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Changes in the surface charge properties of isolated cardiac sarcolemmal vesicles measured by light scattering. II. Characteristics of rabbit preparations

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Numerous studies suggest that cation–sarcolemmal interactions play an essential role in the excitation/contraction/relaxation cycles of cardiac muscle cells. To help elucidate the molecular mechanisms involved in these processes the cation binding characteristics of isolated rabbit cardiac sarcolemmal vesicles were investigated. Cation–membrane interactions were studied by examining the cation-induced aggregation properties of the vesicles. The results obtained were qualitatively very similar to those previously reported for rat and canine cardiac sarcolemmal vesicle preparations (Leonards, K.S. (1988) Biochim. Biophys. Acta 938, 293–309), indicating that all three species have a shared set of basic membrane characteristics. Specifically the results indicate that cations, such as Ca^{2+}, bind to the sarcolemmal surface, and suggest that two (or more) interacting sites are involved in the process. The selectivity series for the cation-induced aggregation of the sarcolemmal vesicles was: $La^{3+} \geq Cd^{2+} \gg Mn^{2+} > Ca^{2+} > Ba^{2+} = Sr^{2+} = Mg^{2+}$. Protons ($H^+$) could also induce massive vesicle aggregation at pH 5.60–5.75. However, the results obtained also show that the interactions of cations with the rabbit cardiac sarcolemmal membrane surface are quantitatively distinct from those obtained in either rat or canine sarcolemmal vesicle preparations, thereby confirming the species specific nature of cation–sarcolemmal interactions in cardiac cells.

Introduction

A primary function of the cardiac sarcolemmal membrane is to transport ions into and out of the cell during muscle excitation/contraction/relaxation cycles [1–11]. Although the membrane proteins responsible for these ion movements (i.e., ion channels, cation pumps, the Na^+/Ca^{2+}-exchanger, etc.) are known to be quite sensitive to their membrane environment, the physical-chem-

Abbreviations: Mes, 4-morpholine ethanesulfonic acid; Mops, 4-morpholine propanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Ches, 2-(*N*-cyclohexylamino)ethanesulfonic acid; DTT, DL-dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; SUVs, sonicated unilamellar vesicles.

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ical properties of the membrane involved in regulating these processes remain unknown [12–19].

In order to understand the molecular mechanisms involved in these processes and how the physical-chemical properties of the sarcolemmal membrane may regulate them it is first necessary to: (1) ascertain the basic characteristics of this membrane, (2) determine if cation–membrane interactions affect its physical-chemical properties, and (3) identify the components participating in these interactions. To this end, we have begun a systematic examination of the interactions of cations with the sarcolemmal surface. In the present study cation–sarcolemmal membrane interactions were examined by analyzing the cation-induced aggregation behavior of the isolated sarcolemmal vesicles. A major advantage of this experimental approach is that the interactions we are observing (aggregation) are directly related to the specific adsorption (binding) of cations to the sarcolemmal surface and not diffuse double layer effects [20,21]. We are therefore able to clearly distinguish between these two types of membrane association and ascertain the contributions of ion binding (adsorption) to cation–sarcolemmal interactions.

In a previous study [22] we reported upon the results obtained using both canine and rat cardiac sarcolemmal vesicle preparations. The purpose of the present study is to extend this examination to include rabbit cardiac sarcolemmal preparations, thereby covering the three animal species most commonly employed in cardiovascular research. Our specific aims are three fold: (1) to determine whether cations actually bind (adsorb) to the sarcolemmal surface, rather than being present only in the diffuse double layer, (2) to characterize these interactions in order to help define a physical-chemical baseline for future quantitative analysis, and (3) to compare the cation binding properties of the isolated rabbit cardiac sarcolemmal vesicle preparations to those obtained with the canine and rat vesicle preparations.

Materials and Methods

Materials. Phospho*enol*pyruvate (trisodium salt), pyruvate kinase (type III), L-lactate dehydrogenase (type XI), Na_2-ATP (vanadium free), β-NADH (from yeast, grade III, disodium salt), *p*-nitrophenyl phosphate (diTris salt), Mes, Hepes, Ches, Tris-maleate, Mops, EDTA, PMSF, DTT, sodium pyrophosphate, and EGTA were obtained from Sigma Chemical Co. Chloride salts (vesicle isolation buffers, enzyme assays) were from Mallinckrodt Chemical Co. Ultrapure mono- and divalent salts for vesicle aggregation experiments (Gold Label 99.999%) were from Aldrich Chemical Co. DNAase I (bovine pancreas, lyophilized) was from Worthington Biomedical. Distilled, deionized water was further purified (18 MΩ) with a Water-I apparatus (Gelman Sciences).

Procedures. Sarcolemmal vesicles were isolated from rabbit hearts using the same procedures employed in the rat and canine studies, as previously described [22]. Enzymatic activities of the isolated vesicles were measured spectrophotometrically using a Hewlett-Packard model 8450A Photodiode Array Spectrophotometer equipped with a thermoelectric temperature controller. K^+-stimulated *p*-nitrophenylphosphatase activity was measured according to Bers and Langer [23]; K^+-stimulated Na^+/K^+-ATPase with the coupled enzyme assay according to Forgag [24] and Luciani [25]; and Ca^{2+}-stimulated Ca^{2+}/Mg^{2+}-ATPase using the coupled enzyme assay procedure described by Madden et al. [26]. Protein concentrations were determined according to the method of Lowry et al. [27], as modified for membrane proteins by Wang and Smith [28], using bovine serum albumin as standard. Phosphate was measured according the Bartlett [29].

The aggregation behavior of the vesicle suspensions was measured turbidometrically over the wavelength range 200–800 nm as a function of cation concentration or at 300 nm as a function of time at constant cation concentration using the Hewlett-Packard 8450A Photodiode Array Spectrophotometer. Although the spectrophotometer recorded the entire spectrum (200–800 nm) of the vesicle suspension simultaneously, 300 nm was used to generate the absorbance vs. [cation] and absorbance vs. time curves because of its sensitivity to small changes in aggregation behavior. All vesicle aggregation experiments were conducted under identical, 'standard', conditions (100 mM NaCl; 5.0 mM buffer (Mes, Hepes, Ches); 0.05 mM Na_2-EDTA; 25 °C) at constant protein concentration (60 $\mu g \cdot ml^{-1}$). The absorbance vs. [cat-

ion] experiments were conducted as described by Ohki et al. [30,31]. Briefly, the ion concentration of the specific cation being tested was raised in stepwise increments by injecting aliquots of the concentrated salt solution (60 mM to 3.0 M depending on salt and desired size of increment) into the sample and mixing. The absorbance was measured 2 min after each injection, the injection procedure being repeated until the absorbance began to decrease. The most likely reason for the observed decrease in absorbance at A_{300nm} at higher [cation] in the 2-min assay is that the vesicle aggregates are reaching a threshold beyond which they break down due to the cation-induced destabilization of the bilayer. The kinetics of cation-induced vesicle aggregation were also determined for each cation by injecting an aliquot of the concentrated salt solution (final concentration injected test cation = 10 mM) into the vesicle suspension, mixing, and measuring the change in A_{300nm} vs. time as previously described [22]. In contrast to the 2-min assay method, any observed decreases in the $A_{300\ nm}$ values obtained in the aggregation kinetics experiments have been traced to the formation of larger and larger aggregates. With time these aggregates become so large that they begin to fall out of solution. In both cases the concentration of light scattering centers is significantly reduced. For a more detailed description of these procedures see Leonards [22] and Ohki et al. [30,31].

Results

Biochemical assays

The average enzymatic activities of the isolated rabbit sarcolemmal vesicles were: K^+-stimulated *p*-nitrophenylphosphatase, 5.79 μmol *p*-nitrophenol · (mg protein)$^{-1}$ · h^{-1}; K^+-stimulated Na^+/K^+-ATPase activity, 50.87 μmol · (mg protein)$^{-1}$ · h^{-1}; Ca^{2+}-stimulated Ca^{2+}, Mg^{2+}-ATPase, 0.35 μmol · (mg protein)$^{-1}$ · h^{-1} ($n = 4$ for all above values).

Ca^{2+}-induced vesicle aggregation

In the first set of experiments the effects of pH (range 6.0–9.0) on the Ca^{2+}-induced aggregation behavior of isolated rabbit sarcolemmal vesicles were determined as a function of [Ca^{2+}]. If the

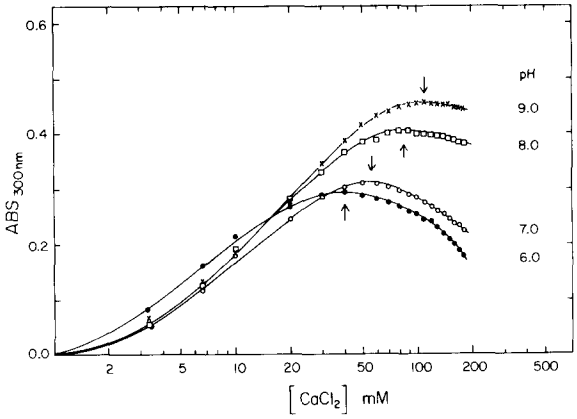

Fig. 1. Aggregation behavior (A_{300nm}) of isolated rabbit cardiac sarcolemmal vesicles at different pH values as a function of [$CaCl_2$]. ●, aggregation of vesicles suspended in pH 6.0 buffer; ○, aggregation of vesicles suspended in pH 7.0 buffer; □, aggregation of vesicles suspended in pH 8.0 buffer; ×, aggregation of vesicles suspended in pH 9.0 buffer. Buffers: 100 mM NaCl; 5.0 mM (Mes (pH 6.0), Hepes (pH 7.0 and 8.0) or Ches (pH 9.0)); 0.05 mM Na_2-EDTA. Temp. 25°C. Protein concentration all samples, 60 $\mu g \cdot ml^{-1}$. Arrows indicate $A_{300nm,max}$ values. Curves are averages of $n > 3$ experiments each curve. Aggregation assay conducted using the two minute procedure described in Methods.

sarcolemmal membrane surface contains ionizable sites which dissociate over this pH range, there should be an increase in the surface charge density of the vesicle membrane as the pH is raised. We have previously shown that this is analogous to increasing the mole% PS present in PC/PE/PS SUVs [22] and, assuming that Ca^{2+} can bind to these sites, should result in an increase in the degree of aggregation ($A_{300nm,max}$ ↑) and a shift of the aggregation curves to lower [Ca^{2+}]. Fig. 1 illustrates the results obtained. Two points are evident. The first is that Ca^{2+} did induce the aggregation of the isolated sarcolemmal vesicles, indicating that Ca^{2+} does bind to the sarcolemmal surface. The second major observation was that vesicle aggregation was sensitive to the pH of the suspension buffer, with the [Ca^{2+}] corresponding to the $A_{300nm,max}$ values shifting from 40 mM Ca^{2+} (pH 6.0), to 60 mM Ca^{2+} (pH 7.0), 80 mM Ca^{2+} (pH 8.0), and 110 mM Ca^{2+} (pH 9.0). However, although the degree of aggregation increased ($A_{300nm,max}$ ↑) as the pH of the suspension buffer was raised, the aggregation curves were shifted to higher, not lower [Ca^{2+}]. This is the opposite

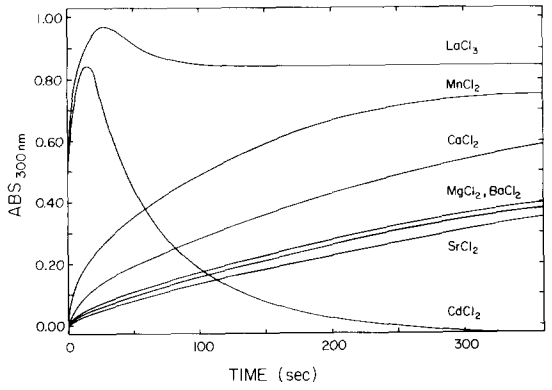

Fig. 2. Aggregation kinetics of isolated rabbit sarcolemmal vesicles for various di- and trivalent cations under identical experimental conditions. Buffer: 100 mM NaCl; 5.0 mM Hepes; 0.05 mM Na_2-EDTA (pH 7.0). Temp. 25 °C. Protein concentration all samples, 60 $\mu g \cdot ml^{-1}$. An aliquot of the respective di- or trivalent cation salt was injected into the vesicle suspension to give a final test cation concentration of 10 mM. Changes in turbidity (A_{300nm}) were then monitored as a function of time. Since these experiments were conducted under identical conditions at a constant protein concentration, the differences observed for these curves correspond to differences in cation binding to the sarcolemmal vesicle surface. Aggregation curves were obtained for La^{3+}, Cd^{2+}, Mn^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, and Mg^{2+}. The overall selectivity series for cation binding is: $La^{3+} \geq Cd^{2+} \gg Mn^{2+} > Ca^{2+} > Ba^{2+} = Sr^{2+} = Mg^{2+}$. Curves are averages of three experiments each curve.

result of that expected given our previous PC/PS and PC/PE/PS SUV data [22,30], but is similar to the results obtained with both rat and canine sarcolemmal vesicle preparations [22].

Mg^{2+}-induced vesicle aggregation

To determine if the effects of pH on the Mg^{2+}-induced aggregation of the sarcolemmal vesicles were similar to those obtained for Ca^{2+}, above, the aggregation experiments were repeated under identical conditions, but as a function of $[Mg^{2+}]$. The results obtained (not shown) were similar to those observed with Ca^{2+}, except that the absolute absorbance ($A_{300nm,max}$) values measured were always significantly lower. For Mg^{2+} the cation concentrations corresponding to the $A_{300nm,max}$ values were: 60 mM Mg^{2+} (pH 7.0), 80 mM Mg^{2+} (pH 8.0), and 90 mM (pH 9.0) (pH 6.0 not tested).

Selectivity series for cation binding

The reduced degree of vesicle aggregation ($A_{300nm,max}$ values) observed as a function of $[Mg^{2+}]$, relative to that seen as a function of $[Ca^{2+}]$, suggested that Mg^{2+} does not bind to the rabbit sarcolemmal membrane as well as Ca^{2+}. To further examine the cation binding behavior of the sarcolemmal surface the kinetics of cation-induced vesicle aggregation were measured for a series of di- and trivalent cations. Previous studies [20,21,37] have shown that for vesicles having negatively charged surfaces the initial increase in turbidity is essentially governed by the aggregation rate constant C_{11}, which is direct function of cation binding (i.e., the dissociation rate constant, D_{11}, equals zero). Consequently, under identical experimental conditions (kinetic energy constant), the faster the vesicles aggregate (rate A_{300nm} increases) the greater the binding affinity to the cation for the vesicle surface. The results obtained for the rabbit sarcolemmal vesicles from such kinetic experiments are shown in Fig. 2. They indicate that the selectivity series for cation binding to the sarcolemmal membrane is $La^{3+} \geq Cd^{2+} \gg Mn^{2+} > Ca^{2+} > Ba^{2+} = Sr^{2+} = Mg^{2+}$ *.

Effects of protons (H^+) on vesicle aggregation

The previous studies conducted using rat or canine sarcolemmal vesicles preparations demonstrated that protons (H^+) were the only monovalent cations capable of inducing vesicle aggregation [22]. To examine the proton (H^+)-induced aggregation behavior of the rabbit sarcolemmal vesicles, vesicle preparations were suspended in either pH 8.0 (Hepes) or pH 9.0 (Ches) buffers under standard conditions. The [HCl] of the vesicle suspensions was then increased in a stepwise fashion, using the same protocol employed in the

* It should be noted that vesicle aggregation depends on the charge of the cation as well as its binding affinity. In a strict sense mixing di- and trivalent cations into one binding series is quantitatively questionable. However, given the large differences in aggregation kinetics found it is unlikely that the qualitative order of cation binding would be changed under these conditions. Moreover by keeping all of the cation concentrations constant we insure that all of the experiments are run under identical conditions (kinetic energy constant) and can be compared.

divalent cation experiments. Fig. 3 illustrates the results obtained with both buffers. (Note: the abscissa [HCl] values used in this figure correspond to the total [HCl] added to the suspension, not the pH of the suspension.) Adding HCl to the vesicles suspended in pH 9.0 Ches buffer resulted in a very sharp increase in A_{300nm} over a very small range of [HCl] ($A_{300nm,max} \equiv 2.0$ mM H^+; mid-point $\equiv 1.6$ mM H^+), indicating massive aggregation of the vesicles. When this procedure was repeated using the pH 8.0 Hepes buffer, the resultant aggregation behavior of the sarcolemmal vesicles was similar, but shifted to higher [HCl] ($A_{300nm,max} \equiv 4.45$ mM H^+; mid-point $\equiv 3.9$ mM H^+).

To ascertain the actual pH of the suspensions, and how it changed as HCl was added, both the pH 8.0 Hepes and pH 9.0 Ches buffers were titrated with HCl using the same conditions employed in the sarcolemmal vesicle experiments. These curves are shown in Fig. 4. When the [HCl] corresponding to the mid-points of the vesicle aggregation curves were plotted onto these titration curves, the pH induced aggregation behavior of the vesicles could be determined for both buffers. As Fig. 4 demonstrates, the pHs at which the vesicles were induced to aggregate were the almost the same in both buffers (pH 5.75 in the pH 8.0 Hepes buffer, and pH 5.60 in the pH 9.0 Ches buffer) indicating that the sarcolemmal vesicles were aggregating in direct response to the actual pH of the suspension.

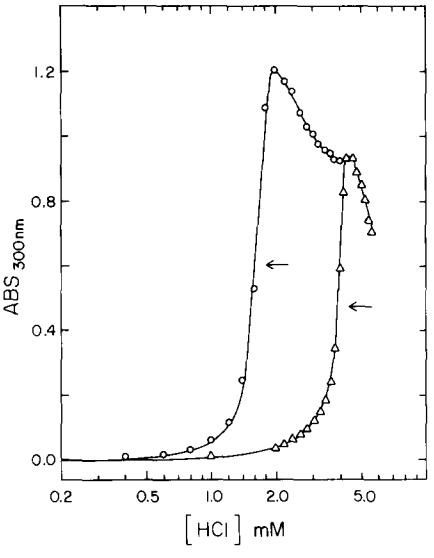

Fig. 3. Effects of protons (H^+) on the vesicle aggregation behavior A_{300nm} of rabbit cardiac sarcolemmal vesicles. ○, aggregation of vesicles suspended in pH 9.0 buffer; △, aggregation of vesicles suspended in pH 8.0 buffer. Buffers: 100 mM NaCl; 5.0 mM Hepes (pH 8.0) or Ches (pH 9.0); 0.05 mM Na_2-EDTA. Temp. 25 °C. Protein concentration 60 $\mu g \cdot ml^{-1}$. Arrows indicate mid-points of each curve. Curves are averages of $n > 3$ experiments per curve.

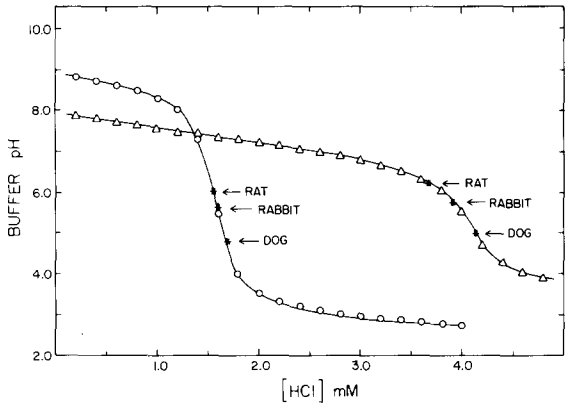

Fig. 4. Titration curves of the pH 8.0 and pH 9.0 buffers used to determine the effects of protons (H^+) on rabbit cardiac sarcolemmal vesicle aggregation (Fig. 3). The pH values corresponding to the mid-points of the proton-induced vesicle aggregation curves (Fig. 3) are indicated by the arrows. The titration experiments were conducted using the same buffers, and under the same conditions, employed in the sarcolemmal vesicle aggregation experiments. The results obtained for rat and canine sarcolemmal vesicle preparations are included for comparison (from Ref. 22). Buffers: 100 mM NaCl; 5.0 mM Hepes (pH 8.0) (△) or Ches (pH 9.0) (○) 0.05 mM Na_2-EDTA. Temp. 25 °C. Note that the pH values corresponding to these mid-points are essentially the same.

Discussion

Numerous physiological studies suggest that cation–sarcolemmal interactions play an essential role in the excitation/contraction/relaxation cycles of cardiac muscle cells [5,11,23,38–46]. However, while these studies have demonstrated a close correlation between sarcolemmal associated Ca^{2+} and cardiac cell contractility, they have not been able to ascertain if Ca^{2+} was actually binding (adsorbing) to the sarcolemmal surface under these conditions or only present in the diffuse double layer. To clarify this issue was the first aim

of our study. As our results demonstrate (Fig. 1), there is a significant degree of Ca^{2+} binding to the sarcolemmal surface. In addition, the results shown in Fig. 2 indicate that cation binding to the sarcolemmal surface varies from cation to cation, with the overall selectivity series for cation binding being: $La^{3+} \geq Cd^{2+} \gg Mn^{2+} > Ca^{2+} > Ba^{2+} = Sr^{2+} = Mg^{2+}$. The fact that such a selectivity series exists confirms the conclusion that these cations are actually binding to the vesicle surface, since all divalent cations are identical, and indistinguishable, in their double layer effects. A comparison of the results obtained in the Ca^{2+} and Mg^{2+}-induced vesicle aggregation experiments also indicates that cation binding selectivity is maintained over the entire pH range tested. Finally, it should also be noted that this cation binding selectivity series is identical to that reported to progressively uncouple excitation from contraction in neonatal rat papillary muscle ($La^{3+} \geq Cd^{2+} > Mn^{2+} > Mg^{2+}$) [41,44]. Thus our results are consistent with Langer's proposal [5,11] that sarcolemmal bound Ca^{2+} plays an essential role in cardiac muscle cell contraction.

The second goal of this study was to describe some of the basic characteristics of this binding, and to compare these results to those obtained previously with the rat and canine cardiac sarcolemmal vesicle preparations [22]. One of the major conclusions of the rat and canine vesicles study [22] was that Ca^{2+} binding to the sarcolemmal surface could not be explained by a model involving a simple pH induced increase in surface charge density of a membrane containing a single type of charge molecule (i.e., phospholipid like phosphatidylserine). Instead, the results suggested that two or more interacting sites are involved, one of which binds Ca^{2+} and a second which modifies this Ca^{2+} binding as a function of pH, but does not itself bind Ca^{2+}. One of the bases for this conclusion was that the sarcolemmal vesicle aggregation curves obtained with both rat and canine preparations were shifted to higher, not lower, $[Ca^{2+}]$ as the pH was raised [22]. (Control experiments confirmed that the observed results could not be explained by the presence of a mixed population of right side out and inside out vesicles [22].) The results obtained in this study (such as Fig. 1) indicate that the bahavior of the rabbit

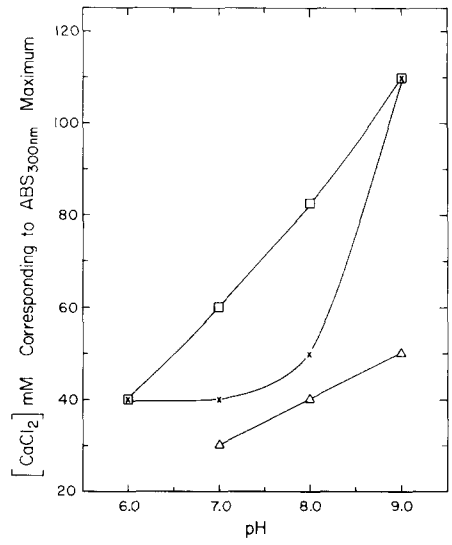

Fig. 5. Comparison plot of the $[CaCl_2]$ corresponding to the $A_{300nm,max}$ values obtained at pH 6.0–9.0 for isolated rabbit (□), rat (△), and canine (X) sarcolemmal vesicle preparations, indicating the species specific nature of cation binding to the sarcolemmal membrane surface. The rat and canine sarcolemmal vesicle values are taken from Ref. 22.

vesicle preparations is the same as that seen in the rat and canine sarcolemmal vesicles, indicating that the same 'working hypothesis' for cation–sarcolemmal interactions is appropriate for the rabbit sarcolemmal vesicles. Thus, the involvement of two, or more, interacting sites seems to be a common characteristic of the cardiac sarcolemmal membrane. This 'working hypothesis' is similar to that proposed by Langer [5,11] to explain the relationship between $^{45}Ca^{2+}$ 'binding' and contraction in isolated cardiac myocyte studies.

Although the basic characteristics of Ca^{2+}–sarcolemmal membrane interactions seem to be the same in rabbit, rat, and canine sarcolemmal vesicle preparations, the actual molecular interactions involved appear to be quite species specific. This specificity is apparent from the curves shown in Fig. 5, where the $[CaCl_2]$ corresponding to the $A_{300nm,max}$ values obtained at pH 6.0–9.0 for isolated rabbit, rat, and canine sarcolemmal vesicle preparations are plotted as a function of aggregation buffer pH. The results indicate that of the three species evaluated, rat sarcolemmal vesicles are the least affected by pH changes in the en-

vironment, with $A_{300nm,max}$ values only shifting from 30 to 50 mM [Ca^{2+}] over the pH range 7.0 to 9.0. In contrast, rabbit and canine vesicle preparations are markedly sensitive to buffer pH, with both species displaying $A_{300nm,max}$ values shifting from 40 to 110 mM [Ca^{2+}] over a similar pH range (6.0 to 9.0). However, even though both rabbit and canine vesicle preparations have the same [$CaCl_2$] endpoint values (40 and 110 mM Ca^{2+} at pH 6.0 and 9.0, respectively), they are very different from each other in how their $A_{300nm,max}$ values shift within this range. Whereas rabbit sarcolemmal vesicles showed a steady increase in the [$CaCl_2$] corresponding to the $A_{300nm,max}$ value as the suspension pH was raised, canine vesicle preparations displayed little change over the pH range 6.0 to 8.0, and a large change between pH 8.0 and 9.0. In this context it is worthwhile noting that previous studies have established that the basic characteristics of sarcolemmal vesicles isolated from a number of species are essentially the same with respect to leaky vs. sealed, inside-out vs. right side-out vesicle populations, etc. when purified using this isolation procedure [43,47–49]. Consequently, any differences observed between these three species can be attributed to the species specific properties of the membranes themselves.

Species specific differences in cation–sarcolemmal membrane interactions are also evident from the results obtained in the aggregation kinetics experiments. A comparison of the aggregation curves shown in Fig. 2 for rabbit sarcolemmal vesicles, to those found for rat (Fig. 3 [22]) and canine vesicle preparations [22] indicate that although the selectivity series for cation binding is the same for all three species, the relative relationship between cations varied from species to species. This is especially true for Ca^{2+} relative to other cations. In rat vesicle preparations Ca^{2+} binding is much less effective than Mn^{2+}, and is similar to, though still greater than, Ba^{2+}, Sr^{2+}, and Mg^{2+}. In contrast, in both rabbit and canine sarcolemmal vesicles Ca^{2+} binding is much closer to that found for Mn^{2+}, and is significantly greater than that observed for Ba^{2+}, Sr^{2+}, and Mg^{2+}.

Finally, these species specific differences are apparent in the relative ability of protons (H^+) to induce vesicle aggregation. When rabbit sarcolemmal vesicles were employed massive vesicle aggregation occurred when the pH of the suspension was reduced to 5.60–5.75 (Figs. 4 and 5). Under the same experimental conditions, rat vesicles aggregated at pH 6.0–6.2, and canine vesicle preparations at pH 4.8–5.0.

Taken together, the results obtained in this and our preceding study [22] indicate that the membrane surfaces of rat, rabbit, and canine cardiac sarcolemmal membranes can be characterized as sharing a general set of membrane properties, with respect to cation–membrane interactions. These include: (1) the presence of low-affinity cation binding sites on the vesicle surface, (2) the same selectivity series for cation binding, and (3) the finding that Ca^{2+}–sarcolemmal interactions seem to involve two, or more, interacting sites, one of which binds Ca^{2+}, and a second which modifies this Ca^{2+} binding as a function of pH, but does not itself bind Ca^{2+}. Our results also suggest however, that the specific molecular interactions involved vary from species to species in a very specific manner. Whether these interactions, and their variations, are due to compositional or organizational differences between the sarcolemmal membranes of rat, rabbit, and canine cardiac cells remains to be determined. Nonetheless, such species specific interactions could help to explain the large variability in transsarcolemmal ion transport activities observed from species to species, even though the membrane proteins themselves are the same in each cardiac cell. We are presently dismantling the sarcolemmal membrane in order to identify the molecules participating in these cation–sarcolemmal membrane interactions, and to quantitatively analyze their binding properties.

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