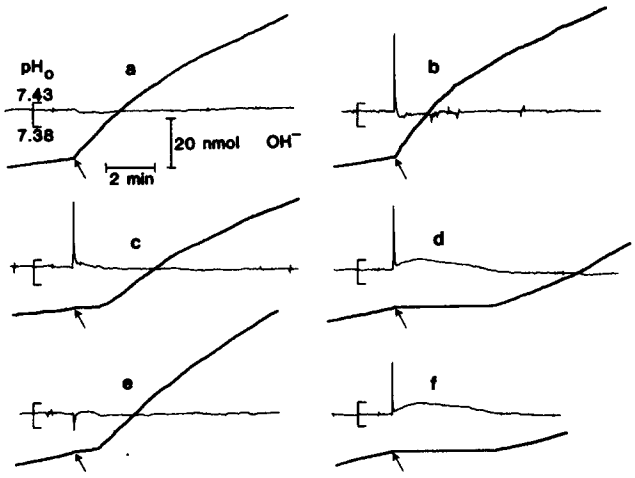

FIGURE 7. Rate of acid release by cultured heart cells under pH stat conditions. Polystrands were preincubated for 15 min in pH stat control solution alone (solution 6; Table I), modified as indicated below and by addition of 5 mM HEPES to maintain pH 7.4. At the arrow, polystrands were transferred to the pH stat cuvette containing lightly buffered control solution alone (solution 6) or modified as indicated, and the rate of acid release was recorded as the rate of KOH addition to maintain pH 7.40 ± 0.01. The initial portion of each pH stat tracing shows background CO_2 absorption by the solution. The thinner tracing in each panel is pH_o. The vertical and horizontal scales apply to all panels. Each panel is representative of four to six identical experiments on different preparations, except panel *e*, which was one observation (see Table II for confirmation of DIDS effect). The final acid extrusion rates are normalized to tissue dry weight (see text). (*a*) Preincubation: control solution; pH stat: control solution. (*b*) Preincubation: control solution plus amiloride (10^{-4} M); pH stat: control solution plus amiloride (10^{-4} M). (*c*) Preincubation: 20 mM NH_4Cl solution; pH stat: control solution. (*d*) Preincubation: 20 mM NH_4Cl solution plus amiloride (10^{-4} M); pH stat: control solution plus amiloride (10^{-4} M). (*e*) Preincubation: 20 mM NH_4Cl solution plus DIDS (10^{-4} M); pH stat: control solution plus DIDS (10^{-4} M). (*f*) Preincubation: 20 mM NH_4Cl, Na_o-free solution; pH stat: Na_o-free solution.

Transmembrane H^+ Transport

pH stat experiments confirmed that pH_i regulation following an acid load involved the transmembrane extrusion of H^+ equivalents rather than an Na-dependent intracellular buffering mechanism. Preparations preincubated for 15 min in control solution, when transferred to the pH stat cuvette containing control solution, showed a rapid phase of acid release of unknown origin (Fig. 7*a*); after ~5 min, a steady rate of acid extrusion was achieved (22.8 ± 1.4 nmol

H^+ equivalents/mg dry weight·min; $n = 12$). Amiloride (10^{-4} M) did not inhibit the initial acid transient (Fig. 7*b*), but reduced the steady rate to 16.4 ± 1.7 nmol H^+ equivalents/mg dry weight·min ($n = 5$). Preparations preincubated for 15 min in NH_4Cl did not show a rapid phase of acid release (Fig. 7*c*); almost no net acid extrusion occurred during the first 20–40 s in control solution (Fig. 7). NH_4Cl preincubation with amiloride (10^{-4} M) or Na_o-free solution completely inhibited net acid extrusion for an extended period (Fig. 7, *d* and *f*, respectively); an amiloride- or Na_o-insensitive acid release appeared only after 5–6 min in control solution containing amiloride or in Na_o-free solution. DIDS (10^{-4} M) had no effect on the pattern of acid release following NH_4Cl preincubation (Fig. 7*e*).

pH_o remained at ~7.41 in control solution with or without amiloride, drifted briefly alkaline after NH_4Cl preincubation alone, and shifted strongly alkaline for an extended period following NH_4Cl preincubation in the presence of amiloride or Na_o-free solution (Fig. 7). NH_4Cl preincubation experiments were interpreted as follows: the preparation and external solution in the cuvette represented a closed two-compartment system. Thus, on return to control solution, brief alkaline drifts in pH_o indicated that NH_3 diffused across the cell membrane to bind extracellular H^+ at a rate only slightly faster than H^+ was extruded by Na/H exchange; the combined effect during the first minute resulted in little net extrusion of acid equivalents (Fig. 7*c*). Inhibiting Na/H exchange with amiloride (or Na_o-free solution) revealed the external alkalinizing effect of transmembrane NH_3 efflux acting alone (Fig. 7, *d* and *f*). Therefore, the difference in the maximum pH_o changes under each condition, combined with information on the buffer capacity of the solutions, yielded the net amiloride-sensitive acid extrusion. Correcting for background CO_2 absorption, this method gave 70.9 ± 9.1 nmol/mg dry weight ($n = 5$) of amiloride-sensitive H^+ equivalents extruded across the cell membrane in the first minute after transfer to NH_4^+-free (control) solution. This value, compared with 68.5 ± 8.7 nmol/mg dry weight of amiloride-sensitive net Na uptake (see above), indicates a 1:1 Na/H stoichiometry.

The pH stat technique detected a Na_o-insensitive acid release (Fig. 8*a*) 6–8 min after NH_4Cl preincubation in Na_o-free solution of 9.4 ± 0.7 nmol/mg dry weight·min ($n = 9$).[2] Acid extrusion could be stimulated by addition of Na^+ to the extracellular solution; this effect was amiloride sensitive (Fig. 8*a*). Li^+, but not K^+ or choline$^+$, could substitute for Na^+ in augmenting acid extrusion. The dose response for Na_o-dependent acid extrusion showed a half-maximal effect of ~15 mM Na_o (Fig. 8*b*). The reduced maximum rate of H^+ extrusion under this condition compared with that immediately after NH_4 pretreatment probably reflects partial dissipation of the acid load by amiloride- or Na_o-insensitive mechanisms.

[2] Assuming steady state values for cell H_2O and intracellular buffering capacity (see Discussion), this would be equivalent to a change in pH_i at the rate of ~0.035 pH unit/min; the relatively insensitive single-wavelength fluorescence technique could not detect this slow rate of Na_o-insensitive pH_i recovery over the short times tested (Fig. 1).

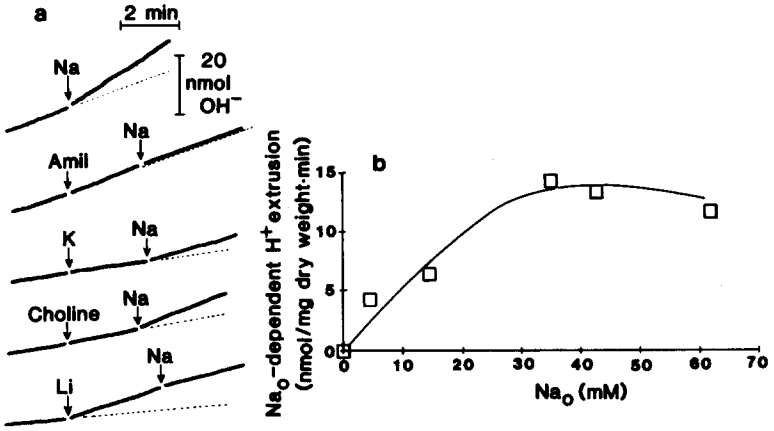

FIGURE 8. Na_o-dependent H^+ extrusion in cultured heart cells. (*a*) Preparations were preincubated for 15 min in 20 mM NH_4Cl, Na_o-free (TMA) solution (based on solution 6, Table I), with 5 mM HEPES to maintain pH 7.4, and transferred to Na_o-free pH stat solution (pH 7.4). The initial portion of each tracing shows Na_o-independent acid release (plus background CO_2 absorption) ~5–6 min after transfer; the dashed line is an extension of this rate. At the arrow, concentrated NaCl, LiCl, KCl, or choline Cl in 0.05 mM HEPES (pH 7.4) was added such that the final concentration of cation was 40 mM; the final concentration of amiloride was 10^{-4} M. The break in each tracing represents 10–20 s. The vertical and horizontal scales apply to each tracing. (*b*) Na_o-dependent H^+ extrusion based on results obtained by the method illustrated in panel *a*. Each point in the series represents a single observation corrected for background CO_2 absorption.

Electrical Response to pH_i Regulation

The electroneutrality of Na/H exchange was confirmed by membrane potential recordings (Fig. 9). Application of NH_4Cl depolarized polystrands, possibly because of NH_4^+ permeability through K^+ channels (Roos and Boron, 1981). Application of ouabain (10^{-4} M) inhibited secondary activation of the electrogenic Na/K pump during pH_i regulation. The lack of a significant depolarization

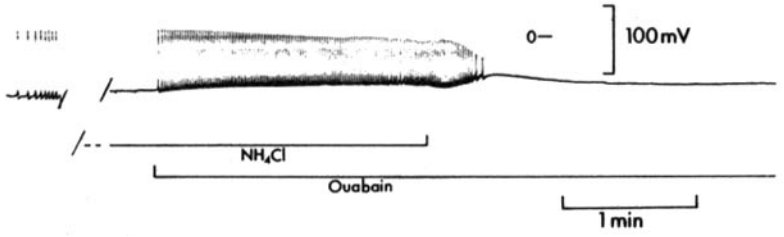

FIGURE 9. Continuous recording of membrane potential of cultured heart cells during pH_i regulation. The initial tracing shows a polystrand in control solution. The solution was then switched to one containing 20 mM NH_4Cl for 15 min; ouabain (10^{-4} M) was added 2 min before the switch back to NH_4^+-free (control) solution. The break in the record represents 13 min.

in the presence of a massive uptake of Na^+ (see Table II) provided direct evidence for electroneutral Na/H exchange.

Ca_i Content Changes

The interaction of H^+ and Na^+ with Ca^{2+} is of considerable interest in cardiac muscle (Bers and Ellis, 1982; Vaughan-Jones et al., 1983; Piwnica-Worms and Lieberman, 1983). Therefore, total cell Ca (Ca_i) was also determined during an NH_4^+-induced acid load. Steady state Ca_i was 6.6 ± 0.6 nmol/mg dry weight (n = 5), and declined by ~30% during NH_4^+ exposure. Switching to NH_4^+-free solution produced a rise in Ca_i back toward the control value (n = 3) with a $t_{1/2}$

TABLE IV

Effect of Ca_o on Acid-induced Na Uptake

Ca_o^{2+}	Mg^{2+}	HPO_4^{2-}	Ouabain	Acid-induced ΔNa_i
mM	*0.8 mM*	*0.8 mM*	*10^{-4} M*	*nmol/mg dry wt·1 min*
2.7	+	+	−	73.7±9.6 (9)
	+	−	−	43.7±6.6 (3)
	+	−	+	64.8±13.0 (3)
21.8	+	−	+	44.9±7.6 (3)
10.8	+	−	−	58.4±16.9 (3)
0.27	+	+	−	78.3±10.4 (3)
0	+	−	+	125.6±5.7 (3)*
0	−	+	−	145.8±18.3 (3)*

Values are means ± SEM. n is the number of determinations. The asterisk indicates a significant difference from the control value (top of table) at $P < 0.05$. Acid-induced net Na uptake was determined as shown in Fig. 3. Both control and NH_4Cl-containing solutions had the indicated HPO_4 concentrations; Ca and Mg were adjusted on return to control solution; ouabain, if present, was added on return to control solution. Amiloride (10^{-3} M) inhibited ~90% of acid-induced Na uptake in Ca_o-free solution and at Ca_o concentrations of 21.8, 10.8, and 2.7 mM (not tested at 0.27 mM Ca_o).

on the order of 4 min. The response time of Ca_i changes indicated that net Ca uptake correlated best with net Na extrusion following peak Na_i values (see Fig. 2), which suggests that the Na/Ca exchange that exists in the polystrand preparation (Lieberman et al., 1984) may assist the Na/K pump in re-establishing the Na gradient subsequent to pH_i regulation.

External Ca

The effects of the external divalent ions Ca^{2+}, Mg^{2+}, and HPO_4^{2-} on acid-induced Na uptake are shown in Table IV. Of the various conditions tested, only nominally Ca_o-free or Mg_o-free, Ca_o-free solutions significantly increased acid-induced Na uptake; on the other hand, the presence of these external cations showed an inhibitory trend. The net increase in acid-induced Na uptake in Ca_o-free compared with control Ca_o was more than could be accounted for by Na/Ca exchange, even if all steady state Ca_i exchanged for Na_o at a 4:1 stoichiometry (hypothetical increment in Na uptake: 26 nmol Na/mg dry weight·1 min vs. measured increment: 52 nmol Na/mg dry weight·1 min).

DISCUSSION

Characterization of Na/H Exchange in Heart Cells

Several experiments demonstrate the coupled movements of Na^+ and H^+ consistent with Na/H exchange: following the introduction of an NH_4^+-induced acid load, (*a*) Na_o-sensitive pH_i regulation mediated by extrusion of H^+ across the cell membrane results in a return of pH_i toward control values, (*b*) pH_i regulation promotes a rapid net uptake of Na^+, (*c*) larger decreases in pH_i produce larger increases in net Na^+ uptake, and (*d*) decreasing pH_o inhibits acid-induced Na uptake. Also, without an acid load, (*e*) reduction of Na_o, which reverses the Na^+ chemical gradient, causes intracellular acidification, and (*f*) alkalinization of pH_i occurs when an inward Na^+ chemical gradient is imposed across cell membranes of Na_i-depleted preparations.

Several conclusions regarding the kinetics of Na/H exchange are suggested by these studies. (*a*) The external Na^+ binding site is saturated at Na_o above 72 mM and, on switching to low Na_o solution, a half-maximal rate of cytosolic acidification occurs between 20 and 30 mM Na_o. (*b*) Similarly, the external Na^+ binding site, evaluated with the pH stat method by addition of Na_o to Na_i-depleted cells, shows a half-maximal, Na_o-dependent rate of H^+ extrusion at ~15 mM Na_o. (*c*) The amiloride-sensitive rate of acid-induced Na^+ uptake, evaluated at saturating Na_o concentrations, is pH_o dependent. (*d*) Internal acidification activates Na/H exchange. These data are consistent with the kinetic mechanisms of Na/H exchange described in sheep cardiac Purkinje fibers (Ellis and MacLeod, 1983) and in other tissues (Rindler and Saier, 1981; Villereal, 1981; Aronson et al., 1982; Vigne et al., 1982; Boron and Boulpaep, 1983; Aronson et al., 1983; Grinstein et al., 1984).

Effect of Amiloride

Corroborative evidence for the existence of Na/H exchange in cardiac muscle relies on inhibitory actions of the diuretic amiloride. In the polystrand preparation, amiloride (10^{-3} M) blocks: (*a*) pH_i recovery following an acid load, (*b*) net Na^+ uptake induced by an acid load, (*c*) cytosolic acidification induced by low Na_o solution, and (*d*) cytosolic alkalinization produced by re-establishment of an inward Na^+ gradient in Na_i-depleted cells. Maximal inhibition of Na/H exchange by amiloride is similar in dose and reversibility to that reported for several other tissue types (for review, Benos, 1982; Aicken and Thomas, 1977; Moolenaar et al., 1981; Aronson et al., 1983). Furthermore, the half-maximal inhibitory dose (ID_{50}) of amiloride is ~3 μM in 144 mM Na_o, a value that correlates with ID_{50} for amiloride inhibition of Na/H exchange in Chinese hamster lung fibroblast cells (Pouyssegur et al., 1982), renal membrane vesicles (Kinsella and Aronson, 1981), chick skeletal muscle cells in culture (Vigne et al., 1982), and dog red blood cells (Parker, 1983).

Na/H Exchange vs. Na/Ca Exchange

Distinguishing the effects of Na/Ca exchange from Na/H exchange in cardiac muscle has received recent attention (Bers and Ellis, 1982; Vaughan-Jones et al.,

1983; Piwnica-Worms and Lieberman, 1983). In this study, the acid-induced net Na uptake is too large to be attributed to Na/Ca exchange, a mechanism also known to exist in the cell membrane of polystrand preparations (Lieberman et al., 1984), as well as other preparations of cardiac muscle (for review, Langer, 1982). The Na/Ca exchange hypothesis suggests that increasing the intracellular H^+ concentration by an acid load would increase cytosolic free Ca^{2+} both through direct displacement of Ca^{2+} from intracellular binding sites and through H^+ exchange for Ca^{2+} across mitochondrial membranes (Vercesi et al., 1978). The resulting increase in cytosolic free Ca would favor Na entry via Na/Ca exchange. However, Ca_i measurements show, on a mass balance basis, that the initial net Na uptake cannot result from Na/Ca exchange across the cell membrane. The control Ca_i content was 6.6 nmol/mg dry weight, the majority being highly compartmentalized within subcellular organelles (Murphy et al., 1983). Assuming (*a*) that all depots of Ca_i exchange rapidly with external Na, and (*b*) an Na/Ca 4:1 stoichiometry (Mullins, 1981), then the maximum net Na uptake possible from Na/Ca exchange following the acid load would be 26.4 nmol/mg dry weight. This value represents only 35% of the acid-induced Na uptake (Fig. 2). Because the most positive value observed for E_m was −50 mV (Fig. 9; E_m actually remained far negative to that value throughout most of the return to NH_4^+-free solution), Ca uptake via "slow" channels (for review, McDonald, 1982) is unlikely to enable further Na uptake to occur via the consequent Na/Ca exchange. The purpose of these calculations is not to address the electrogenicity or stoichiometry of Na/Ca exchange, but to illustrate that Na/Ca exchange could not be the mechanism mediating the rapid amiloride-sensitive Na uptake induced by an acid load.

On the other hand, because direct measurement of the membrane potential indicated electroneutral Na uptake following an acid load, an Na/H stoichiometry of 1:1 can be used to estimate the extent of the pH_i recovery. For example, assuming the mean intracellular buffering capacity of adult chicken cardiac muscle (58 mmol/liter·pH unit; Lykkeboe and Johansen, 1975) is comparable to chick embryo heart cells, the intracellular pH change expected from the 1-min H^+ extrusion of 103.8 nmol/mg dry weight (Table II) is 0.42 pH unit. This calculated pH_i change is a reasonable value and is in good agreement with that observed in other preparations of muscle under similar conditions (Aickin and Thomas, 1977; Reber and Weingart, 1982).

The presence of Ca_o is partially inhibitory to Na/H exchange in cultured heart cells (Table IV); a similar effect has been reported in human fibroblasts (Villereal, 1982) and A431 epidermoid cells (Rothenberg et al., 1983; however, see Cala, 1983). Whether the stimulatory effect produced by removing external Ca^{2+} is by direct action on Na/H exchange or indirect via Ca_i is unproven. Nevertheless, these data raise a point of caution to investigators evaluating Na/Ca exchange in cardiac muscle. Na uptake promoted by Ca_o-free solution may be due in part to stimulation of Na/H exchange rather than to Na/Ca exchange alone.

Comparison with Other Cardiac Tissues

Evidence suggesting Na/H exchange has been described in one other cardiac preparation, the quiescent sheep Purkinje fiber (Deitmer and Ellis, 1980). Major

differences in the magnitude and rate of Na/H exchange exist between the polystrand preparation of cultured heart cells and sheep Purkinje fibers. For example, Na_o-sensitive regulation of pH_i following a 17-min NH_4^+-induced acid load in Purkinje fibers occurs with a $t_{1/2}$ of ~10 min, whereas pH_i regulation in polystrands, under similar conditions, occurs with a $t_{1/2}$ of ~2.9 min. In addition, with a 1:1 exchange stoichiometry, the maximum rate of acid-induced net Na^+ uptake in Purkinje fibers is 2.5 mmol/liter·min (Deitmer and Ellis, 1980), 10 times less than the acid-induced net Na uptake found in polystrands (24.2 mmol/liter·min; Table II). The rapidity of Na/H exchange in polystrands may reflect the minimal extracellular diffusion delays of the preparation. Complex extracellular spaces of naturally occurring cardiac muscle tend to lessen experimentally measured transport rates of ionic fluxes (Horres and Lieberman, 1977; Attwell et al., 1979). In addition, spontaneously contracting polystrands could have an enhanced rate of H^+ production and consequently require a greater rate of Na/H exchange compared with quiescent Purkinje fibers.

Steady State pH_i Maintenance

Data obtained following an acid load in heart cells strongly indicate the activation of Na/H exchange during pH_i regulation. Less conclusive is whether Na/H exchange is active at normal pH_i values to participate in steady state pH_i maintenance. Data obtained from cultured heart cells in support of this include: (*a*) the Na_i content decreases a small but significant amount upon application of amiloride (10^{-3} M), (*b*) transmembrane acid extrusion rates in HCO_3-free solution decrease by 6.4 nmol/mg dry weight·min in amiloride (10^{-4} M) (Fig. 7), and (*c*) from control pH_i levels, exposure to low Na_o solutions promotes cytosolic acidification (Fig. 6). In addition, pH_i and Na_i activity decline in sheep Purkinje fibers upon application of amiloride (10^{-3} M) (Deitmer and Ellis, 1980). Thus, the evidence tentatively suggests that a small basal rate of Na/H exchange may participate in steady state pH_i maintenance in cardiac muscle. Small but detectable steady state rates of Na/H exchange also exist in neuroblastoma cells (Moolenaar et al., 1981) and *Necturus* gallbladder (Weinman and Reuss, 1982); in contrast, steady state Na/H exchange is undetectable in cultured skeletal muscle cells (Vigne et al., 1982) and thymic lymphocytes (Grinstein et al., 1984).

Relative Magnitude of Na/H Exchange

The ability of Na/H exchange to mediate rapid net Na influx can be examined by calculating net transmembrane Na flux from Na_i content changes. For example, the most rapid net Na uptake rate measured in normal Ca_o was 131 nmol/mg dry weight·min (Fig. 5). Assuming no major changes in the volume-to-surface area ratio under these conditions (1.06×10^{-4} cm; Horres et al., 1977), the net transmembrane Na influx associated with Na/H exchange is 54 pmol/cm^2·s. This value is 3.5 times larger than steady state ouabain-sensitive (Na/K pump-mediated) unidirectional ^{24}Na efflux (16 pmol/cm^2·s; Wheeler et al., 1982) and ~25 times the estimated steady state unidirectional Na influx through passive conductive channels (1–2 pmol/cm^2·s; Wheeler, 1981). The data illustrate that stimulation of Na/H exchange in cardiac muscle can mediate remarkably rapid transmembrane Na movements.

In conclusion, a rapid, electroneutral, amiloride-sensitive Na/H exchange has been demonstrated in the polystrand preparation of cultured heart cells. The exchange is stimulated by intracellular acidification and appears to be a major pathway for pH_i regulation in these cardiac muscle cells.

We appreciate the comments and suggestions of Dr. Paul De Weer and thank Dr. Laz Mandel for use of the pH stat apparatus. We thank Charles Henry, Owen Oakeley, Dora Lyons, and Connie Ganong for their technical assistance. We gratefully acknowledge the secretarial assistance of Carole Marin.

This study was supported by grants from the National Institute of Health (HL-27105, HL-17670, and HL-07101), the American Heart Association, North Carolina Affiliate (1982–1983-A-39), and the Medical Scientist Training Program (GM-07171).

Preliminary reports of this work have appeared (1982, *J. Gen. Physiol.*, 80:20*a*; 1983, *J. Gen. Physiol*, 82:18*a*).

Original version received 20 October 1983 and accepted version received 17 September 1984.

REFERENCES

Aickin, C. C., and R. C. Thomas. 1977. An investigation of the ionic mechanism of intracellular pH regulation in mouse soleus muscle fiber. *J. Physiol. (Lond.)*. 273:295–316.

Aronson, P. S., J. Nee, and M. A. Suhm. 1982. Modifier role of internal H^+ in activating the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Nature (Lond.)*. 299:161–163.

Aronson, P. S., M. A. Suhm, and J. Nee. 1983. Interaction of external H^+ with the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *J. Biol. Chem.* 258:6767–6771.

Attwell, D., D. Eisner, and I. Cohen. 1979. Voltage clamp and tracer flux data: effects of a restricted extracellular space. *Q. Rev. Biophys.* 12:213–261.

Benos, P. J. 1982. Amiloride: a molecular probe of sodium transport in tissues and cells. *Am. J. Physiol.* 242:C131–C145.

Benos, D. J., J. Reyes, and D. G. Shoemaker. 1983. Amiloride fluxes across erythrocyte membranes. *Biochem. Biophys. Acta.* 734P:99.

Bers, D. M., and D. Ellis. 1982. Intracellular calcium and sodium activity in sheep heart Purkinje fibers. Effects of changes of external sodium and intracellular pH. *Pflügers Arch. Eur. J. Physiol.* 393:171–178.

Boron, W. F., and E. L. Boulpaep. 1983. Intracellular pH regulation in the renal proximal tubule of the salamander. Na-H exchange. *J. Gen. Physiol.* 81:29–52.

Boron, W. F., and P. De Weer. 1976. Active proton transport stimulation by CO_2/HCO_3^-, blocked by cyanide. *Nature (Lond.)*. 259:240–241.

Boron, W. F., W. C. McCormick, and A. Roos. 1981. pH regulation in barnacle muscle fibers: dependence on extracellular sodium and bicarbonate. *Am. J. Physiol.* 240:C80–C89.

Boron, W. F., and J. M. Russell. 1983. Stoichiometry and ion dependencies of the intracellular pH-regulating mechanism in squid giant axons. *J. Gen. Physiol.* 81:373–399.

Breitwieser, G. E., and J. M. Russell. 1983. Sidedness of pH dependence of the sodium pump in dialyzed squid giant axons. *Biophys. J.* 41:71*a*. (Abstr.)

Cala, P. M. 1983. Cell volume regulation by *Amphiuma* red blood cells. The role of Ca^{2+} as a modulator of alkali metal/H^+ exchange. *J. Gen. Physiol.* 82:761–784.

Deitmer, J. W., and D. Ellis. 1980. Interactions between the regulation of the intracellular pH and sodium activity of sheep cardiac Purkinje fibers. *J. Physiol. (Lond.)*. 304:471–488.

Ellis, D., and K. T. MacLeod. 1983. The dependence of intracellular pH regulation on extracellular Na in sheep heart Purkinje fibres. *J. Physiol. (Lond.)*. 336:69P–70P.

Ellis, D., and R. C. Thomas. 1976. Direct measurement of the intracellular pH of mammalian cardiac muscle. *J. Physiol. (Lond.).* 262:755–771.

Gonzalez, N. C., and R. L. Clancy. 1981. Myocardial intracellular pH regulation during chloride depletion. *J. Appl. Physiol.* 51:1630–1634.

Grinstein, S., S. Cohen, and A. Rothstein. 1984. Cytoplasmic pH regulation in thymic lymphocytes by an amiloride-sensitive Na^+/H^+ antiport. *J. Gen. Physiol.* 83:341–369.

Horres, C. R. 1975. Potassium tracer kinetics of growth oriented heart cells in tissue culture. Ph.D. Thesis. Duke University, Durham, NC. 203 pp.

Horres, C. R., J. F. Aiton, and M. Lieberman. 1979. Potassium permeability of embryonic avian heart cells in tissue culture. *Am. J. Physiol.* 236:C163–C170.

Horres, C. R., and M. Lieberman. 1977. Compartmental analysis of potassium efflux from growth-oriented heart cells. *J. Membr. Biol.* 34:331–350.

Horres, C. R., M. Lieberman, and J. E. Purdy. 1977. Growth orientation of heart cells on nylon monofilament. *J. Membr. Biol.* 34:313–329.

Kenyon, J. L., and W. R. Gibbons. 1977. Effects of low-chloride solutions on action potentials of sheep cardiac Purkinje fibers. *J. Gen. Physiol.* 70:635–660.

Kinsella, J. L., and P. S. Aronson. 1981. Interaction of NH_4^+ and Li^+ with the renal microvillus membrane Na^+-H^+ exchanger. *Am. J. Physiol.* 241:C220–C226.

Lakowicz, J. R. 1983. Principles of Fluorescence Spectroscopy. Plenum Press, New York. 260–265.

Langer, G. A. 1982. Sodium-calcium exchange in the heart. *Annu. Rev. Physiol.* 44:435–449.

Lieberman, M., C. R. Horres, J. P. Aiton, N. Shigeto, and D. M. Wheeler. 1982. Developmental aspects of cardiac excitation: active transport. *In* Normal and Abnormal Conduction in the Heart. A. Paes de Carvalho, B. F. Hoffman, and M. Lieberman, editors. Futura Publishing Co., New York. 313–326.

Lieberman, M., C. R. Horres, R. Jacob, E. Murphy, D. Piwnica-Worms, and D. M. Wheeler. 1984. Physiologic criteria for electrogenic transport in tissue-cultured heart cells. *In* Electrogenic Transport: Fundamental Principles and Physiological Implications. M. Blaustein and M. Lieberman, editors., Raven Press, New York. 181–191.

Lykkeboe, G., and K. Johansen. 1975. Comparative aspects of buffering capacity in muscle. *Respir. Physiol.* 25:353–361.

McDonald, T. F. 1982. The slow inward calcium current in the heart. *Annu. Rev. Physiol.* 44:425–434.

McManus, T. J., and W. F. Schmidt III. 1978. Ion and co-ion transport in avian red cells. *In* Membrane Transport Processes. J. F. Hoffman, editor. Raven Press, New York. 1:79–106.

Moody, W. J., Jr. 1981. The ionic metabolism of intracellular pH regulation crayfish neurons. *J. Physiol. (Lond.).* 316:293–308.

Moolenaar, W. H., J. Boonstra, P. T. van der Saag, and S. W. deLaat. 1981. Sodium/proton exchange in mouse neuroblastoma cells. *J. Biol. Chem.* 256:12883–12887.

Mullins, L. J. 1981. Ion Transport in Heart. Raven Press, New York. p. 136.

Murphy, E., J. F. Aiton, C. R. Horres, and M. Lieberman. 1983. Calcium elevation in cultured heart cells: its role in cell injury. *Am. J. Physiol.* 245:C316–C321.

Parker, J. C. 1978. Sodium and calcium movements in dog red blood cells. *J. Gen. Physiol.* 71:1–17.

Parker, J. C. 1983. Volume-responsive sodium movements in dog red blood cells. *Am. J. Physiol.* 244:C324–C330.

Piwnica-Worms, D. 1983. Electroneutral ion gradient-coupled membrane transport in cultured chick heart cells. Ph.D. Thesis. Duke University, Durham, NC. 198 pp.

Piwnica-Worms, D., R. Jacob, C. R. Horres, and M. Lieberman. 1983. Transmembrane chloride flux in tissue-cultured chick heart cells. *J. Gen. Physiol.* 81:731–748.

Piwnica-Worms, D., and M. Lieberman. 1983. Microfluorometric monitoring of pH_i in cultured heart cells: Na^+-H^+ exchange. *Am. J. Physiol.* 244:C422–C428.

Pouyssegur, J., J. C. Chambard, A. Franchi, S. Paris, and E. Van Obberghen-Schilling. 1982. Growth factor activation of an amiloride-sensitive Na/H exchange system in quiescent fibroblasts: coupling to ribosomal protein S6 phosphorylation. *Proc. Natl. Acad. Sci. USA.* 79:3935–3939.

Reber, W. R., and R. Weingart. 1982. Ungulate cardiac Purkinje fibers: the influence of intracellular pH on the electrical cell-to-cell coupling. *J. Physiol. (Lond.).* 328:87–104.

Remington, R. D., and M. A. Schork. 1970. Statistics with Applications to the Biological and Health Sciences. Prentice-Hall Inc., NJ. 210–215.

Rindler, M. J., and M. H. Saier, Jr. 1981. Evidence for Na^+/H^+ antiport in cultured dog kidney cells (MDCK). *J. Biol. Chem.* 256:10820–10825.

Roos, A., and W. F. Boron. 1981. Intracellular pH. *Physiol. Rev.* 61:296–434.

Rothenberg, P., L. Glaser, P. Schlesinger, and D. Cassel. 1983. Epidermal growth factor stimulates amiloride-sensitive ^{22}Na uptake in A431 cells. *J. Biol. Chem.* 258:4883–4889.

Russell, J. M., W. F. Boron, and M. S. Broderick. 1983. Intracellular pH and Na fluxes in barnacle muscle with evidence for reversal of the ionic mechanism of intracellular pH regulation. *J. Gen. Physiol.* 82:47–78.

Thomas, R. C. 1976. Ionic mechanism of the H^+ pump in a snail neurone. *Nature (Lond.).* 262:54–55.

Thomas, R. C. 1977. The role of bicarbonate, chloride and sodium ions in the regulation of intracellular pH in snail neurones. *J. Physiol. (Lond.).* 273:317–338.

Vanheel, B., A. deHemptinne, and I. Leusen. 1984. Analysis of Cl-HCO_3 exchange during recovery from intracellular acidosis in sheep cardiac Purkinje strands. *Am. J. Physiol.* 246:C391–C400.

Vaughan-Jones, R. D. 1982. Chloride activity and its control in skeletal and cardiac muscle. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 299:537–548.

Vaughan-Jones, R. D., W. J. Lederer, and D. A. Eisner. 1983. Ca^{2+} ions can affect intracellular pH in mammalian cardiac muscle. *Nature (Lond.).* 301:522–524.

Vercesi, A., B. Reynafurje, and A. L. Lehninger. 1978. Stoichiometry of H^+ ejection and Ca^{2+} uptake coupled to electron transport in rat heart mitochondria. *J. Biol. Chem.* 253:6379–6385.

Vigne, P., C. Frelin, and M. Lazdunski. 1982. The amiloride-sensitive Na^+/H^+ exchange system in skeletal muscle cells in culture. *J. Biol. Chem.* 257:9394–9400.

Villereal, M. L. 1981. Sodium fluxes in human fibroblasts: effect of serum, Ca^{2+}, and amiloride. *J. Cell. Physiol.* 107:359–369.

Villereal, M. L. 1982. Inhibition of the serum-dependent, amiloride-sensitive sodium transport pathway in human fibroblasts by external divalent cations. *J. Cell. Physiol.* 111:163–170.

Weinman, S. A. and L. Reuss. 1982. Na^+-H^+ exchange at the apical membrane of *Necturus* gallbladder. *J. Gen. Physiol.* 80:299–321.

Wheeler, D. M. 1981. Sodium tracer kinetics and transmembrane flux in tissue-cultured chick heart cells. Ph.D. Thesis. Duke University, Durham, NC. 231 pp.

Wheeler, D. M., C. R. Horres, and M. Lieberman. 1982. Sodium tracer kinetics and transmembrane flux in tissue-cultured chick heart cells. *Am. J. Physiol.* 243:C169–C176.