Effects of Cations, Phospholipases, and Neuraminidase on Calcium Binding to “Gas-Dissected” Membranes from Cultured Cardiac Cells

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SUMMARY. Sarcolemmal membranes prepared by ‘gas dissection’ from monolayers of cultured neonatal rat heart cells were studied with respect to their ability to bind calcium. Lanthanum displacement of calcium was 168 ± 7 nmol/mg sarcolemmal protein. This represents 3.21 mmol Ca/kg dry weight original cells on the basis of the measured membrane protein:dry cell weight ratio of 19.1 g/kg. Lanthanum-displaceable calcium from whole cells was essentially equal (3.32 mmol/kg dry weight), which indicates that all calcium displaceable from whole cells by lanthanum is localized to sarcolemmal sites. The potency of a series of divalent cations for calcium displacement from the sarcolemma was according to similarity of their crystal radii to that of calcium (cadmium > manganese > magnesium). This order was the same for the cations’ ability to displace calcium from whole cells and for their ability to uncouple excitation from contraction in neonatal papillary muscle. The membranes were treated with four enzymes: phospholipase A2, phospholipase C, phospholipase D, and neuraminidase. Phospholipase A2 and phospholipase D produced significantly increased calcium-binding. The increased binding secondary to phospholipase A2 treatment was eliminated by an albumin wash which was indicative of binding to the fatty acid product of hydrolysis. The increase after phospholipase D treatment can be attributed to an increase in phosphatidate, with attendant increase in net anionic charge on the membrane. The study indicates that at least 80% of rapidly exchangeable calcium in the cultured cells is sarcolemmal bound, that sarcolemmal-bound calcium is of central importance in maintenance of excitation-contraction coupling, and that anionic phospholipids probably account for a major portion of calcium binding within the sarcolemma of these cells. (Circ Res 53: 482-490, 1983)

IT IS becoming increasingly clear that calcium ion (Ca) bound to the sarcolemmal-glycocalyx complex is of considerable importance in the excitation-contraction coupling (ECC) sequence in the cardiac cell (Burt and Langer, 1982; Philipson and Langer, 1979; Philipson et al., 1980a). It therefore becomes important to learn more about the characteristics of this binding in terms of its relation to contractile function and the nature of the binding sites. Myocardial tissue culture has proven to be useful in these studies, since various probes (cations, enzymes) have direct access to the cellular surface and there are no interstitial binding sites to complicate analysis of cellular binding. Previous studies have examined the effects of various competing cations on Ca binding and exchange (Langer et al., 1974) and of neuraminidase and phospholipases on cation exchange and ultrastructure (Langer et al., 1981). These studies were done on whole, functional cells so that the effects on Ca binding at the sarcolemma, transsarcolemmal movement, and membrane permeability could not be unequivocally separated. The recent development of a technique for rapid isolation of a relatively pure sarcolemmal membrane from cardiac cells in culture (Langer et al., 1978) now permits the interventions applied to the whole cell listed above to be studied in the isolated sarcolemmal membrane fraction from these same cells.

The present study examines (1) the quantity of lanthanum ion (La)-displaceable Ca bound to the membranes with relation to the previously defined La-displaceable fraction from whole cells; (2) the ion affinity sequence for Ca-binding sites in the membrane, compared with the previously defined sequence in whole cells and other sarcolemmal preparations and (3) the effect of phospholipases and neuraminidase on Ca binding by the membranes, compared with previously defined effects of these agents in whole cells. The purpose of the study is to relate sarcolemmal Ca-binding characteristics to previously defined properties of Ca exchange and function in the intact cultured cardiac cell.

Methods

An isotopic monitoring technique has been developed which permits 45Ca exchange to be followed continuously on-line in monolayer myocardial cell cultures (Frank et al., 1977). This technique has been adapted to the measurement of 45Ca binding to sarcolemmal membrane derived from these monolayers (Langer et al., 1978).
Increased in the membrane preparations as compared to Pase, 1.7 ± 0.3 to 11.3 ± 2.3; Na,K-ATPase, 1.0 ± 0.3 to 24.6 ± 4.0. Activity of succinic dehydrogenase (μmol p-iodonitrotetrazolium violet/mg per hour) fell from 0.9 ± 0.2 to nondetectable levels, and protein decreased by over 100-fold. Therefore, sarcolemmal enzyme markers increased 7- to 15-fold in specific activity, no residual mitochondrial activity was detectable, and total protein decreased to less than 1% of the homogenate value.

To document further the small intracellular residuum after gas dissection, whole cell cultures grown on the scintillator disks were labeled with 42K, inserted into the flow cell (see below), and washed out in nonradioactive solution for 10 minutes to remove extracellular 42K, leaving only intracellular 42K in the whole cells (Frank et al., 1977). The cellular 42K activity was recorded, the disks placed in the chamber, and membranes made by gas dissection. The disks, with attached membranes, were reinserted in the flow cell and residual 42K activity recorded. The residual activity, representing retained intracellular material, in six preparations was less than 1%.

The technique permits instantaneous preparation of membranes from living cells in a form amenable to binding studies, with exposure only to inert N2 in the preparative process. The membranes remain active enzymatically, and there is evidence for little cellular contamination.

Membrane-Binding Studies

The membrane sheets are tightly adherent to the scintillator disks, although their configuration, as seen by transmission electron microscopy (Fig. 1), indicates that both inner and outer surfaces of the membrane are accessible to perfusate. 42Ca-binding studies are performed on the membranes by the same technique employed for cellular monolayers. This technique has been described previously in detail (Frank et al., 1977). Two disks with membranes attached are mounted so that they form the transparent walls of a flow cell, the membrane-covered surfaces facing inward. The flow cell is then inserted into the well of a modified Beta-Mate II spectrometer (Beckman Instruments) so that the disks are within 4.5 mm of the spectrometer's opposed photomultiplier tubes. Perfusate, as it passes through the flow cell, bathes the membranes.

The standard perfusate had the following composition (mM) (identical to that used for perfusion of whole cells, except for deletion of 0.3 mM Mg): NaCl, 133; KCl, 3.6; CaCl2, 1.0; glucose, 16.0; N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) buffer, 3.0 (pH 7.1). For isotopic studies 42Ca (ICN Corp.) was added at 1.0 μCi/ml (1.0 μCi/μmol 42Ca). Membranes were perfused at 24°C.

42Ca taken up by the cell layer counts with an efficiency of 44.4%. This efficiency is calculated by labeling the cells under conditions which produce a slow phase component of 42Ca exchange (Ponce Hornos et al., 1982). The flow cell is washed out to remove all noncellular radioactivity, and the counting level from the cells is recorded. The cells are then scraped from the disks, solubilized, and the solution counted in a scintillation spectrometer of known counting efficiency. The counting efficiency of the flow cell system is then calculated and is used to calculate labeled Ca content of the whole cells. The efficiency used for whole cells is, however, too low for application to the membranes. The absorption of 42Ca emission by the thin membrane layer is less than that for the whole cells and is measured by placement of known isotopic activity directly on the disk. This is measured to count at 15% greater
efficiency than that taken up by the whole cell layer, or 51.1%. Therefore, labeled cell content is calculated using a counting efficiency of 44.4%; membrane content is calculated using an efficiency of 51.1%.

Competing cations (La**, Cd**, Mn**, Mg**) were added to the standard perfusate at 0.5 mM concentration as their chloride salts. Phospholipases were obtained from Boehringer-Mannheim. Membranes were exposed to all enzymes for 30 minutes at 37°C. Phospholipase A$_2$ (PLA$_2$, Crotalus adamanteus) was used at 20 U/ml at pH 7.5 in standard perfusate; Phospholipase C (PLC, Bacillus cereus) at 4 U/ml at pH 7.1 in standard perfusate; Phospholipase...
D (PLD, cabbage) at 0.1 U/ml in sodium acetate 80 mM, 20 mM Ca at pH 5.6. PLA2 and PLC were previously shown to be active under these conditions and had less than 0.002 U of protease activity (Langer et al., 1981). The buffer conditions required of PLD were found not to effect Ca binding (see Results).

Neuraminidase (Worthington Biochem.) was a highly purified preparation (Hatton and Regoeczi, 1973). It was used at 0.25 U/ml concentration, which released 61% of cellular sialic acid within 15 minutes (Frank et al., 1977). The enzyme had no detectable proteolytic activity and very low phospholipase activity (< 0.003 U/ml) which has been shown to have no effect on whole cells (Frank et al., 1977).

Protein and Sialic Acid Assay

The membranes are scraped from the disks using 2 × 0.05 ml 1 N NaOH followed by 2 × 0.05 ml 1 N HCl. The "pooled scrapings" are then analyzed by the standard Lowry (1951) protein assay.

For sialic acid analysis, the membranes are scraped as described above and evaporated to dryness; then 0.1 ml H2O + 0.1 ml 0.2 M H2SO4 is added and heated at 80°C for 60 minutes to free bound sialic acid. After cooling, sialic acid is determined by the thiobarbituric analysis, according to the method of Warren (1959).

Experimental Sequence

Membranes on the disks in the flow cell were perfused with standard perfusate for 30 minutes before exposure to 45Ca. After addition of 45Ca, asymptotic labeling levels were achieved. Competitive cations (La+++ Cd++, Mn++, Mg++) were then added at 0.5 M concentration to the 45Ca-containing perfusate, and perfusion continued until a stable count level was achieved. The disks then were analyzed for protein content and displacement expressed as nmol Ca/mg protein.

In the enzyme experiments, the membranes on the disks were exposed to the enzymes for 30 minutes, the enzymes removed by washing with standard perfusate, the disks placed in the flow cell, and 4Ca uptake continued to asymptotic levels. The displacement by 0.5 mM La+++ was then measured, the membranes analyzed for protein, and the normalized displacement calculated.

Results

La-Displaceable Ca

Figure 2 illustrates the pattern of 45Ca uptake and La displacement in a whole cell monolayer and in gas-dissected membranes made from a similar monolayer. The patterns are very similar. The experiments shown were done with a flow rate of 10 ml/min through the flow cell, at which rate cellular or membrane exchange is perfusion limited. Lower perfusion rates are used to conserve isotope. Previous studies at perfusion rates up to 24 ml/min indicate a t1/2 of cellular 45Ca exchange of 9 seconds. Rates above 24 ml/min tend to dislodge the cells from the disks. It is likely that the rate of cellular or membranous exchange remains limited at the perfusion rates possible in this system.

To compare the quantity of Ca displaced from the membranes (Fig. 2B) with that displaced from whole cells, a membrane protein:dry cell weight ratio was measured. This was done by scraping whole cells from one-half the disk surface for cell dry weight. Gas-dissected membranes were then made from the cells attached to the remaining half, and their protein content was measured. For 25 such determinations, a value of 19.1 ± 1.8 (se) μg membrane protein/mg dry cell weight was calculated.

In the experiment illustrated, the La-displaceable 45Ca from whole cells (Fig. 2A) represented 3.92 mmol Ca/kg dry weight original cells. 45Ca displaced from the membranes (Fig. 2B) represented 189 nmol/mg membrane protein or 3.61 mmol Ca/kg dry weight original cells. In a series of 10 exper-
La displaced 168 ± 7 nmol/mg protein from the membranes. Using 19.1 μg membrane protein/mg dry weight original cells, this indicates 3.21 ± 0.13 mmol Ca/kg dry weight cells for the amount of Ca which is membrane bound and La displaceable. In a series of seven experiments in whole cells, 0.5 mM La displaced 3.32 ± 0.37 mmol Ca/kg dry weight cells—an amount virtually equal to that calculated from the membrane series.

Displacement by Other Cations

Figure 3 illustrates the displacement of membrane-bound Ca by 0.5 mM cadmium (Cd**), manganese (Mn**), and magnesium (Mg**), as compared to La**+. Displacement by Cd (166 ± 30 nmol/mg protein) was not significantly different from that by La. Mn displaced 68 ± 10 nmol/mg protein, significantly (P < 0.02) less than either Cd or La. Mg was still less potent—displacing 21 ± 4 nmol/mg protein, significantly (P < 0.025) less than Mn. Analysis of variance among La, Mn, and Mg indicates that the displacements are different at a high level of significance (P < 0.001).

The displacement amounts are plotted in Figure 3 vs. the crystal radii of the ions with the crystal radius of Ca indicated (0.99 Å). It is clear that displacement potency decreases as radius decreases significantly from that of Ca.

Effect of Enzymes

Phospholipase A2

After exposure of the membranes to 20 U/ml PLA2 for 30 minutes, La displaced 297 ± 25 nmol/mg protein, compared with 178 ± 16 nmol/mg protein from controls exposed only to buffer under otherwise identical conditions. This is a highly significant (P < 0.005) difference in La-displaceable Ca. Since PLA2 treatment liberates fatty acid, leaving lyso phosphatidylcholine vesicles, a series of membranes were washed with 1% albumin to remove the fatty acid product. This reduced the La displacement after PLA2 treatment to 138 ± 25 nmol/mg protein, not significantly different from control.

Phospholipase C

Exposure of membranes to 4 U/ml PLC for 30 minutes reduced La-displaceable Ca to 139 ± 19 nmol/mg protein or to 83% of control. This was not a significant (P > 25) reduction. The PLC used was derived from B. cereus. Its activity is negligible on pure phosphatidylcholine, phosphatidylserine, and sphingomyelin vesicles, slight on phosphatidyl-ethanolamine (14% hydrolysis), and very high (90% hydrolysis) on phosphatidylcholine vesicles (personal communication, Dr. K.D. Philipson). Therefore, the predominantly anionic, as opposed to the neutral, phospholipids are resistant to the action of PLC derived from B. cereus.

Phospholipase D

The sequence of 45 Ca-binding response and subsequent La displacement is illustrated in Figure 4. The sequence was the same for the other enzymes, which were all active in standard HEPES buffer. PLD, by contrast, requires a high Ca, low pH environment, and therefore the control series for this enzyme measured the effect of exposure of the membranes to 80 mM acetate, 20 mM Ca buffer at pH 5.6 for 30 minutes (Fig. 4A). After 30 minutes of exposure to this buffer, La-displaceable Ca was 172 ± 24 nmol/mg protein, or no different from the value in standard HEPES buffer. As shown in Figure 4B, 30 minutes of exposure to 0.1 U/ml PLD increased Ca binding. This pattern occurred in a total of 10 preparations. La-displaceable Ca increased by 56% to 269 ± 29 nmol/mg protein—a highly significant increase (P < 0.005).

Neuraminidase

The sialic acid content of the gas-dissected membranes was measured at 111 ± 19 nmol/mg protein. This is somewhat higher than the level obtained for highly purified sarcolemmal vesicles derived from rabbit heart by sucrose gradient technique (Philipson et al., 1980b). This is further evidence of the relative purity of the gas-dissected membranes.) Neuraminidase was used at a concentration of 0.25 U/ml, for 30 minutes. Previous evaluation of this enzyme's activity indicated that this concentration removed over 60% of cellular sialic acid within 15 minutes (Frank et al., 1977). La-displaceable Ca, after neuraminidase treatment, was 151 ± 8 nmol/mg—amount nonsignificant reduction of 10%.

Figure 5 summarizes the effect of the phospholipases and neuraminidase on the La-displaceable Ca from the gas-dissected membranes.
**FIGURE 4.** Part A: control sequence $^{45}$Ca uptake and La displacement: a 30-minute period of uptake in standard buffer; a 30-minute nonisotopic exposure to acetate buffer (80 mM acetate, 20 mM Ca, pH = 5.6) at 37°C; 30-minute exposure to $^{45}$Ca-labeled standard buffer; 30-minute exposure to 0.5 mM La in $^{45}$Ca-labeled standard buffer. Note the lack of significant effect on Ca uptake of the 30-minute exposure to acetate buffer. Part B: identical $^{45}$Ca uptake and displacement sequence as described in part A, except that acetate buffer contained 0.1 U/ml PLD. Note significant increase in Ca binding and large La displacement which followed exposure to PLD.
Localization of Exchangeable Ca

In 1979, Langer et al. demonstrated two distinct Ca compartments in cultured cells derived from neonatal rat heart and perfused with HEPES-buffered solution. Of a total 7.4 mmol Ca/kg weight cells, 58% remained unlabeled with 45Ca, despite prolonged isotopic exposure. The remaining 42% was exchanged as rapidly as the perfusate could be exchanged in the perfusion system (t1/2 = 10-20 seconds). Seldis et al. (1983) have recently shown, in culture derived from neonatal rat heart, no difference in the amount of 45Ca exchanged within 2 minutes as compared with 6 hours—therefore confirming compartmentalization into very rapidly and very slowly exchangeable fractions.

The labeled Ca within the rapidly exchangeable compartment of the cell is readily displaced by La+++ ion which has been conclusively shown not to penetrate beyond the sarcolemma of intact cells (Langer and Frank, 1972; Burton et al., 1977; Langer et al., 1979). La displaces between 80 and 90% of the rapidly exchangeable fraction (Langer et al., 1979; Burt and Langer, 1982). This suggested that this Ca was bound within the sarcolemmal-glycocalyx complex of the cell. The present results clearly indicate that such is the case. La-displaceable Ca from whole cells was measured to be 3.32 ± 0.37 nmol/kg dry weight. La displacement from gas-dissected membranes was 168 ± 7 nmol/mg protein. This represents 3.21 ± 0.13 mmol Ca/kg dry weight original cells, on the basis of the measured membrane protein:dry cell weight ratio of 19.1 g/kg. These values are essentially equal. Since gas dissection removes more than 99% of intracellular material, all cellular Ca displaceable by La is localized to the sarcolemmal-glycocalyx structure. When one considers that both surfaces of the gas-dissected membrane appear to be accessible to La and that Ca displacement by La from intact cells and membranes is essentially equal, it follows that La displaces Ca from the inner as well as the outer surface of the sarcolemma in the intact cell.

Further support for the sarcolemmal locus of La-displaceable Ca comes from comparison of 45Ca uptake and La-displacement patterns in whole cells and in gas-dissected membranes (see Fig. 2). The patterns of uptake and the response to La for whole cells and membranes are essentially identical and prove that a major fraction of the rapidly exchangeable Ca in these cells is membrane bound and displaced by La. This is not consistent with the interpretation of Barry and Smith (1982) that La displacement of Ca from whole cells is only an "apparent" one, due to a more rapid depression of Ca influx than efflux, with no evidence for a component of superficial La-displaceable Ca. The gas-dissected membranes used in the present study are capable of Ca binding only; i.e., no transsarcolemmal cellular exchange is present. The only possible action of La is displacement, and the amount is essentially equal to that found in whole cells. Barry and Smith (1982) state that superficially bound Ca in their cells (chick embryo culture) probably exchanges as fast as the extracellular fluid space. Since they wash their cells for 16 seconds after 45Ca labeling, it appears likely that most of the rapidly exchangeable bound 45Ca is removed before exposure to La, in their experiments.

It is of interest to compare the amount of La displaceable Ca from the gas-dissected membranes with values from other preparations. Tibbits et al. (1981) measured 155 nmol Ca/mg protein bound to sarcolemmal vesicles isolated from adult rat ventricle at 1.0 mM external [Ca]o. Isolated rat liver plasma membranes (Shlatz and Marinetti, 1972) bind considerably less (30 nmol/mg protein), and the binding is not affected by the presence or absence of Na. This is in contrast to cardiac sarcolemma where, at 1 mM external [Ca]o, decrease of [Na]o, from 140 to 0 mM increases Ca binding 3-fold in sarcolemmal vesicles prepared from rabbit ventricle (Philipson et al., 1980a). The point to be emphasized is that myocardial sarcolemma has a very large Ca-binding capacity. The value of 3.2 mmol/kg dry weight cells for membrane binding converts to 550 μmol/kg wet weight on the basis of the wet weight:dry weight ratio of 5.85 for these cells (Langer et al., 1979). This value is for cells in the presence of physiological levels of [Na]o. It represents more than 15 times the amount of Ca required for interaction at the contractile proteins to produce 90% activation of contractile force in dog ventricle, according to Solaro et al. (1974).

The magnitude of Ca binding (168 nmol/mg protein) essentially rules out significant contribution by possible sarcotubular contamination. Katz and Repke (1967) indicate maximal microsomal Ca binding (in absence of ATP, as in present study) of 8
nmol/mg protein, less than 5% that of the gas-dissected membranes.

**Displacement of Ca by Cations**

Figure 3 illustrates the Ca-displacing potency from gas-dissected membrane by divalent cations relative to La. In Figure 6, this sequence is compared to the cation’s ability to displace Ca from whole cultured cells and to their ability to uncouple excitation from contraction in neonatal ventricle (Langer et al., 1974) relative to La. It is evident that, in general, cations affect each of the parameters in the same way. Cd, the ion with its crystal radius the closest to Ca, is the most effective displacer and uncoupler, and Mg, with its radius farthest from Ca, is the least effective. This confirms the proposal (Bers and Langer, 1979) that Ca bound to sarcolemmal sites plays an important role in control of the amount of Ca available to the myofilaments and, thus, in the control of myocardial contractility.

It is of interest that the cation-displacement sequence for Ca in the gas-dissected cultured cell membrane is identical to that of inside-out vesicles made from the red blood cell (Cohen and Solomon, 1976). In this system, La and Cd were of greatest potency and essentially equal, Mn was intermediate, and Mg was better than only last-place Hg. This suggests that the membrane sites responsible for Ca binding may be common to a variety of tissues. In this respect, it is worth noting that the cation sequence demonstrated for Ca displacement and the uncoupling of excitation to contraction is identical to the sequence for the effect on the temperature of phospholipid phase transition (Rainier et al., 1979) and to the sequence for cation ability to enhance fusion of phospholipid vesicles (Liao and Prestegard, 1980). These relationships and the fact that phospholipids account for 80–85% of Ca binding to sarcolemmal vesicles extracted from rabbit heart (Philipson et al., 1980a) indicates an important role for these membrane constituents in the Ca-mediated control of myocardial force development.

**Enzyme Effects on Ca Binding**

Of the four enzymes applied to the gas-dissected membranes, two induced significant effect on Ca binding. PLA₂ cleaves the acyl group from the C2 position of phospholipids, leaving lysophosphoglyceride and a fatty acid. This enzyme produced no effect on either Ca or K permeability; neither did it cause any change in bilayer integral protein when applied to whole cells (Langer et al., 1981), but produced a significant increase in Ca binding (Fig. 5) in the present study. This increase in binding was, however, completely eliminated by an albumin wash (see Fig. 5) which indicated that the fatty acid product was responsible for the enhanced binding and not the formation of lysophosphoglyceride within the membrane. This is to be expected, since this alteration would produce no change in net anionic charge attributable to the phospholipid moieties.

Treatment with PLD increased La-displaceable Ca binding significantly—by 56%. PLD cleaves the nitrogenous base from the phospholipids, producing phospha tidic acid, and thereby increasing the net anionic charge on the membrane. If anionic phospholipids play a role in Ca binding, the demonstrated increase in binding with the PLD modification is to be expected. It is consistent with the proposal that anionic phospholipids play a significant role in binding, but, taken alone, does not prove the case.

PLA₂ cleaves the whole phosphorylated base from the C3 position. This decreased Ca binding by 17%, and this was not statistically significant (Fig. 5). If the enzyme had been effective on the anionic phospholipids, one might have expected a larger decrease in binding but the B. cereus PLA₂ used in the present study was found to have its major effect on neutral phosphatidylycerine and negligible effect on the natural anionic phospholipids. This suggests that, as might be expected, and as supported by the effect of PLD, the anionic phospholipids account for the major part of Ca binding to this class of molecules within the membrane. This is consistent with the finding of Philipson et al. (1980b) in which pure phosphatidyserine and phosphatidylinositol vesicles bound significantly more Ca, compared with phosphatidylethanolamine.

Although the present study shows little effect of B. cereus PLC on sarcolemmal Ca binding, our previous work on whole cells demonstrated that this enzyme caused marked increase of Ca and K permeability and marked aggregation of integral proteins within the lipid bilayer (Langer et al., 1981). Therefore, removal of the whole phosphorylated base...
from C3 of the neutral phospholipid has minimal effect on Ca binding, but has a major disruptive effect on selective Ca and K permeability.

Neuraminidase specifically cleaves terminal sialic acid from polysaccharides within the surface coat of the sarcotubule and from the external lamina. In the whole cell, this produces a specific increase in Ca permeability without change in K permeability and without change in bilayer integral protein pattern (Langer et al., 1978, 1981). Since sialic acid is a particularly acid sugar, we thought that, in addition to its role in control of Ca permeability, it might play a significant role in Ca binding at the cellular surface (Langer, 1976). However, Philipson et al. (1980b) showed that removal of sialic acid from sarcotubule vesicles had only minimal effect on Ca binding at physiological levels of [Ca]. The present results confirm this finding in the gas-dissected membrane preparation. Neuraminidase treatment caused a nonsignificant reduction in Ca binding of 10%. Therefore, it seems that sialic acid within the glycocalyx plays little role in Ca binding at the cellular surface, but plays a major role in the control of selective Ca permeability.

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References


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Hutton MWC, Regoeczi E (1973) A simple method for the purification of commercial neuraminidase preparation free from proteases. Biochim Biophys Acta 327: 144-120


Philipson KD, Bers DM, Nishimoto AY (1980b) The role of phospholipids in the Ca**+ binding of isolated cardiac sarcotubule. J Mol Cell Cardiol 12: 1159-1173


Schädt L, Marinetti GV (1972) Calcium binding to the rat liver plasma membrane. Biochim Biophys Acta 290: 70-83


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