Effects of pH on Na⁺-Ca²⁺ Exchange in Canine Cardiac Sarcolemmal Vesicles

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SUMMARY Using highly purified sarcolemmal vesicles isolated from dog ventricles, we examined the effects of pH on Na⁺-Ca²⁺ exchange. The initial rate of Na⁺-dependent Ca²⁺ uptake is a sigmoid function of pH. The Ca²⁺ uptake is inhibited at pH 6 and stimulated at pH 9 (as compared with uptake at pH 7.4). This dependence on pH suggests that the ionization state of a histidine residue may be important in Na⁺-Ca²⁺ exchange. The effects of H⁺ on Na⁺-dependent Ca²⁺ uptake are partially competitive with Ca²⁺, although this relationship is complex. Na⁺-dependent Ca²⁺ efflux is also sensitive to H⁺ and increases monotonically with pH. These effects of pH appear to be due to intrinsic interactions with the Na⁺-Ca²⁺ exchange system and are not due to an alteration of Na⁺-H⁺ exchange or membrane permeability. The effects of pH on vesicular Na⁺-Ca²⁺ exchange are apparent only at low Ca²⁺ and Na⁺ concentrations. Thus modulation of vesicular Na⁺-Ca²⁺ exchange by pH is manifest only under ionic conditions which exist intracellularly in intact myocardium. Since the negative inotropy caused by acidosis is thought to reflect a fall in internal pH, these results suggest that alteration of sarcolemmal Ca²⁺ transport (mediated by Na⁺-Ca²⁺ exchange) by internal pH may contribute to the regulation of myocardial contractility by pH. Circ Res 50: 287-293, 1982

THE contractility of cardiac muscle is sensitive to the pH of its environment. Acidosis results in negative inotropy, whereas alkalosis produces a positive effect. The greater contractile response evoked by respiratory acidosis as compared to metabolic acidosis suggests that the effects of altered pH are primarily mediated by changes in intracellular pH (Steinberger et al., 1977; Fry and Poole-Wilson, 1981). pH modulates multiple cellular processes, and the effects of H⁺ on contractility may involve myofilaments (Schadler, 1967; Fabiato and Fabiato, 1978), sarcoplasmic reticulum (Nakamura and Schwartz, 1972; Fabiato and Fabiato, 1978), sarcolemmal Ca²⁺ binding (Williamson et al., 1975), the slow inward Ca²⁺ current (Chesnais et al., 1975; Fry and Poole-Wilson, 1981) or metabolic processes. Intracellular acidosis may be responsible for the rapid initial decline in contractility which accompanies myocardial ischemia (Steinberger et al., 1977; Cobbe and Poole-Wilson, 1980).

There is considerable evidence that Na⁺-Ca²⁺ exchange across the sarcolemmal membrane is a mechanism responsible for the control of myocardial contractility by extracellular Ca²⁺ [for reviews, see Mullins (1979) and Langer (1976)]. Considering the central role of Na⁺-Ca²⁺ exchange in cardiac excitation-contraction coupling, the regulation of this system by pH could be of major significance.

In the past, the methodology for evaluating the interaction of pH with Na⁺-Ca²⁺ exchange in cardiac muscle did not exist. Recently Reeves and Sutko (1979) described a technique for measuring Na⁺-Ca²⁺ exchange in isolated cardiac sarcolemmal vesicles. Using this technique, several reports on the properties of sarcolemmal Na⁺-Ca²⁺ exchange have appeared (e.g., Pitts, 1979; Caroni et al., 1980; Reeves and Sutko, 1980; Philipson and Nishimoto, 1981). It has become clear that myocardial Na⁺-Ca²⁺ exchange is a specific property of the sarcolemma and that this exchange is electrogenic (involving the exchange of three or more Na⁺ for each Ca²⁺). Na⁺-Ca²⁺ exchange activity co-purifies with other sarcolemmal markers (Reeves and Sutko, 1979; Bers et al., 1980) and is present in vesicles that possess Na⁺, K⁺-ATPase activity (Pitts, 1979). Na⁺-induced Ca²⁺ fluxes are not present in cardiac sarcoplasmic reticulum-enriched fractions (Trumble et al., 1980; Caroni and Carafoli, 1981).

We report here that the Na⁺-Ca²⁺ exchange in a highly purified sarcolemmal fraction from canine ventricles is extremely sensitive to pH. This sensitivity is most apparent under ionic conditions similar to those present in vivo at the intracellular surface of the sarcolemma. These findings imply that reduction of intracellular pH (but not extracellular pH) will markedly reduce Ca²⁺ transport mediated by the Na⁺-Ca²⁺ exchange system. The interactions between pH and Na⁺-Ca²⁺ exchange are complex, and we report on the properties of Na⁺-Ca²⁺ exchange under different experimental conditions.

Methods

Sarcolemmal vesicles were isolated from dog ventricles, as described previously (Philipson and Nish-
imoto, 1981). The only modifications were that Tris-maleate buffer instead of Tris buffer was used and that no buffer or dithiothreitol was included in the sucrose gradient. Some characteristics of the sarcolemmal preparation are presented in Results.

We have developed a technique for measuring Na\(^+\)-dependent Ca\(^{2+}\) uptake at short time periods. Usually, 0.0025 ml of sarcolemmal vesicles (~3-5 mg/ml; preincubated for 20–30 minutes at 37°C) suspended in 140 mM NaCl (or 140 mM KCl for blanks), 5 mM Tris-maleate buffer (pH 7.4 at 37°C) was suspended on the side of a plastic culture tube above 0.2475 ml of uptake medium at 37°C. The uptake medium contained 140 mM KCl, 1.25 µCi of \(^{45}\)CaCl\(_2\), \(^{40}\)CaCl\(_2\) (concentration as specified in figure legends), 5 mM Tris-maleate buffer (pH as specified in figure legends at 37°C). The uptake reaction was initiated by rapid mixing of the culture tube with a Vortex mixer (Van Lab). A signal from the Vortex mixer simultaneously started a clock in a custom-built timing device. At a preset time, a signal from the timing device caused a solenoid-driven syringe to inject 0.03 ml of 140 mM KCl, 25 mM LaCl\(_3\) into the culture tube to stop the Na\(^+\)-Ca\(^{2+}\) exchange reaction. La\(^{3+}\) has been used previously to inhibit Ca\(^{2+}\) transport in sarcolemmal vesicles (Philipson and Nishimoto, 1980; Bartschatt and Lindenmayer, 1980). The reaction mixture (0.22 ml) was applied to a Millipore filter (0.45 pm) under suction and the filter was washed with two aliquots of 3 ml 140 mM KCl, 1 mM LaCl\(_3\). The \(^{45}\)Ca\(^{2+}\) remaining on the filter was then measured with a liquid scintillation counter as described previously (Philipson et al., 1980). For all experiments we ran blanks in which KCl-loaded vesicles were used. This ensured that only Na\(^+\)-dependent Ca\(^{2+}\) movements were measured. The use of La\(^{3+}\) both to stop the uptake reaction and to wash the filtered vesicles removed superficial bound Ca\(^{2+}\) and left only intravesicular Ca\(^{2+}\). Other experiments to confirm the intravesicular nature of the measured Ca\(^{2+}\) are discussed by Philipson and Nishimoto (1981).

Ca\(^{2+}\) efflux experiments were performed as described previously (Philipson and Nishimoto, 1981). Specific conditions are given in the legend to Figure 5.

For Ca\(^{2+}\) uptake experiments, the media were treated with Chelex 100 (Bio Rad) to remove heavy metals and Ca\(^{2+}\) contamination. Using a Ca\(^{2+}\)-selective electrode (Orion), we determined that the Ca\(^{2+}\) contamination in our KCl uptake media was less than 2 µM.

All data are presented as the mean ± standard error of the mean. Error bars in figures also represent standard errors.

The notation Na\(^+\) and Na\(^0\) refers to intravesicular and extravesicular Na\(^+\), respectively.

**Results**

**Measurement of Sarcolemmal Na\(^+\)-Ca\(^{2+}\) Exchange**

Na\(^+\)-dependent Ca\(^{2+}\) uptake was measured by diluting Na\(^+\)-preloaded sarcolemmal vesicles into a Na\(^+\)-free isosmotic medium containing labeled Ca\(^{2+}\) (see Methods). Under these conditions, a rapid uptake of Ca\(^{2+}\) occurs in exchange for intravesicular Na\(^+\). All data were corrected for the Ca\(^{2+}\) uptake that occurred in the same experiments performed using K\(^+\)-preloaded vesicles; thus only Na\(^+\)-dependent Ca\(^{2+}\) movements are reported. We have devised an apparatus (see Methods) for the rapid measurement of Ca\(^{2+}\) uptake into sarcolemmal vesicles. Using this apparatus, we are able to measure initial rates of Na\(^+\)-dependent Ca\(^{2+}\) uptake into sarcolemma isolated from canine ventricles. An example of the time course of Na\(^+\)-Ca\(^{2+}\) exchange in an especially active preparation is shown in Figure 1. In most preparations, the maximum Na\(^+\)-dependent Ca\(^{2+}\) uptake was 60–100 nmol/mg protein. The earliest time point obtained was 0.5 second and linearity was maintained to about 6 seconds. In many preparations, nonlinearity was apparent by 2 seconds. For this reason, in all Na\(^+\)-dependent Ca\(^{2+}\) uptake measurements described below, the initial rate was measured as the Ca\(^{2+}\) uptake after 1.5 seconds of reaction. At pH 9.0 the uptake was also linear to 1.5 seconds.

Most of the data reported here were obtained using cardiac sarcolemma with the following characteristics: K\(^+\)-p-nitrophenylphosphatase (K\(^+\)-pNPase) activity, 26.5 ± 2.4 µmol p-nitrophenol/mg protein x hr; purification factor (sarcolemmal K\(^+\)-pNPase activity/homogenate K\(^+\)-pNPase activity) was 150.
activity), 80.8 ± 10.8; Na⁺, K⁺-ATPase activity, 31.5 ± 3.3 μmol Pi/mg protein × hr; n = 6. Alamethicin (12 μg/assay tube; generously supplied by Dr. J. E. Grady, Upjohn Co.) stimulated Na⁺, K⁺-ATPase activity to 210 ± 30% of this initial value. This is consistent with some sarcolemmal vesicles being in a right-side out configuration (Jones et al., 1980). A yield of approximately 10–15% of these sarcolemmal markers was recovered in the sarcolemmal fraction.

Fluoride-stimulated adenylate cyclase activity was 1893 ± 93 pmol/mg protein × min; n = 3. Less than 0.5% of the succinate dehydrogenase activity was recovered in the sarcolemmal fraction.

Dependence of Na⁺⁺-Dependent Ca²⁺ Uptake on pH

Figure 2 demonstrates the marked effects of pH on the initial rate of Na⁺⁺-Ca²⁺ exchange. The Ca²⁺ concentration in the uptake medium was 15 μM for this experiment. The sarcolemmal vesicles were initially suspended in 140 mM NaCl (or KCl for blanks), 5 mM Tris-maleate buffer, pH 7.4 at 37°C, and were exposed to the altered pH only during the 1.5 seconds of the uptake reaction. The uptake medium into which the vesicles were diluted contained (in addition to Ca²⁺) 140 mM KCl, 5 mM Tris-maleate buffer at the appropriate pH. The data in Figure 2 are normalized so that Na⁺⁺-dependent Ca²⁺ uptake at pH 7.4 is taken as 100% activity. Ca²⁺ uptake activity increased steeply from pH 6 to 9. The initial rate of Na⁺⁺-dependent Ca²⁺ uptake was diminished to 50% at approximately pH 6.7. The observed effects are opposite to what might be expected if the Ca²⁺ activity of the uptake medium were reduced by increasing pH. The data could be explained if the passive Ca²⁺ permeability of the vesicles was substantially altered by pH. However, we found that passive Ca²⁺ flux was unaffected by pH in this range (see below). The sigmoidal shape of the curve in Figure 2 (pK ~ pH 8) is suggestive that the ionization state of a histidine (the only amino acid with an appropriate pK) residue may be important in regulating Na⁺⁺-Ca²⁺ exchange.

Another possible explanation for the data in Figure 2 would be the presence of a highly active Na⁺⁻H⁺ exchange system in cardiac sarcolemma. At low pH, external H⁺ would exchange for internal Na⁺. Less internal Na⁺ would then be available to participate in Na⁺⁺-Ca²⁺ exchange. However, we found that 0.1 mM amiloride, a reported inhibitor of Na⁺⁻H⁺ exchange (Aickin and Thomas, 1977; Kinsella and Aronson, 1980), resulted in a small (6%) stimulation of Na⁺⁺-dependent Ca²⁺ uptake at pH 6. In addition, in preliminary experiments using ²²Na⁺, we have not found evidence for a sarcolemmal Na⁺⁻H⁺ exchange of high activity under these conditions.

To investigate further the interactions between H⁺ and Ca²⁺, Na⁺⁺-dependent Ca²⁺ uptake was measured as a function of Ca²⁺ concentration (3–200 μM) at both pH 7.4 and pH 9.0 (Fig. 3). The difference between the two curves decreases as Ca²⁺ concentration increases. At lower Ca²⁺ concentrations ([Ca²⁺] ≤ 25 μM), the Ca²⁺ uptake at pH 9.0 is about 300% greater than the uptake at pH 7.4. At [Ca²⁺] = 200 μM, the difference is less than 70%.
This pattern was reproducible in several experiments. The data in Figure 3 are shown as an Eadie-Hofstee plot in Figure 4. The points at pH 9.0 can be fitted by a single line indicating a $K_i/2$ for Ca$^{2+}$ of 38 $\mu$M. The data obtained at pH 7.4 are more complex and cannot be fit by one line. At low Ca$^{2+}$ concentrations ([Ca$^{2+}$] $\leq$ 25 $\mu$M), the data parallel that at pH 9.0, whereas, at higher Ca$^{2+}$, the data fall on a line with a slope of 140 $\mu$M. Although straightforward interpretation of the data is difficult, it appears that some competitive interaction between H$^+$ and Ca$^{2+}$ exists at the higher Ca$^{2+}$ concentrations. At a Ca$^{2+}$ concentration similar to that present in extracellular fluid (~1.5 mM), pH would have little effect on Na$^+$/Ca$^{2+}$ exchange.

**Dependence of Na$^+$/Ca$^{2+}$ Efflux on pH**

Efflux experiments were performed in which cardiac sarcolemmal vesicles were first preloaded with Ca$^{2+}$ by Na$^+$/Ca$^{2+}$ exchange at pH 7.4. After 2 minutes of Ca$^{2+}$ uptake (when a plateau of uptake was obtained), the vesicles were diluted 15-fold into a solution containing EGTA (to inhibit any further Ca$^{2+}$uptake) and KCl $\pm$ NaCl at various pH values. The efflux of Ca$^{2+}$ from the vesicles was then followed by Millipore filtration. This technique has been discussed in detail previously (Philipson and Nishimoto, 1981). Under these conditions, Na$^+$/Ca$^{2+}$ exchange is readily demonstrated. This manifestation of Na$^+$/Ca$^{2+}$ exchange is the reverse reaction of Na$^+$/Ca$^{2+}$ uptake described in the experiments above. The stimulation of Ca$^{2+}$ efflux induced by Na$^+$ as a function of pH was determined. For each point, the passive efflux (in the absence of added Na$^+$) was also measured. No effect of pH from 6 to 9 on the passive rate of Ca$^{2+}$ efflux was observed (data not shown). The effects of pH on Na$^+$/dependent Ca$^{2+}$ efflux are shown in Figure 5. On the left half of the figure is displayed the percentage of the Ca$^{2+}$ load lost from the vesicles after 2 minutes of efflux. The efflux medium contained 8 mM Na$^+$ and pH was varied. At this low Na$^+$ concentration ($K_i/2$ for Na$^+$ induced Ca$^{2+}$ efflux is 12.5 mM (Philipson and Nishimoto, 1981)), Na$^+$/dependent Ca$^{2+}$ efflux increases as pH increases. The process was almost completely inhibited at pH 6.0. In the right half of Figure 5, the effects of 70 mM Na$^+$ on Ca$^{2+}$ efflux are shown after 0.3 minute of efflux. Less effect of pH on Na$^+$/dependent Ca$^{2+}$ efflux is observed at this saturating Na$^+$ concentration. Just as the effects of pH on Na$^+$/dependent Ca$^{2+}$ uptake are most dramatic at low [Ca$^{2+}$] (see above), the effects of pH on Na$^+$/dependent Ca$^{2+}$ efflux are most manifest only at low values of external Na$^+$. It should also be noted that the pH sensitivity of Na$^+$/dependent Ca$^{2+}$ efflux cannot be explained by either a Na$^+$/H$^+$/ exchange or a Ca$^{2+}$/H$^+$ exchange which would tend to stimulate Ca$^{2+}$ efflux at low pH. Initial rates were not measured in these efflux experiments, but the qualitative results are still likely to be valid.
Na\textsuperscript{+}-Dependent Ca\textsuperscript{2+} Uptake in Different Uptake Media

We have described above that Na\textsuperscript{+}-dependent Ca\textsuperscript{2+} uptake at pH 7.4 has a complex dependence on [Ca\textsuperscript{2+}] (Fig. 4). At low [Ca\textsuperscript{2+}], the slope is similar to the slope at pH 9.0 (38 \textmu M). The value measured will depend on the range of Ca\textsuperscript{2+} concentrations investigated. This may explain the different values reported in the literature (K\textsubscript{1/2} = 18 \textmu M, Reeves and Sutko, 1979; K\textsubscript{1/2} ~ 30 \textmu M, Bers et al., 1980). In more recent experiments we obtain a K\textsubscript{1/2} for Ca\textsuperscript{2+} of 28 \textmu M (pH 7.4, measured over a Ca\textsuperscript{2+} concentration range of 6 to 40 \textmu M). Caroni et al. (1980), however, have reported a much lower K\textsubscript{1/2} for Ca\textsuperscript{2+} (~1.5 \textmu M) which varied with the composition of the uptake medium. To investigate the effect of variations in the uptake medium, we have examined the initial rate of Ca\textsuperscript{2+} uptake under different conditions (Table 1). Shown are the Na\textsuperscript{+}-dependent Ca\textsuperscript{2+} uptakes at [Ca\textsuperscript{2+}] = 10 and 100 \textmu M in KCl, KCl plus valinomycin, and sucrose. The stimulation of Ca\textsuperscript{2+} uptake induced by valinomycin (~100%) was identical at both Ca\textsuperscript{2+} concentrations. This confirms earlier results from this laboratory (Philipson and Nishimoto, 1980) and directly contrasts with the results of Caroni et al. (1980), who reported that the stimulatory effects of valinomycin were greatly diminished at 100 \textmu M Ca\textsuperscript{2+}. In addition, Na\textsuperscript{+}-dependent Ca\textsuperscript{2+} uptake was similar in both sucrose and KCl uptake media independent of [Ca\textsuperscript{2+}]. This again contrasts with the data of Caroni et al. (1980) who reported that Ca\textsuperscript{2+} uptake in a sucrose medium is saturated by 1 \textmu M Ca\textsuperscript{2+} and is significantly less than the uptake in a KCl medium. The reasons for these discrepancies are unclear. The stimulation of Na\textsuperscript{+}-dependent Ca\textsuperscript{2+} uptake by increased pH occurs in several buffer systems (Tris-maleate, Tris, MOPS) and in other uptake media (KCl, KCl plus valinomycin, sucrose) (data not shown). Thus, H\textsuperscript{+} itself was probably responsible for the modulation of Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange with variations in pH.

Discussion

We find that Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange in a highly enriched preparation of cardiac sarcolemmal vesicles has a striking sensitivity to pH. Na\textsuperscript{+}-Ca\textsuperscript{2+} countertransport (operating in either direction) is severely inhibited at pH 6 and markedly stimulated at pH 9 (Figs. 2 and 5). These effects are not due to an effect of pH on medium Ca\textsuperscript{2+} activity, on passive membrane permeability, or on some other transport system (e.g., Na\textsuperscript{+}-H\textsuperscript{+} or Ca\textsuperscript{2+}-H\textsuperscript{+} exchange). We conclude that the effects of pH on Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange are due to an intrinsic interaction between H\textsuperscript{+} and the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange system.

Since Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange is sensitive to membrane potential (Philipson and Nishimoto, 1980), it is possible that pH could be affecting Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange activity indirectly by altering potential. This is unlikely for two reasons. First, increased pH stimulates both Na\textsuperscript{+}-dependent Ca\textsuperscript{2+} influx and Na\textsuperscript{+}-dependent Ca\textsuperscript{2+} efflux. A pH-induced change in membrane potential would not be expected to affect both directional modes of Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange in the same way. Second, we report that valinomycin-induced membrane potentials alter Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange independent of pH. This would also not be expected if the primary effect of pH was on membrane potential.

The effects of pH on Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange are most apparent at low Ca\textsuperscript{2+} (Fig. 3) and low Na\textsuperscript{+} (Fig. 5) concentrations. This suggests that there may be some competitive interaction between H\textsuperscript{+} and Na\textsuperscript{+} and Ca\textsuperscript{2+} at some important ion binding sites (Fig. 4). In the intact myocardial cell, the extracellular fluid has high concentrations of both Na\textsuperscript{+} and Ca\textsuperscript{2+}, whereas the intracellular environment has low concentrations of these ions. The implication is that, in a myocardial cell, an altered pH will exert significant influence on Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange only under the ionic conditions at the intracellular surface of the sarcolemmal membrane. It has previously been concluded from experiments using intact cardiac muscle preparations that the negative inotropic effect of acidosis is due primarily to a fall in intracellular pH (e.g., Steenbergen et al., 1977; Fry and Poole-Wilson, 1981). Thus, the characteristics of the pH-modification of vesicular Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange are consistent with a role for this process in the pH-modification of myocardial contractility. That is, a change in intracellular pH (but not extra-

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Na\textsuperscript{+}-dependent Ca\textsuperscript{2+} uptake was measured at pH 7.4, 1.5 seconds after dilution of the sarcolemmal vesicles in the uptake medium. [KCl] = 140 mM, [valinomycin] = 0.4 \textmu M, [sucrose] = 280 mM in the different uptake media. n = 3.
cellular pH) could affect myocardial excitation-contraction coupling by affecting Ca\(^{2+}\) transport (mediated by Na\(^+-\)Ca\(^{2+}\) exchange) across the sarcolemma.

Most of the Na\(^+-\)Ca\(^{2+}\) exchange measurements reported here were not made under physiological ionic conditions (high Na\(^+\) and Ca\(^{2+}\) outside, low Na\(^+\) and Ca\(^{2+}\) inside). Using sarcolemmal vesicles it is not technically feasible to measure Na\(^+\)-dependent Ca\(^{2+}\) uptake under physiological conditions due to the extremely small volume-to-surface area ratio of vesicles. Intravesicular Ca\(^{2+}\) must rise to a relatively high level to be measurable. This requires an initially high intravesicular Na\(^+\) level. In this study we approach this problem by examining effects of pH on only one side of the membrane under controlled conditions of external Na\(^+\) or Ca\(^{2+}\) concentrations. The data are clearly consistent with an interaction of H\(^+\) with Na\(^+\) and Ca\(^{2+}\) binding sites on the exchange mechanism. It is possible, although unlikely, that this result would be altered by the ionic conditions on the opposite side of the membrane. We feel our extrapolation of the data to the intact cell is valid, although limitations of the techniques should be recognized.

A recent study (Coraboeuf et al., 1981) included an investigation of the effects of acidosis on low Na\(^+\) contractures in dog Purkinje fibers. Effects attributable to Na\(^+-\)Ca\(^{2+}\) exchange were abolished at low pH if the acidosis was maintained for a sufficient time. This suggests that a fall in intracellular pH can inhibit Na\(^+-\)Ca\(^{2+}\) exchange in vivo consistent with the data presented here. In another recent report, Wakabayashi and Goshima (1981) found that, in a myocardial cell culture system, pH affects Ca\(^{2+}\) transport in a manner consistent with the present results. They also suggest that a histidine residue may be essential in Na\(^+-\)Ca\(^{2+}\) exchange.

The sarcolemmal preparation contains both inside out (ouabain-sensitive, ATP-dependent Na\(^+\) uptake can be demonstrated (unpublished observation)) and right side out (Bers et al., 1980) vesicles, although the proportion of each is uncertain. When the Na\(^+-\)Ca\(^{2+}\) exchange reaction takes place, it is occurring in both populations of vesicles. The correlation (given above) of these results with pH experiments performed with intact cardiac muscle assumes that the exchange properties of these two types of vesicles are similar. That is, we assume that pH can exert a regulatory influence on the Na\(^+\) and Ca\(^{2+}\) binding sites at both surfaces of the exchange mechanism. Because of the high extracellular Na\(^+\) and Ca\(^{2+}\) levels, this regulatory influence of pH will occur in vivo only at the intracellular sarcosomal surface.

Many intracellular systems are affected by a change in pH. Negative inotropy during acidosis probably is a response to multiple interactions. The results presented here may be of special significance, considering the possible central role of Na\(^+-\)Ca\(^{2+}\) exchange in cardiac excitation-contraction coupling. Intracellular acidosis accompanies myocardial ischemia, and this may account for the initial rapid decline in contractility induced by ischemia (Steenbergen et al., 1977; Cobbe and Poole-Wilson, 1980). Our results may be relevant to the design and interpretation of future experiments concerning pH, ischemia, and myocardial contractility.

Acknowledgments

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