Na/H Exchange in Cultured Chick Heart Cells

pHi 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 pHi regulation. The kinetics of pHi changes in cultured chick heart cells were monitored microfluorometrically with 6-carboxyfluorescein and correlated with Na 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 NH4Cl, a regulatory cytoplasmic alkalinization occurred with a t1/2 of 2.9 min. pHi regulation required external Na+ and was concomitant with transmembrane H+ extrusion as well as a rapid rise in Na content in an Na/H ratio of 1:1. Microelectrode recordings of membrane potential demonstrated directly the electroneutral character of pHi regulation. Acid-induced net Na+ uptake could be either stimulated by further decreasing pHi or inhibited by decreasing pHi; Na+ uptake was unaffected by tetrodotoxin (10 μg/ml), quinidine (10⁻³ M), DIDS (10⁻⁴ M), Cl⁻-free solution, or HCO₃⁻-free solution. Amiloride (10⁻³ M) maximally inhibited both pHi regulation and Na+ uptake; the ID₅₀ for amiloride inhibition of Na+ uptake was 3 μM. Na⁺-dependent H⁺ extrusion showed half-maximal activation at 15 mM Na⁺; Li⁺, but not K⁺ or choline⁺, could substitute for Na⁺ to support H⁺ extrusion. Ca⁺-free solution also stimulated acid-induced Na+ uptake. We conclude that pHi 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/cm²·s indicates the rapidity of this exchange across cardiac cell membranes. Na/H exchange may also participate in steady state maintenance of pHi.

INTRODUCTION

The intracellular pH (pHi) of cardiac muscle in steady state is ~7.1 (Ellis and Thomas, 1976; Roos and Boron, 1981), 1 pH unit more alkaline than the pHi expected for electrochemical equilibrium of H⁺. This gradient implies the existence of an H⁺ extrusion system; however, mechanisms of pHi maintenance in heart cells in steady state have not yet been resolved. Recent evidence in non-
steady state indicates that Cl/HCO₃ exchange may regulate pH₄ following cytosolic alkalinization (Vaughan-Jones, 1982), but does not appear to participate significantly in pH₄ regulation following cytosolic acidification (Gonzalez and Clancy, 1981; Vaughan-Jones, 1982; Piwnica-Worms, 1983; Vanheel et al., 1984). We recently reported changes in pH₄ 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 pH₄ 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 pH₄ regulation. Monitoring pH₄ with the fluorescent dye 6-carboxyfluorescein in combination with measurements of transmembrane H⁺ movements, Na⁺ content, and membrane potential demonstrates that electroneutral Na/H exchange regulates pH₄ following an acid load in heart cells and may participate in steady state pH₄ 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. NH₄ solution was made by equimolar substitution of 20 mM NH₄Cl for NaCl; low Naₐ solution was produced by equimolar substitution of tetramethylammonium-Cl (TMA-Cl) for NaCl; Cl⁻ 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% CO₂, 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 H₂O before addition to solutions.

Intracellular Ion Content

Experiments were carried out at 37°C in an incubator containing either 96% air/4% CO₂ or 100% air at ~95% humidity (Horres et al., 1977). To follow rapid Na⁺ content changes, preparations were rinsed continuously in 250 ml of solution for 30-180 s. To follow Na⁺
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 Co., Portland, OR), and weighed on an electrobalance (Cahn G-2, Vention 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 HNO₃ (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.

### Table I

| Component | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|-----------|---|---|---|---|---|---|---|
| *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 | — |
| *Mg²⁺*    | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
| *Ca²⁺*    | 2.7 | 2.7 | 2.7 | 2.7 | 2.7 | 2.7 | — |
| *Choline* | 26.0 | —   | —   | —   | —   | —   | 145.0 |
| NH₄⁺      | —   | —   | 20.0 | —   | —   | —   | — |
| CH₃COO⁻   | 128.8 | 128.8 | 128.8 | —   | 135.8 | 156.4 | 150.4 |
| X⁻        | —   | —   | —   | 128.0 | —   | —   | — |
| HCO₃⁻     | 26.0 | 26.0 | 26.0 | 26.0 | —   | —   | — |
| H₂PO₄⁻    | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | —   | — |
| SO₄²⁻     | 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 | —   | — |
| pH₄       | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 5.5–8.5 | 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-HCO₃ from Sigma Chemical Co., St. Louis, MO. HCO₃⁻, HPO₄²⁻ solution was made from solution 5 omitting NaH₂PO₄; some Na-free solutions were made from solution 2 by substituting KH₂PO₄ for NaH₂PO₄, while adjusting KCl; Ca-free rinse was made from solution 7 by omitting CaCl₂.
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 \(\text{Ca}^2\) 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, \(^{47}\)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. 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, Omniscibe 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 \(pH\) continued to be recorded. This Δ\(pH\), 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., 1985); 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. 1A 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 $\sim$2.9 ± 0.5 min ($n = 6$). Regulation of $pH_i$ was inhibited by Na$^+$-free solution (Fig. 1B); $pH_i$ 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$^+$ is unknown; a similar transient, under these conditions, has also been recorded in renal proximal tubules with $pH_i$-selective microelectrodes (Boron and Boulaep, 1983). The effect of amiloride ($10^{-5}$ M), a rapid and reversible inhibitor of Na/H exchange in several cell types (for review, Benos, 1982), is illustrated in Fig. 1C. 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$^+$

Figs. 2 and 3 show changes in Na$^+$ 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 $\mu$M 6CF in control solution was reduced 24% by $10^{-3}$ M amiloride, 4% by $10^{-4}$ M amiloride, and $\sim$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\textsubscript{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\textsubscript{i} in polystrands (Wheeler et al., 1982). After 15 min in \(\text{NH}_4^+\) solution, Na\textsubscript{i} was 48.7 ± 4.2 nmol/mg dry weight (n = 5). Acidifying the cytosol by shifting \(\text{NH}_4^+\)-loaded preparations to \(\text{NH}_4^+\)-free (control) solution for 1 min caused a rapid increase in Na\textsubscript{i} to almost twice the steady state value. The difference of 75.6 ± 6.9 nmol/mg dry weight between the latter two values of Na\textsubscript{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\textsubscript{i} content peaked at this time. The decline in Na\textsubscript{i} content over longer times (Fig. 2) was ouabain inhibitable (10\textsuperscript{-4} M; data not shown), which suggests that the Na/K pump secondarily responded to the acid-induced increase in Na\textsubscript{i}.

pH\textsubscript{i} regulation was previously found to be amiloride sensitive, and therefore Na\textsubscript{i} changes during pH\textsubscript{i} regulation were similarly examined. Amiloride (10\textsuperscript{-5} M) did not significantly affect the fall in Na\textsubscript{i} during \(\text{NH}_4^+\) exposure; however,
Figure 2. Changes in Na\(_4\) content during NH\(_4\)\(^+\)-induced acid load. Bars represent means ± SEM of three to six determinations; individual points are means of two experiments. Na\(_4\) 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\(_4\) content of cultured heart cells produced by NH\(_4\)\(^+\)-induced changes in pH. Na\(_4\) 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 NH₄⁺-free solution containingamiloride blocked net Na uptake. The difference of 68.5 ± 8.7 nmol 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 NH₄⁺ exposure and present throughout the return to NH₄⁺-free solution or applied simultaneously with a return to NH₄⁺-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⁺ 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 NH₄⁺ (C: 48.7 ± 4.2 [n = 5] vs. NC: 36.7 ± 3.8 [n = 3] nmol/mg dry weight; P > 0.05). However, Na⁺ content in noncontractile polystrands only approached control values 1 min after return to NH₄⁺-free solution (75.3 ± 10.6 nmol/mg dry weight [n = 3]), and was significantly less than Na⁺ 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⁺ 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 NH₄⁺ solution (except as noted) and then switched for 1 min to NH₄⁺-free solutions altered as indicated. Net Na uptake is presented as a percent of Na uptake induced by a return to NH₄⁺-free (control) solution. Amiloride (10⁻⁵ 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⁺ 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 (10⁻⁵ M), a putative blocker of Na/Ca exchange (Parker, 1978), applied 1 min
before the return to control solution, caused Na\textsubscript{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 ofamiloride (10\textsuperscript{-3} M), Na\textsubscript{i} declined to 57.8 ± 3.6 nmol/mg dry weight (n = 3); after 5 min, Na\textsubscript{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 ofamiloride (10\textsuperscript{-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\textsuperscript{+} conductance and suggested that part of the steady state Na\textsuperscript{+} “leak” may actually be mediated by Na/H exchange.

**Na/H Exchange vs. Na + HCO\textsubscript{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\textsubscript{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\textsuperscript{-} gradient and is inhibited by removal of HCO\textsubscript{3} as well as by the application of stilbene derivatives (Russell et al., 1983). Therefore, to rule out the possibility of Na + HCO\textsubscript{3}/Cl + H exchange, Na uptake following NH\textsubscript{4}Cl preincubation was measured under the following conditions (Table II): (a) in nominally HCO\textsubscript{3}-free solution throughout, (b) with a return to Cl\textsuperscript{-}-free solution (which might stimulate Na uptake), and (c) in the presence of DIDS (10\textsuperscript{-4} M), at a dose 10 times that necessary to inhibit 90\% of pH\textsubscript{r} regulation mediated by Na + HCO\textsubscript{3}/Cl + H

![Image](https://via.placeholder.com/150)

**TABLE II**

| Condition          | ΔNa\textsubscript{i} (% control) | n   | \( P \) |
|--------------------|----------------------------------|-----|--------|
| NH\textsubscript{3}-free control | 100±9                           | 6   | —      |
| + Amiloride (10\textsuperscript{-3} M) | 10±4                            | 10  | <0.001 |
| + Ouabain (10\textsuperscript{-4} M)  | 138±13                          | 7   | 0.05   |
| + Amiloride (10\textsuperscript{-3} M) + ouabain (10\textsuperscript{-4} M) | 56±15                           | 4   | <0.02  |
| + TTX (10 µg/ml)  | 86±6                            | 3   | >0.20  |
| + DIDS (10\textsuperscript{-4} M)  | 82±14                           | 4   | >0.30  |
| HCO\textsubscript{3}-free | 96±20                           | 3   | >0.50  |
| Cl\textsuperscript{-}-free | 109±22                          | 4   | >0.50  |

Polystrands were exposed to 20 mM NH\textsubscript{4}Cl solution for 15 min and then incubated for 1 min in NH\textsubscript{3}-free (control) solution altered as indicated (see text for details). Net Na uptake in NH\textsubscript{3}-free solution was normalized to the control NH\textsubscript{3}-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.
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\(_{\text{o}}\) concentration of 144 mM, half-maximal inhibition of amiloride-sensitive net Na uptake occurred at 3 \(\mu\)M.

![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\(^+\) 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\(_{\text{o}}\) was 7.4; Na\(_{\text{o}}\) 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 \(\mu\)M.](image)

\(pH_{\text{e}}\) Effects on Acid-induced Na Uptake

The response of Na uptake to changes in pH\(_{\text{e}}\) (maintaining pH\(_{\text{i}}\) approximately constant) is summarized in Table III. Preparations were exposed to HCO\(_3^-\)-free, HPO\(_4^{2-}\)-free NH\(_4^+\) solution (pH\(_{\text{e}}\) 7.4) for 15 min before switching to HCO\(_3^-\)-free, HPO\(_4^{2-}\)-free, NH\(_4^+\)-free solutions of various pH\(_{\text{e}}\)'s. The absence of HCO\(_3^-\) and HPO\(_4^{2-}\) in the solutions allowed pH\(_{\text{e}}\) 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\(_{\text{e}}\) 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\(_{\text{e}}\) 6.5 decreased amiloride-sensitive net Na uptake; solutions at pH\(_{\text{e}}\) 5.5 further decreased net Na uptake. Although the decrease in net Na uptake upon decreasing pH\(_{\text{e}}\) 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, Effects on Acid-induced Na Uptake**

The dependence of Na uptake on pH (at constant pH) was examined by exposing polystrands to NH₄ solution (pH 7.4) for various times before returning to NH₄-free solution (pH 7.4). Prolonging the duration of NH₄ exposure (but less than the time required for NH₄ to reach electrochemical equilibrium) increases the magnitude of the acidification produced on switching to NH₄-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 NH₄-free incubation, changes in net Na uptake could not be explained by pH sensitivity of the Na/K pump-mediated Na efflux (Breitwieser and Russell, 1983).

**Table III**

*The Influence of pH₆ on Acid-induced Na Uptake in Cultured Heart Cells*

| pH₆  | Amiloride | ΔNaᵢ | Amiloride-sensitive | ΔNaᵢ |
|------|-----------|------|--------------------|------|
|      | 10⁻⁴ 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 (5)  |                    |
| 6.5  | O         | 65.7±5.5 (5) | 56.6±5.0* |
|      | +         | 9.1±3.6 (5)  |                    |
| 5.5  | O         | 37.9±15.2 (5) | 29.9±15.5*† |
|      | +         | 8.0±3.4 (5)  |                    |

Values are means ± SEM of n determinations. Net Na uptake was defined as the difference in Na; after incubating in HCO₃⁻, HPO₄⁻, 20 mM NH₄ solution for 15 min, and Na; after a subsequent incubation for 1 min in HCO₃⁻, HPO₄⁻, NH₄-free solution containing either ouabain* (10⁻⁴ M) or amiloride (10⁻³ M).

* Comparison with pH₆ 7.4, P > 0.50.
† Comparison with pH₆ 7.4, P < 0.05 (one-tailed).
‡ Comparison with pH₆ 6.5, P = 0.07 (one-tailed).

**Naᵢ-induced pH₆ Changes**

To examine the relationship between Naᵢ and pH₆, Na gradients were systematically varied while rates of pH₆ changes were monitored with 6CF (Fig. 6). From a stable pH₆ in control solution, lowering Naᵢ resulted in increased rates of intracellular acidification up to a minimum t₁/₂ of ~20 s in 0.8 mM Naᵢ solution. Responses occurred only when Naᵢ was reduced below 72 mM and, within the accuracy of this technique, a half-maximal response occurred between 20 and 30 mM Naᵢ. On switching to progressively lower Naᵢ, 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 Naᵢ-depleted by preincubation in low Naᵢ, 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 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 1). In this series, the diameter of each monitored strand was 100 μm. In the last tracing, amiloride (10^{-5} M) was added 3 min before the switch to low Na_{o} solution.
Amiloride (10^{-5} M) completely and reversibly inhibited both low Na\textsubscript{i} induced acidification (Fig. 6; n = 3) and, in Na\textsubscript{i}-depleted cells, normal Na\textsubscript{i}-induced alkalinization. Ouabain (10^{-4} M) did not inhibit Na\textsubscript{i}-induced pH\textsubscript{i} changes (data not shown).

Transmembrane H\textsuperscript{+} Transport

pH stat experiments confirmed that pH\textsubscript{i} regulation following an acid load involved the transmembrane extrusion of H\textsuperscript{+} equivalents rather than an Na\textsuperscript{+}-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. 7a); 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. 7b), 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$_4$Cl did not show a rapid phase of acid release (Fig. 7c); almost no net acid extrusion occurred during the first 20–40 s in control solution (Fig. 7). NH$_4$Cl 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$_4$Cl preincubation (Fig. 7e).

$\text{pH}_0$ remained at ~7.41 in control solution with or without amiloride, drifted briefly alkaline after NH$_4$Cl preincubation alone, and shifted strongly alkaline for an extended period following NH$_4$Cl preincubation in the presence of amiloride or Na$_o$-free solution (Fig. 7). NH$_4$Cl 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 $\text{pH}_0$ 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. 7c). 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 $\text{pH}_0$ 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. 8a) 6–8 min after NH$_4$Cl preincubation in Na$_o$-free solution of 9.4 ± 0.7 nmol/mg dry weight·min ($n = 9$). Acid extrusion could be stimulated by addition of Na$^+$ to the extracellular solution; this effect was amiloride sensitive (Fig. 8a). 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$^+$ (Fig. 8b). 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 $\text{pH}_0$ 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$_0$ recovery over the short times tested (Fig. 1).
Electrical Response to pH\textsubscript{i} Regulation

The electroneutrality of Na/H exchange was confirmed by membrane potential recordings (Fig. 9). Application of NH\textsubscript{4}Cl depolarized polystrands, possibly because of NH\textsubscript{4}\textsuperscript{+} permeability through K\textsuperscript{+} channels (Roos and Boron, 1981). Application of ouabain (10\textsuperscript{-4} M) inhibited secondary activation of the electrogenic Na/K pump during pH\textsubscript{i} regulation. The lack of a significant depolarization
in the presence of a massive uptake of Na⁺ (see Table II) provided direct evidence for electroneutral Na/H exchange.

Ca⁺ Content Changes

The interaction of H⁺ and Na⁺ with Ca²⁺ 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 (Ca₀) was also determined during an NH₄⁺-induced acid load. Steady state Ca₀ was 6.6 ± 0.6 nmol/mg dry weight (n = 5), and declined by ~30% during NH₄⁺ exposure. Switching to NH₄⁻ free solution produced a rise in Ca₀ back toward the control value (n = 3) with a t₁/₂.

| TABLE IV |
|-----------------|----------------|-----------------|-----------------|-----------------|
| Effect of Ca₀ on Acid-induced Na Uptake |
| Ca₀⁺ | Mg²⁺ | HPO₄²⁻ | Ouabain | Acid-induced ∆Na⁺ |
| 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±15.0 (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 NH₄Cl-containing solutions had the indicated HPO₄²⁻ 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 Ca₀-free solution and at Ca₀ concentrations of 21.8, 10.8, and 2.7 mM (not tested at 0.27 mM Ca₀).

The effect of the external divalent ions Ca²⁺, Mg²⁺, and HPO₄²⁻ on acid-induced Na uptake are shown in Table IV. Of the various conditions tested, only nominally Ca₀-free or Mg₀-free, Ca₀-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 Ca₀-free compared with control Ca₀ was more than could be accounted for by Na/Ca exchange, even if all steady state Ca₀ exchanged for Na₀ 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 NH₄⁺-induced acid load, (a) Na⁺-sensitive pHᵢ regulation mediated by extrusion of H⁺ across the cell membrane results in a return of pHᵢ toward control values, (b) pHᵢ regulation promotes a rapid net uptake of Na⁺, (c) larger decreases in pHᵢ produce larger increases in net Na⁺ uptake, and (d) decreasing pHᵢ inhibits acid-induced Na⁺ uptake. Also, without an acid load, (e) reduction of Na⁺, which reverses the Na⁺ chemical gradient, causes intracellular acidification, and (f) alkalinization of pHᵢ occurs when an inward Na⁺ chemical gradient is imposed across cell membranes of Na⁺-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⁺ above 72 mM and, on switching to low Na⁺ solution, a half-maximal rate of cytosolic acidification occurs between 20 and 30 mM Na⁺. (b) Similarly, the external Na⁺ binding site, evaluated with the pH stat method by addition of Na⁺ to Na⁺-depleted cells, shows a half-maximal, Na⁺-dependent rate of H⁺ extrusion at ~15 mM Na⁺. (c) The amiloride-sensitive rate of acid-induced Na⁺ uptake, evaluated at saturating Na⁺ concentrations, is pHᵢ 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 (10⁻⁵ M) blocks: (a) pHᵢ recovery following an acid load, (b) net Na⁺ uptake induced by an acid load, (c) cytosolic acidification induced by low Na⁺ solution, and (d) cytosolic alkalinization produced by re-establishment of an inward Na⁺ gradient in Na⁺-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 (ID₅₀) of amiloride is ~3 μM in 144 mM Na⁺, a value that correlates with ID₅₀ 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.,
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 polystranded 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 Ca²⁺ both through direct displacement of Ca²⁺ from intracellular binding sites and through H⁺ exchange for Ca²⁺ across mitochondrial membranes (Vercesi et al., 1978). The resulting increase in cytosolic free Ca would favor Na entry via Na/Ca exchange. However, Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ 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 Eₘ was −50 mV (Fig. 9; Eₘ actually remained far negative to that value throughout most of the return to NH₄⁺-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 electrogenticity 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; 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 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 Ca²⁺ 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 Ca²⁺ is by direct action on Na/H exchange or indirect via Ca is unproven. Nevertheless, these data raise a point of caution to investigators evaluating Na/Ca exchange in cardiac muscle. Na uptake promoted by Ca₂⁺-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⁺-sensitive regulation of pH, following a 17-min NH₄⁺-induced acid load in Purkinje fibers occurs with a $t_{1/2}$ of ~10 min, whereas pH, 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, Maintenance**

Data obtained following an acid load in heart cells strongly indicate the activation of Na/H exchange during pH, regulation. Less conclusive is whether Na/H exchange is active at normal pH, values to participate in steady state pH, maintenance. Data obtained from cultured heart cells in support of this include: (a) the Na⁺ content decreases a small but significant amount upon application of amiloride (10⁻⁵ M), (b) transmembrane acid extrusion rates in HCO₃⁻-free solution decrease by 6.4 nmol/mg dry weight-min in amiloride (10⁻⁵ M) (Fig. 7), and (c) from control pH, levels, exposure to low Na⁺ solutions promotes cytosolic acidification (Fig. 6). In addition, pH, and Na⁺ activity decline in sheep Purkinje fibers upon application of amiloride (10⁻⁵ 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, 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⁺ content changes. For example, the most rapid net Na uptake rate measured in normal Ca⁺ 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 × 10⁻⁶ cm²; Horres et al., 1977), the net transmembrane Na influx associated with Na/H exchange is 54 pmol/cm²-s. This value is 3.5 times larger than steady state ouabain-sensitive (Na/K pump-mediated) unidirectional ⁴⁴Na efflux (16 pmol/cm²-s; Wheeler et al., 1982) and ~25 times the estimated steady state unidirectional Na influx through passive conductive channels (1–2 pmol/cm²-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 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., 80:20a; 1983, J. Gen. Physiol, 82:18a).

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. 12:215–261.

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. 734:99.

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. 395:171–178.

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 CO₂/HCO₃⁻ 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. 81:375–399.

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

Cala, P. M. 1983. Cell volume regulation by Amphiura red blood cells. The role of Ca²⁺ 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.). 356:69P–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.* 51:1650-1654.

Grinstein, S., S. Cohen, and A. Rothstein. 1984. Cytoplasmic pH regulation in thymic lymphocytes by an amiloride-sensitive Na⁺/H⁺ antiport. *J. Gen. Physiol.* 83:341-359.

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.* 70:655-660.

Kinsella, J. L., and P. S. Aronson. 1981. Interaction of NH₄⁺ 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. F. 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.* 25:355-361.

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. de Laat. 1981. Sodium/proton exchange in mouse neuroblastoma cells. *J. Biol. Chem.* 256:12885-12887.

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. 1985. Transmembrane chloride flux in tissue-cultured chick heart cells. J. Gen. Physiol. 81:731–748.

Piwnica-Worms, D., and M. Lieberman. 1985. Microfluorometric monitoring of pH 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. 79:3985–3989.

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 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.). 273:317–358.

Vanheel, B., A. deHemptinne, and I. Leusen. 1984. Analysis of Cl-HCO₃ 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. Ca²⁺ 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 Ca²⁺ 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, Ca²⁺, 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.