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Na^+-H^+ exchange in cardiac sarcolemmal vesicles

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The transport of Na^+ by a purified sarcolemmal vesicular preparation from canine ventricular tissue was studied as a function of both internal and external pH. The uptake of Na^+ into sarcolemmal vesicles increased upon raising the extravesicular pH of the reaction medium. Half-maximal uptake of Na^+ was observed at a pH_o of about 8.1 and maximal uptake occurred at pH 8.6. The uptake of Na^+ by sarcolemma was also dependent upon the intravesicular pH. Na^+ uptake into sarcolemmal vesicles was greatly attenuated in the absence of a H^+ gradient across the membrane. Transport of Na^+ was potently inhibited by amiloride, a known blocker of Na^+-H^+ exchange. LiCl was also an effective inhibitor of Na^+ transport. In the presence of optimal H^+ gradients, Na^+ uptake was linear for the first 5 seconds of the reaction and exhibited a V_{max} of 290 nmol Na^+/mg per min and a K_{Na} of 3.5 mM. These experiments strongly indicate the presence of a Na^+-H^+ exchange system in cardiac sarcolemma. This activity appeared to be relatively specific for this membrane fraction. The identification of Na^+-H^+ exchange activity in a sarcolemmal vesicular fraction from the heart will permit extensive characterization of the regulation and kinetics of this antiporter in future investigations.

Introduction

The cardiodepressive effects of intracellular acidosis are well established [1–3]. Calcium flux at a tissue [3] and subcellular level [4–6] are significantly affected by changes in pH. Acidosis has received considerable attention as a theory to explain contractile failure of the heart after ischemia or hypoxia [7]. Although intracellular pH in the heart is of obvious importance, little is known concerning its regulation.

Recent investigations with cultured heart cells have indicated the presence of a Na^+-H^+ exchange system [8,9]. This transport system may be an important mechanism for Na^+ entry into the cell [9]. However, the data obtained from studying Na^+-H^+ exchange in whole cells are limited because of several factors. First, there are technical problems involved in measuring Na^+-H^+ flux in whole cells [10]. Second, it is difficult to separate out the effects of other intracellular transport systems (such as the Na^+-Ca^{2+} exchange system) from Na^+-H^+ exchange movements [8]. Third, intracellular compartmentation of Na^+ [11] or H^+ may influence transsarcolemmal Na^+-H^+ exchange kinetics. Therefore, it becomes essential to study Na^+-H^+ transport in isolated, purified sarcolemmal vesicles.

Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid; Caps, 3-(cyclohexylamino)-1-propanesulfonic acid; Ches, 2-(cyclohexylamino)ethanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; Taps, 3-[tris(hydroxymethyl)methyl]aminopropanesulfonic acid.

We report here the identification and partial characterization of the Na^+-H^+ exchange system in cardiac sarcolemma. The exchanger has a relatively high activity and should prove valuable in the study of Na^+-H^+ movements in the heart.

Methods and Materials

Sarcolemmal membrane preparations. Sarcolemmal vesicles were isolated from trimmed canine ventricles as described in detail previously [12]. The membranes exhibit high activities of markers associated with sarcolemma. Na^+-Ca^{2+} exchange activity was 16.9 ± 1.9 nmol Ca^{2+}/mg per s at 40 μM [Ca^{2+}] ($n = 6$). ($Na^+ + K^+$)ATPase activity was 21.1 ± 0.9 and 97.3 ± 17.0 μmol P_i/mg per h in the absence and presence of 12.5 μg/ml alamethicin, respectively ($n = 5$). Marker enzyme activities for sarcoplasmic reticular and mitochondrial contamination of this sarcolemmal membrane preparation were low or undetectable [13] (see Results). Electron microscopic analysis of this preparation further supports its origin as being predominantly sarcolemmal [12].

Membrane fractions were separated by overnight discontinuous sucrose gradient centrifugation as outlined elsewhere [12]. A light fraction (F1) was collected from the top 11% (w/w) sucrose medium. At the 11–26% interface was a concentrated protein band identified as sarcolemma (F2). Unless otherwise specified, all Na^+-H^+ exchange activities were measured using the F2 or sarcolemmal fraction. For some experiments, further fractions were collected from the 26–29% (F3), 29–34% (F4) and 34–45% (F5) sucrose fractions (w/w). Each fraction was diluted, centrifuged for 75 min at $160\,000 \times g$ and resuspended in suspension medium containing 200 mM sucrose, 25 mM Mes, 8 mM KOH (pH 5.5). The fractions were centrifuged again as above and pellets resuspended in the above suspension medium at a protein concentration of 1–3 mg/ml. Samples were stored in liquid N_2.

Na^+ flux studies. $^{22}Na^+$ transport was measured at 22°C in a total incubation volume of 50 μl. The method employed was an adaption of that described by Aronson and co-workers [14]. Unless otherwise stated in the figure legends, the standard protocol was as follows. A 25 μl aliquot of uptake medium (200 mM sucrose, 35 mM Ches, 26 mM KOH, pH 9.42, 0.1 mM EGTA and varying NaCl concentrations) was placed on the bottom of a 12×55 mm clear polystyrene tube already containing 5 μl of $^{22}Na^+$ (0.1 μCi). A 20 μl aliquot of sarcolemmal membrane protein (20–30 μg) (prewarmed to 22°C) was placed on the side of the tube and the uptake assay initiated by vortexing the mixture. The assay medium, therefore, typically contained final concentrations of (mM): 180 sucrose, 10 Mes, 17.5 Ches, 17 KOH, 0.05 EGTA and varying Na^+ at pH 8.6. In control experiments, pH measurements of the suspension medium, uptake medium and the final assay medium were carefully checked with a Beckman 39505 pH electrode to ensure accuracy. The length of time of the reaction was monitored by a timing device which started when the vortexing was initiated [15]. After a preset time (1–6 s), an auditory signal from the timing device triggered the manual addition of 3 ml of ice-cold stop solution (100 mM KCl, 20 mM Hepes, pH 7.5) to the assay tube. Longer incubation periods were timed with a stopwatch. The assay volume was rapidly filtered through 0.45 μm pore size filters under vacuum. The filters were further washed with two more 3 ml additions of ice-cold stop solution. The total wash volume, therefore, was 9 ml. More than 9 ml of wash did not alter the ^{22}Na retained by the vesicles. If only 3 or 6 ml total wash volume was used, background counts were high and intra-assay variability increased. KCl was required in the stop solution to reduce non-specific Na^+ binding to the filters and to the extravesicular surface of the sarcolemmal membranes. The inclusion of amiloride (0.1 mM) in the stop solution had no effect on values. Filtration was complete in approx. 15 s. Filters were placed in vials, dried, immersed in 6 ml scintillation fluid and counted by scintillation spectroscopy. Control samples were included to subtract non-specific retention of ^{22}Na to the filters. These samples were treated in a similar manner as described above except that 3 ml of ice-cold stop solution was added to the assay medium immediately prior to the addition of membrane protein. Background cpm were typically $\leqslant 10\%$ of total cpm under conditions of maximal $^{22}Na^+$ uptake by sarcolemmal vesicles.

Other assay procedures. Membrane protein was

quantitated by the method of Lowry et al. [16]. Bovine serum albumin was used as a standard. K^+-stimulated *p*-nitrophenylphosphatase and succinate dehydrogenase activities were measured as described previously [17].

Materials. The filters used to harvest vesicles were either from Millipore Corporation (Bedford, MA) or Sartorius (West Coast Scientific, Inc.). We found no difference between the two filter brands for measuring Na^+ uptake. All chemicals were obtained from Sigma (St. Louis, MO). $^{22}Na^+$ was obtained from Amersham International. Alamethicin was kindly donated by Dr. J.E. Grady, The Upjohn Co.

Results

Sarcolemmal vesicles were suspended in a medium with a pH of 5.5. If Na^+ uptake into the vesicles was dependent upon an outwardly directed H^+ gradient then Na^+ transport should be augmented as the extravesicular pH was increased. As shown in Fig. 1, Na^+ influx into the sarcolemmal vesicles was stimulated by decreasing the $[H^+]$ in the extravesicular medium. Half maximal activity was observed at a pH of 8.1. Maximal Na^+ uptake was observed at pH 8.6 and further increases in pH resulted in no further stimulation of Na^+ transport.

These results are consistent with the the hypothesis that the sarcolemma is capable of Na^+-H^+ exchange. We also examined the dependence of Na^+ transport on internal pH using two different types of experimental protocols. In the first, sarcolemma (pH 5.5) was allowed to equilibrate in normal uptake medium (final pH 8.6) in the absence of any Na^+ for up to 2 h. During the preincubation period the intra- to extravesicular pH gradient dissipated. At selected times during this period NaCl was added and uptake examined. If the internal pH gradient was important for Na^+ influx, one would expect Na^+ uptake to decrease as the preincubation time increased. As shown in Fig. 2, Na^+ uptake did decrease by up to 75% as a function of the preincubation period. This was not due to any inability of the sarcolemma to take up Na^+. Sarcolemmal samples kept at 22°C for a similar amount of time but not preincubated with the pH 8.6 medium maintained Na^+ uptake activity for at least 2 h (Fig. 2).

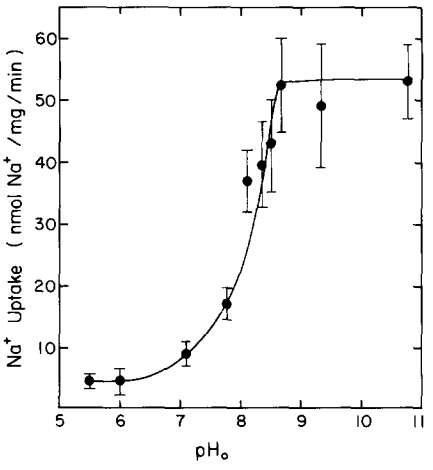

Fig. 1. Na^+ uptake by sarcolemmal vesicles in the presence of varying extravesicular pH (pH_o). 20 μl of sarcolemma at pH 5.5 (as described in Methods and Materials) were incubated with 5 μl ^{22}Na (0.1 μCi) and 25 μl of uptake medium of varying composition to yield 50 μl of final reaction medium composed of (mM): 180 sucrose, 22.5 Mes, 7 KOH (pH 5.5); 192 sucrose, 25 Mes, 18 KOH (pH 6.05); 192 sucrose, 10 Mes, 15 Hepes, 18 KOH (pH 7.11); 160 sucrose, 10 Mes, 20 Hepes, 29 KOH (pH 7.75); 180 sucrose, 10 Mes, 20 Taps, 21 KOH (pH 8.12); 180 sucrose, 10 Mes, 20 Taps, 24 KOH (pH 8.35); 170 sucrose, 10 Mes, 20 Taps, 27 KOH (pH 8.49); 180 sucrose, 10 Mes, 17.5 Ches, 17 KOH (pH 8.60); 180 sucrose, 10 Mes, 15 Ches, 24 KOH (pH 9.33); 170 sucrose, 10 Mes, 15 Caps, 29 KOH (pH 10.70). Final $[Na^+]$ and [EGTA] were always 1 mM and 0.05 mM, respectively. Reaction was carried out for 10 s at 22°C. Each value represents the mean ± S.E. of five experiments.

In a second set of experiments, sarcolemmal vesicles harvested from the sucrose gradient after centrifugation were divided into two aliquots and suspended in pH 5.5 medium or pH 8.8 medium. Again, if a vesicular pH gradient is important, then Na^+ influx should be depressed in the absence of a gradient. Fig. 3 demonstrates Na^+ uptake after diluting sarcolemmal vesicles with different intravesicular pH into assay media with essentially the same final pH. Na^+ uptake was significantly higher in vesicles with an internal pH of 5.5. Sarcolemmal vesicles (pH 8.8) which were diluted into pH 8.8 medium exhibited Na^+ uptake which was only 10–15% of that observed at similar time points using sarcolemma with an internal pH of 5.5. In addition, amiloride could inhibit only 50% of Na^+ uptake in the absence of a pH gradient whereas this agent could block 70–80% of

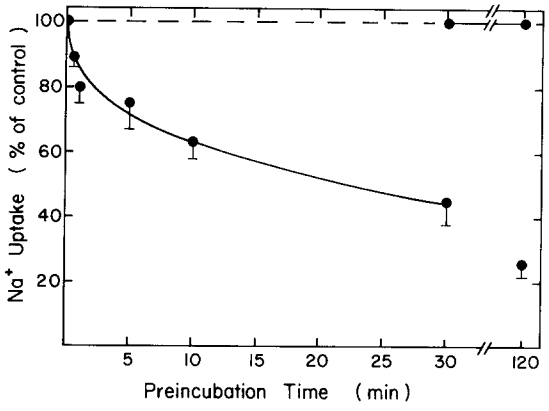

Fig. 2. Effect of the dissipation of proton gradient on Na^+ uptake by sarcolemmal vesicles. Experimental sarcolemmal vesicles (●———●) (pH 5.5) were preincubated for varying times with uptake medium (final pH 8.6; composition described in Methods and Materials) which did not include NaCl or $^{22}Na^+$. Control sarcolemmal vesicles (●- - - - -●) were preincubated in tubes which did not contain NaCl, $^{22}Na^+$ or uptake medium. At selected times, $^{22}NaCl$ was added to the preparations (1 mM [Na^+] final) and the reaction was allowed to occur for 10 s. Control samples were treated in an identical manner except uptake medium was also added at the selected time. Results are presented as a % of Na^+ uptake by control preparations. The absolute value for Na^+ uptake by the control sarcolemmal vesicles was 48.8 ± 5.1 nmol Na^+/mg per min ($n = 4$). Each value represents the mean ± S.E. from three or four experiments.

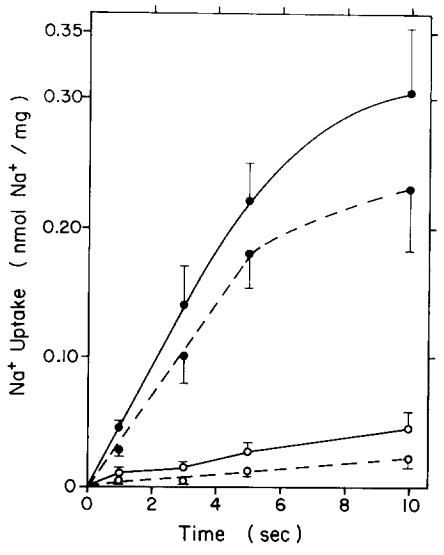

Fig. 3. Na^+ uptake by cardiac sarcolemma examined as a function of intravesicular pH. After isolation, sarcolemmal vesicles were suspended in a medium containing 200 mM sucrose, 25 mM Mes, 8 mM KOH (pH 5.5) or a medium composed of 180 mM sucrose, 25 mM Taps, 30 mM KOH (pH 8.8). Sarcolemmal vesicles (pH 5.5) (●) were diluted into a final reaction medium of 180 mM sucrose, 10 mM Mes, 17.5 mM Ches, 0.05 mM EGTA, 0.05 mM NaCl, 17 mM KOH (pH 8.6). Sarcolemmal vesicles (pH 8.8) (○) were diluted into a final reaction medium of 170 mM sucrose, 30 mM Taps, 0.05 mM EGTA, 30 mM KOH (pH 8.8). Final [Na^+] = 0.05 mM. Reactions were terminated at the times indicated. Total Na^+ uptake activity (————) and amiloride-sensitive Na^+ uptake activity (- - - - -) were measured. Amiloride-sensitive Na^+ uptake activity is defined as that uptake inhibited by amiloride (2.5 mM). In these experiments [amiloride] = 2.5 mM. Each value represents the mean ± S.E. for four experiments.

TABLE I

AMILORIDE INHIBITION OF Na^+ UPTAKE BY CARDIAC SARCOLEMMAL VESICLES

Na^+ uptake was measured in a standard uptake medium as described in Methods and Materials. [Na^+] = 0.05 mM. Reaction time was 6 s. Values are presented as a % of that uptake which occurred in the absence of the drug. Na^+ uptake in the control preparations was 3.5 ± 0.5 nmol Na^+/mg per min ($n = 4$). Each value represents the mean ± S.E. of four analysis.

Amiloride concn. (mM)	Na^+ uptake (% of control)
0.00	100
0.005	74 ± 4
0.01	67 ± 3
0.10	52 ± 6
0.50	37 ± 1
1.00	27 ± 1
2.00	20 ± 1
2.50	20 ± 1

Na^+ uptake in the presence of a gradient.

Amiloride is known to be a potent and relatively selective inhibitor of Na^+-H^+ exchange in other tissues [18,19]. We examined the concentration-dependent capacity of amiloride to block Na^+ influx into cardiac sarcolemmal vesicles (Table I). Amiloride could inhibit Na^+ transport by up to 80%. Approximately half of the Na^+ flux in sarcolemma was inhibited by about 100 μM amiloride.

Lithium chloride is a cation which has been demonstrated to inhibit Na^+-H^+ exchange in renal microvillus membrane vesicles [20]. As demonstrated in Table II, LiCl was capable of inhibiting Na^+ uptake by sarcolemma. In the presence of 1 mM NaCl, Na^+ uptake was inhibited 50% by approx. 3 mM LiCl.

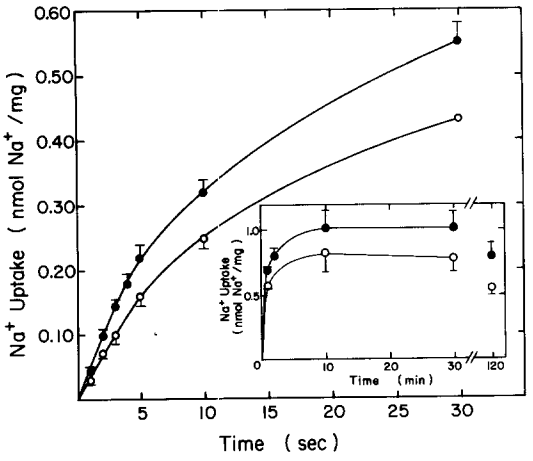

Fig. 4. Time dependence of Na^+ uptake by cardiac sarcolemmal vesicles. Na^+ uptake was measured as described in Methods and Materials. $[Na^+] = 0.05$ mM. Total (●) and the amiloride-sensitive (○) component of Na^+ uptake were measured. [Amiloride], if present, was 2.5 mM. Each value represents the mean ± S.E. of four experiments.

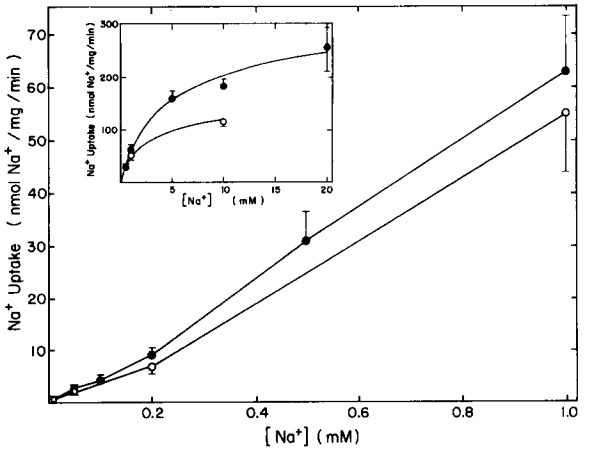

Fig. 5. $[Na^+]$ dependence of Na^+ uptake by cardiac sarcolemma. Na^+ uptake was measured as described in Methods and Materials. Reaction time was 6 s. Total (●) and amiloride-sensitive (○) Na^+ uptake was measured. [Amiloride], if present, was 2.5 mM. Each value represents the mean ± S.E. for four analyses.

In order to more fully characterize the Na^+-H^+ exchange process in cardiac sarcolemma, we also examined Na^+ uptake as a function of reaction time (Fig. 4). Both total and amiloride-sensitive Na^+ uptake appeared linear for the first 5 s. Na^+ uptake increased until plateau values were reached at approx. 10 min (Fig. 4, inset). Since the vesicles could remain 120 min at 22°C in the absence of Na^+ without any significant effect on Na^+ uptake activity (Fig. 2), the lower values at 120 min shown here may reflect a release process. Amiloride inhibited at least 70% of total Na^+ uptake at all time points.

TABLE II

INHIBITION OF CARDIAC SARCOLEMMAL Na^+ UPTAKE BY LITHIUM CHLORIDE

Na^+ uptake was measured in a standard uptake medium as described in Methods and Materials. $[Na^+] = 1$ mM. Reaction time was 6 s. Values are presented as a % of that activity measured in the absence of LiCl. Na^+ uptake in the control samples was 67 ± 13 nmol Na^+/mg per min ($n = 4$). Each value represents the mean ± S.E. of four experiments.

LiCl concn. (mM)	Na^+ uptake (% of control)
0.00	100
0.01	102 ± 2
0.10	95 ± 6
0.50	82 ± 9
1.00	65 ± 11
2.00	59 ± 8
4.00	45 ± 7
5.00	42 ± 8

The Na^+ concentration dependence of Na^+-H^+ exchange in cardiac sarcolemma is presented in Fig. 5. Amiloride-sensitive Na^+ uptake paralleled that of total Na^+ uptake except at high $[Na^+]$ where amiloride inhibition decreased. K_{Na} and V_{max} values of 3.5 mM and 290.3 nmol/mg per min, respectively, were calculated for amiloride-insensitive Na^+ uptake from the data in the inset graph of Fig. 5.

Na^+-H^+ exchange activity was measured in several fractions from the sucrose gradient. This was done in order to determine if Na^+-H^+ exchange was specific to the sarcolemmal membrane. As presented in Table III, the sarcolemmal fraction (F2) and the low density F1 fraction had the highest K^+-phosphatase and Na^+-H^+ exchange activities. The sarcolemmal fraction also had the lowest mitochondrial contamination (as judged from succinate dehydrogenase activity). As the density of the fraction increased, K^+-phosphatase and Na^+-H^+ exchange activities decreased and succinate dehydrogenase activity increased. Frac-

TABLE III

DISTRIBUTION OF SEVERAL ACTIVITIES IN DIFFERENT GRADIENT FRACTIONS

Gradient fractions were separated after overnight centrifugation as described in Methods and Materials. K^+-*p*-Nitrophenylphosphatase activity (μmol/mg per h) and succinate dehydrogenase activity (nmol reduced iodonitrotetrazolium violet per mg per 3 min) were assayed in the respective gradient fractions immediately after isolation. Separate aliquots of each gradient fraction were resuspended in 200 mM sucrose, 25 mM Mes, 8.1 mM KOH (pH 5.5), centrifuged at 177000 × *g* for 75 min, then the pellet was resuspended in the same medium and frozen under liquid N_2 atmosphere for subsequent assay of Na^+-H^+ exchange activity. Na^+-H^+ uptake was carried out at 22°C for 10 s with 1 mM Na^+ ± 2.5 mM amiloride. Other assay conditions were as described in Methods and Materials. Na^+-H^+ exchange is expressed in nmol Na^+ accumulated/mg per min. Total protein is expressed as mg. All values are means ± S.E. for 3–5 samples.

Fraction	Total protein	K^+-*p*-Nitrophenyl-phosphatase activity	Succinate dehydrogenase activity	Na^+-H^+ exchange (total)	Amiloride-sensitive Na^+-H^+ exchange
F1	0.7 ± 0.1	43.1 ± 6.5	122 ± 39	32.3 ± 7.9	25.9 ± 7.9
F2	12.8 ± 0.8	34.3 ± 4.2	69 ± 12	31.3 ± 4.9	23.8 ± 3.9
F3	6.0 ± 0.3	16.1 ± 4.2	184 ± 37	18.4 ± 1.6	13.4 ± 1.3
F4	15.8 ± 1.3	7.3 ± 1.5	328 ± 43	11.7 ± 0.8	7.7 ± 0.7
F5	143.7 ± 19.8	0.7 ± 0.3	764 ± 88	3.4 ± 0.4	1.9 ± 0.4

tion F5, demonstrated high activity of a mitochondrial marker enzyme but low K^+-phosphatase and Na^+-H^+ exchange activities.

Discussion

The results of the present study provide clear evidence for the existence of a Na^+-H^+ exchange system in cardiac sarcolemma. This is supported by several experiments. (1) The Na^+ uptake by sarcolemma was dependent upon extravesicular pH. (2) The Na^+ uptake by sarcolemma was dependent upon intravesicular pH. Less than 10% of Na^+ influx was not dependent upon a pH gradient across the sarcolemmal membrane (Fig. 3). (3) Dissipation of the proton gradient reduced Na^+ uptake by up to 80% (Fig. 2). (4) Amiloride, a relatively specific inhibitor of Na^+-H^+ exchange in membrane preparations from other tissues [18,19], was capable of blocking the Na^+ flux by 80%. This evidence argues strongly in favor of a proton-dependent Na^+ influx process in cardiac sarcolemmal vesicles.

Another interpretation of the data is that we have measured a change in the passive binding of Na^+ to sarcolemma which is dependent upon pH rather than an active accumulation. Cation binding to sarcolemma is known to be pH dependent [21]. However, several arguments support the contention that we are measuring an active flux of Na^+ across the sarcolemmal membrane. First, if we included sodium dodecyl sulfate in the reaction medium to increase membrane permeability, no Na^+ uptake occurred (data not shown). Second, cation binding to cardiac sarcolemma is completed in less than 1 min [22], yet we have demonstrated an increase in Na^+ uptake which reaches a plateau as late as 10 min after the initiation of the reaction. Third, osmotic shock of the vesicles by washing the filters with 6 ml water instead of 6 ml 100 mM KCl, 20 mM Hepes (pH 7.5) reduced the Na^+ uptake by 70% (data not shown). Lastly, the $[K^+]$ of the wash solution was 5000–10000-times the $[Na^+]$ in the assay medium and should have been sufficient to remove Na^+ bound to the extravesicular surface.

The possibility that we may have measured Na^+ influx that was dependent upon endogenous Ca^{2+} in the medium is also unlikely. The intravesicular pH (5.5) was low enough to inhibit cardiac sarcolemmal Na^+-Ca^{2+} exchange almost completely [6]. In addition, EGTA was included in the medium to chelate any endogenous Ca^{2+} and remove the possibility that Na^+ taken into the vesicles may be released by extravesicular Ca^{2+}. In preliminary experiments we found that 50 μM EGTA stimulated Na^+-H^+ exchange by a factor of about 2. Further study of the effects of EGTA on Na^+-H^+ exchange is ongoing. The potent inhibition of Na^+ influx by LiCl is additional evidence that we are measuring a Na^+-H^+ exchange process and not Na^+-Ca^{2+} exchange. Na^+-H^+ ex-

change was 50% inhibited by 3 mM LiCl in the presence of 1 mM Na^+ (Table II), yet Na^+-Ca^{2+} exchange at 10 mM Na^+ is unaffected by 140 mM LiCl in cardiac sarcolemmal vesicles [23].

The activity of cardiac sarcolemmal Na^+-H^+ exchange reported here is similar to that reported for renal microvillus vesicles [20]. At 1 mM Na^+, Kinsella and Aronson [20] reported Na^+ uptake values of about 50 nmol/mg per min. This was largely amiloride sensitive. We report comparable activity and amiloride sensitivity under similar conditions (Fig. 5). Na^+-H^+ exchange activity of about 300 nmol/mg per min in the presence of 15 mM Na^+ has been reported for renal brush-border membrane vesicles [24], a value which is similar to the 256 nmol Na^+/mg per min (amiloride-sensitive) which we observed in the presence of 20 mM Na^+ (Fig. 5). One significant difference noted between the two membranes appears to be that the renal membrane Na^+-H^+ exchanger appears more sensitive to amiloride inhibition than reported here. Na^+ influx in renal microvillus membrane vesicles was inhibited half maximally by 30 μM amiloride [20], whereas our data showed that about 100 μM amiloride was necessary to produce comparable inhibition. However, an amiloride I_{50} of 100 μM is not unusual [18].

Frelin et al. [9] have demonstrated that the Na^+-H^+ exchange pathway may represent an important mechanism for Na^+ entry into cultured heart cells. Our data confirm the presence of a Na^+-H^+ exchange mechanism in the heart and extend these findings to localize the exchanger primarily to the sarcolemmal membrane. We recently became aware of another report identifying the presence of the Na^+-H^+ exchanger in a heart sarcolemmal preparation [24]. Many of the characteristics of the Na^+-H^+ exchange described [24] are in accordance with those reported here even though different cardiac sarcolemmal vesicle preparations were used. The identification and initial characterization of the Na^+-H^+ exchange system in a cardiac vesicular preparation will allow for more complete analyses of kinetics, stoichiometry and regulatory processes in future investigations. Studies of Na^+-proton flux in renal membranes isolated from animals with metabolic acidosis have revealed alterations in the V_{max} for the Na^+-H^+ exchanger [25–27]. Response of the Na^+-H^+ antiporter in the heart to ischemic or pathological states will be of particular interest.

Acknowledgments

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