Distribution of the Na^+-Ca^{2+} Exchange Protein in Mammalian Cardiac Myocytes: An Immunofluorescence and Immunocolloidal Gold-labeling Study

J. S. Frank,* G. Mottino,* D. Reid, R. S. Molday, and K. D. Philipson*

*Cardiovascular Research Laboratory, Departments of Medicine and Physiology, UCLA School of Medicine, Los Angeles, California 90024-1760; and Department of Biochemistry, University of British Columbia, Vancouver, B.C. V6T 1Z3, Canada

Abstract. The present study reports on the location of the Na^+-Ca^{2+} exchanger in cardiac sarcolemma with immunofluorescence and immunoelectron microscopy. Both polyclonal and monoclonal antibodies to the Na^+-Ca^{2+} exchanger were used. The mAb was produced from a hybridoma cell line generated by the fusion of mouse myeloma NS-1 cells with spleen cells from a mouse repeatedly immunized with isolated reconstituted canine cardiac Na^+-Ca^{2+} exchanger (Philipson, K. D. S. Longoni, and R. Ward. 1988. *Biochim. Biophys. Acta.* 945:298–306). The polyclonal antibody has been described previously and reacts with three proteins (70, 120, 160 kD) in cardiac sarcolemma associated with the Na^+-Ca^{2+} exchanger (Nicoll, D. A., S. Longoni, and K. D. Philipson. 1990. *Science (Wash. DC)*. 250:562–565). Both the monoclonal and the polyclonal antibodies appear to react with extracellular facing epitopes in the cardiac sarcolemma. Immunofluorescence studies showed labeling of the transverse tubular membrane and patchy labeling of the peripheral sarcolemma. The immunofluorescent labeling clearly delineates the highly interconnected T-tubular system of guinea pig myocytes. This localization of the exchanger to the sarcolemma, with an apparent high density in the transverse tubules, was also seen with immunoelectron microscopy. It is of great interest that the Na^+-Ca^{2+} exchanger, as the main efflux route for Ca^{2+} in heart cells, would be abundantly located in sarcolemma closest to the release of Ca^{2+}.

One of the most important functions of the cardiac sarcolemma is the control of Ca^{2+} movements. A major pathway for transmembrane flux of Ca^{2+} is via the Na^+-Ca^{2+} exchanger (15). The exchanger is the dominant mechanism of Ca^{2+} efflux from cardiac myocytes. Thus, its role in excitation–contraction coupling is significant (3, 10). The exchange activity of 3 Na^+ for 1 Ca^{2+} has been associated with 70, 120, and 160 kD proteins (16). Recent studies have reported the molecular cloning, expression and deduced amino acid sequence of the canine cardiac Na^+-Ca^{2+} exchange protein (13).

While Na^+-Ca^{2+} exchange activity is present in retinal rod outer segments (6), in brain synaptosomes (1) and in smooth and skeletal muscle sarcolemma, cardiac cell membranes are an especially rich source of activity. The capacity of the exchanger to transport Ca^{2+} across the sarcolemma in the heart is substantial with estimates of 100–150 μmol/kg wet wt/s (18). Biochemical and electrophysiological studies have produced estimates for the density of the Na^+-Ca^{+2} exchanger between 75-500/μm^2 (4, 7). Given that the exchanger is an essential link in excitation–contraction coupling in cardiac muscle, it is of interest to determine the location of the exchanger in the sarcolemma. In the present study we report on the location of the Na^+-Ca^{2+} exchanger in cardiac sarcolemma with immunofluorescence and immunoelectron microscopy.

Materials and Methods

Cell Isolation Procedure

Ca^{2+}-tolerant cells from guinea-pig and rat ventricles were obtained following the methods reported by Mitri and Morad (12). Briefly, the heart was rapidly excised and perfused in a retrograde manner in a modified Langendorff set-up with oxygenated solutions at 37°C. The heart was initially perfused with Ca^{2+}-free Tyrode's buffer (136 mM NaCl, 5.4 mM KCl, 0.33 mM NaH_2PO_4, 1.0 mM $MgCl_2$; 10.0 mM Hepes, pH to 7.4 with NaOH, 10 mM glucose) and then was digested with collagenase and Pronase (Boehringer Mannheim Biochemicals, Indianapolis, IN) for ~18 min or alternatively digested with collagenase alone. This was followed by perfusion with low Ca^{2+} buffer. The ventricles were then cut off and gently minced in buffer. The cells were exposed to a 1-mM Ca^{2+} buffer and were checked under the microscope for yield. This technique typically yields between 50 and 80% Ca^{2+} tolerant rod-shaped cells with normal electrical activity.

Antibody Production

mAb (C-2C12) was obtained from a hybridoma cell line generated by the fusion of mouse myeloma NS-1 cells with spleen cells from a mouse repeat-

© The Rockefeller University Press, 0021-9525/92/04/337/9 $2.00

edly immunized with 1 μg of isolated, reconstituted canine cardiac Na^+-Ca^{2+} exchanger (16) in Freund's adjuvant as previously described (12). Antibody screening was carried out by ELISA assays using detergent-solubilized canine sarcolemmal membrane proteins adsorbed to microtiter wells as the immobilized antigen with goat anti-mouse Ig-alkaline phosphatase as the secondary antibody. Positive clones were further screened for immunochemical specificity by Western blots using both the purified exchanger and sarcolemmal membranes. C-2C12 hybridoma cell line was cloned by limiting dilution. The mAb was classified as an IgM using a mouse monoclonal isotyping kit (Sigma Chemical Co., St. Louis, MO).

Immunoblots

Proteins from SDS-PAGE (7.5% gel) were transferred onto nitrocellulose for 30 min at 100 V in a Bio-Rad mini trans-blot apparatus. Immunoreactions were detected using goat anti-rabbit IgG for the polyclonal antibody (16) or goat anti-mouse IgG conjugated to HRP with DAB as substrate.

Indirect Immunofluorescent Labeling

Immunofluorescent labeling was performed on isolated guinea pig and rat myocytes (both fixed in 1% formaldehyde and unfixed) and on cryosections (5–8 μm) from rat papillary muscles. Isolated myocytes were kept in normal Tyrode's solution. If they were to be fixed, 1% buffered formaldehyde was added to the cells for 10 min. The fixed cells were quenched for aldehyde groups in Na borohydrate (0.2%) for 15 min. All tissue was kept in blocking solution (3% BSA, 5% goat serum) for 1 h. The cells and sections were incubated in monoclonal or polyclonal antibody to the Na^+-Ca^{2+} exchanger for 40–50 min. Antibody dilution for both polyclonal and monoclonal antibodies was varied between 1:10 and 1:50. After washing, the cells were incubated with fluorescein-labeled goat anti-mouse secondary antibody (IgA+IgG+IgM, Cappel 1:100 dilution) for 45 min at room temperature. The cells were washed several times in PBS and mounted on slides with mounting medium (which contained 90% glycerol plus 2% DABCO (1,4-diazabicyclo-(2,2,2)-octane), a photo bleaching inhibitor.

With the cryosections of rat papillary muscles the labeling steps were the same as for isolated cells with the exception that the sections were rinsed 12 times after each incubation.

Conventional fluorescence microscopy was carried out with a Nikon fluorescence microscope. Confocal fluorescence microscopy was carried out with a Nikon photomicroscope provided with a Lasersharp MRC-600 confocal fluorescence imaging system with argon laser for illumination (Bio-Rad Laboratories, Richmond, CA). The distribution of FITC-conjugated secondary antibody was visualized by illumination with a laser line at 488 nm. Photographs were taken from an attached photo-recording system.

Wheat Germ Agglutinin

Wheat germ agglutinin (WGA) coupled to FITC was purchased from Vector Laboratories (Burlingame, CA). Guinea pig myocytes were exposed to WGA-FITC (1:50 dilution) for 30 min. The cells were rinsed three times with normal Tyrode's solution and allowed to settle on a glass slide and covered with mounting solution.

Controls

For the polyclonal antibody, the control substituted the primary antibody with preimmune serum or PBS. For the mAb studies the control cells were incubated with the irrelevant monoclonal antikeyhole limpet hemocyanin at the same dilution and times as cells exposed to antibody to Na^+-Ca^{2+} exchanger. These controls were used since antigen (i.e., pure Na^+-Ca^{2+} exchange protein) to block labeling was not a feasible approach. The Na^+-Ca^{2+} exchange protein is 0.1% of the total myocardial sarcolemma protein (4) and has never been isolated in sufficient quantities for this type of control.

Immunogold Electron Microscopic Localization

For the immunolocalization at the electron microscopic level we used three techniques: (*a*) postembedding labeling on freeze-dried, Lowicryl (K_4M) sections (21); (*b*) pre-embedding labeling on ultra-thin cryosections according to most recent methods of Tokuyasu (24); and (*c*) a variation of the label-fracture technique of Pinto da Silva and Kan (2), which is described below.

Isolated rat papillary muscles were used for the preembedding labeling on cryosections and in the postembedding labeling on Lowicryl sections. Cryo-ultramicrotomy was performed on tissue infused with 2.3 M sucrose before being ultra-rapidly frozen in liquid helium. After immunolabeling the thin sections were embedded in a mixture of 0.2% uranyl acetate and 2% poly (vinyl alcohol) according to methods of Tokuyasu (24). Isolated guinea pig and rat myocytes were used for the label fracture studies. The concentration of antibodies used was 1:10 for the monoclonal, 1:25 for the polyclonal and 1:50, 10-nm gold, either as goat anti-mouse IgG+IgM (H+L) or goat anti-rabbit IgG (Amersham Corp., Arlington Heights, IL).

The variation of the label fraction technique was described by Zampighi et al. (26), but our use of this technique was complicated by the fact that we were using isolated cardiac myocytes instead of isolated membranes. The myocytes (unfixed and uncryoprotected) were allowed to settle on a precut glass coverslip that was made positively charged by immersing the glass in a solution of 0.1% Alcian blue. The myocytes, after settling on the glass, were carefully wicked of extra fluid and then covered with a previously cleaned and etched (nitric acid) copper hat. The cell monolayer gently sandwiched between the copper hat and glass coverslip was then frozen by immersion in liquid propane. Fracturing was accomplished at −150°C at a vacuum of 10^{-7} mbar by removing the copper hat with a single pass of the precooled knife (see Fig. 7). The fractured cells were shadowed with Pt-C at a 45° angle followed by carbon shadowing at 90° angle. The replicas were floated off the glass into distilled water. Occasionally, the replicas did not easily lift off the glass which necessitated the addition of dilute hydrofluoric acid (2.5%). The replicas were washed in distilled water and were then directly picked up on formar-coated grids (without digestion of membranes) and viewed in a JEOL 100 CX operating at 80 KV. This produced clean replicas with extensive areas of the fractured face of the sarcolemma.

Results

Characterization of mAb to the Na^+-Ca^{2+} Exchanger

Fig. 1 shows reactions of both the polyclonal (Fig. 1, lane *A*) and monoclonal (Fig. 1, lane *B*) antibodies to cardiac sarcolemma on immunoblots. The reaction patterns, including fine structure, are strikingly similar. The 120-kD protein band corresponds to the mature exchanger protein whereas the 70-kD protein is a proteolytic fragment and the 160-kD band represents nonreduced exchanger protein (13, 16). The

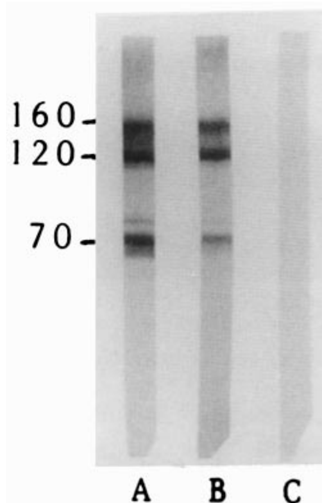
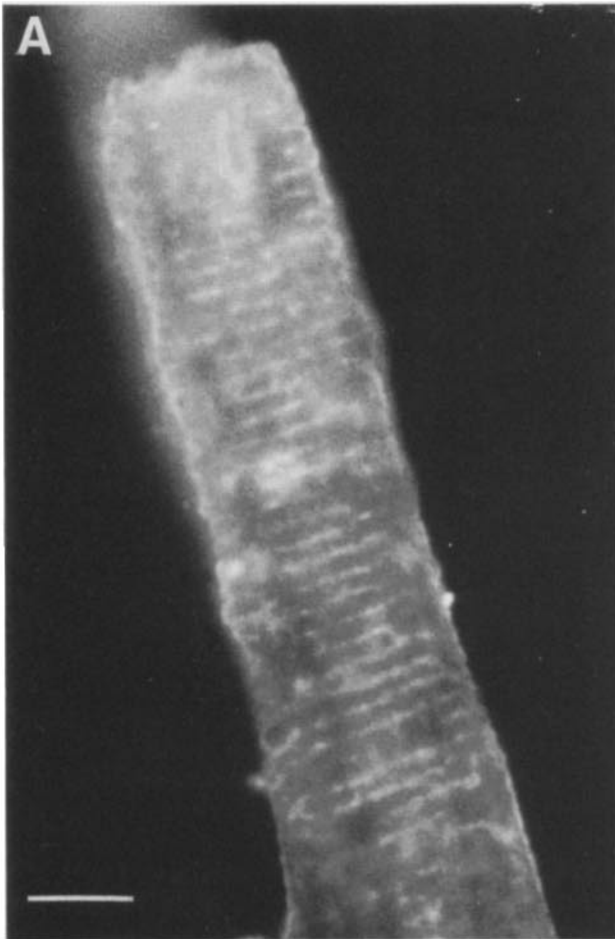
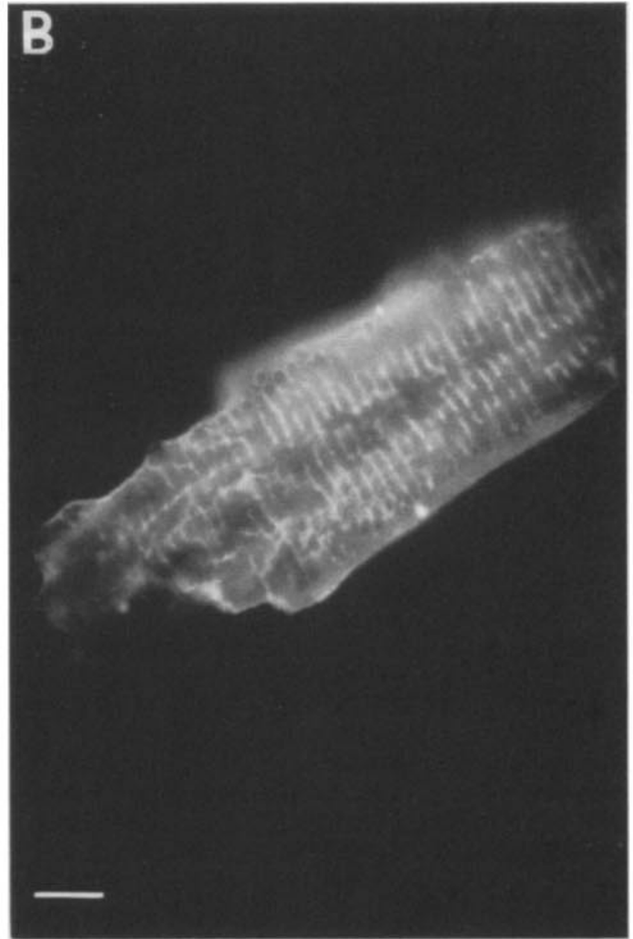


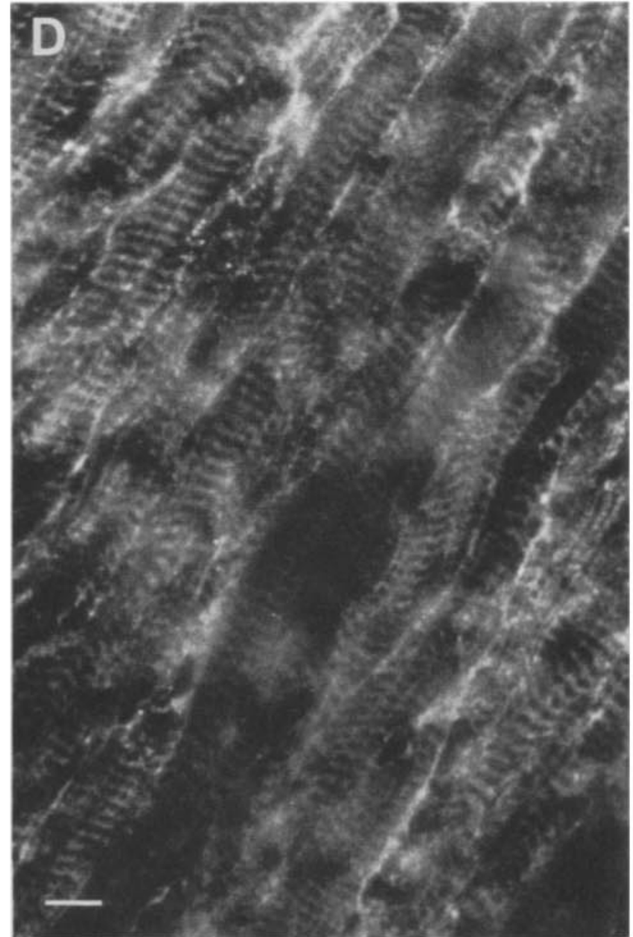
Figure 1. Immunoreactions of cardiac sarcolemma with anti-Na^+-Ca^{2+} exchange antibodies. Each nitrocellulose lane contains ~10 μg of blotted canine cardiac sarcolemmal protein. (*A*) Reactions with polyclonal antibody to the exchanger (1/1,000 dilution). (Lane *B*) Reactions with mAb (C-2C12) to the exchanger (1/7.5 dilution of cell culture supernatant). (Lane *C*) Reactions with mAb (PMe 1B3) (22) to the rod outer segment Na^+-Ca^{2+} exchanger (1/50 dilution of ascites fluid).

Figure 2. Cells in *A–C* are isolated guinea pig myocytes. *D* is a cryosection from rat papillary muscle. The cells in *A*, *B*, and *D* were exposed to mAb against the Na^+-Ca^{2+} exchange protein. The cell in *C* served as a control and was exposed to antikeyhole limpet hemocyanin. All the cells in this figure were exposed to goat antimouse FITC. The cells in *A*, *B*, and *D* demonstrate bright fluorescence in the T tubules. Bar, 10 μm.


A


B

C


D

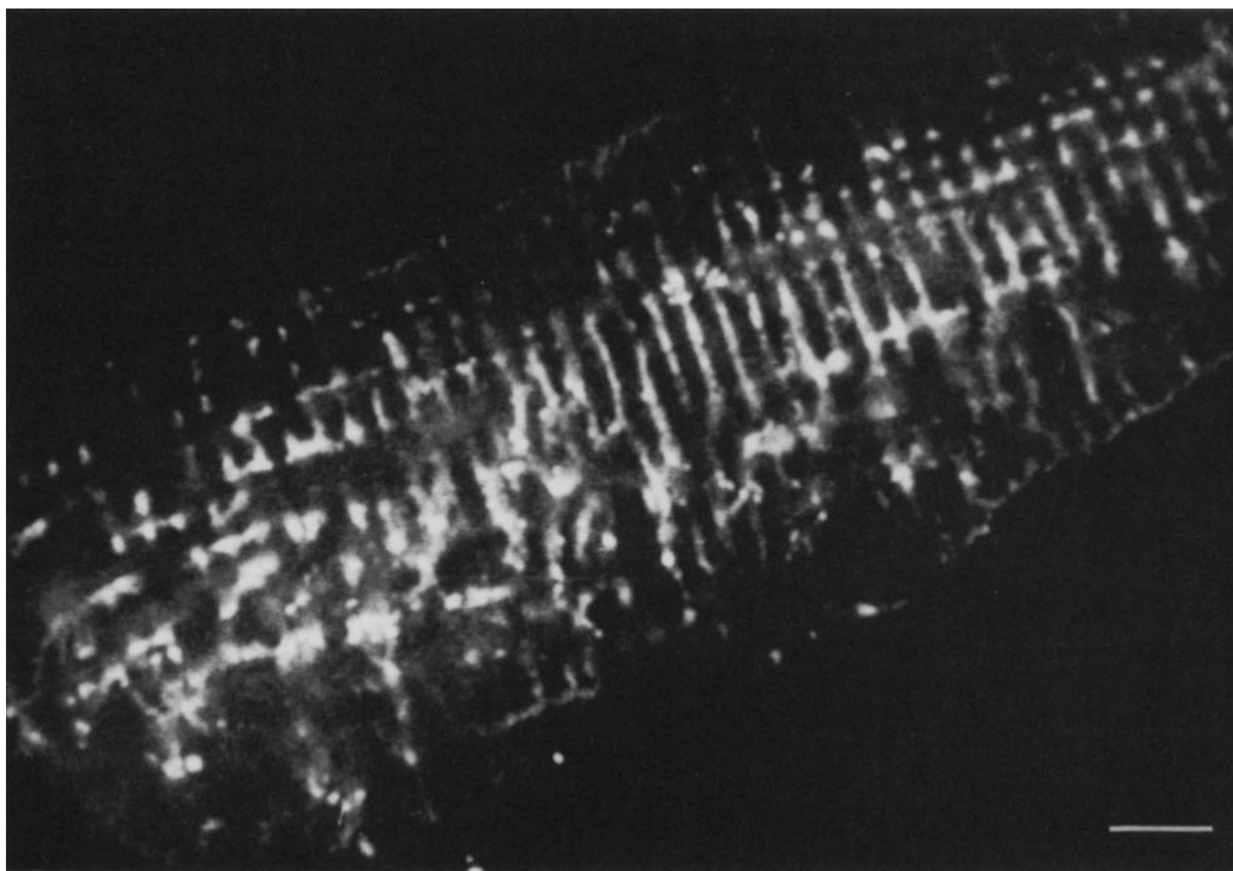


Figure 3. Typical confocal image of a plane through a guinea pig cell labeled with mAb against the Na^+-Ca^{2+} exchanger. The intense labeling of the transverse tubule system is evident. Bar, 5 μm.

results confirm that the mAb is directed against the exchanger and also demonstrates the high specificity of the polyclonal antibody. The polyclonal antibody has been described previously (13, 16) and has some extracellular epitopes (25).

We have previously described (16) the ability of the polyclonal antibody to immunoprecipitate the solubilized Na^2-Ca^{2+} exchanger. Using the same technique, the mAb was also able to immunoprecipitate exchange activity. Only 18% of exchange activity remained in solution following the identical immobilization procedure.

Immunofluorescent Labeling

Both the monoclonal and polyclonal antibodies appear to react with an epitope of the exchanger located on the extracellular side of the sarcolemma. Exposure of isolated myocytes to mAb followed by FITC secondary antibodies resulted in a very reproducible fluorescence staining pattern. Fig. 2 (*A* and *B*) shows a typical distribution of the mAb in guinea pig cells. Fig. 2 *D* is typical of the staining pattern for cryosections. Strong fluorescent lines appeared in a regular striated pattern which coincide with the sarcomeric Z-line pattern of the myofibrils. This is consistent with a distribution of mAb in the transverse tubular membrane of the myocytes. The immunofluorescent labeling appears to have a patchy distribution in the peripheral sarcolemma with areas of intense labeling on some portions of the membrane and little or no labeling in other areas. In contrast, the transverse tubular membrane always exhibited intense fluorescent labeling.

The specificity of the mAb for the exchanger is illustrated in Fig. 2 *C* where the myocyte was exposed to antikeyhole limpet hemocyanin mAb followed by FITC-labeled secondary antibodies. The absence of labeling is striking.

The immunofluorescent labeling with the polyclonal Ab was similar to that seen in Fig. 2 (*A* and *B*), however, nonspecific staining of background between the isolated myocytes required extensive rinsing which resulted in a considerable loss of myocytes. Isolated myocytes incubated with preimmune serum in place of the polyclonal antibodies were free of labeling.

Confocal Imaging of Na^+-Ca^{2+} Exchanger in Isolated Guinea Pig Cells

The distribution of immunofluorescent labeling in 0.5-μm optical sections of isolated myocytes was examined by confocal microscopy. Fig. 3 is a confocal micrograph taken through the center (6 μm from the surface of the cell) of an isolated guinea pig myocyte. It illustrates the intense labeling of the transverse tubules and sparse labeling of peripheral sarcolemma. Fig. 4 shows two images from a series of 58 confocal images taken at 0.25-μm increments through another cell. Fig. 4 *A* is taken at the cell surface, Fig. 4 *B* is 4 μm below this surface section. It is striking that in Fig. 4 *A*

Figure 4. Series of confocal images taken through a guinea pig myocyte labeled with mAb against the Na^{+}-Ca^{2+} exchanger. *A* is at the cell surface. The brightly labeled dots along the fiber are the T tubule openings. The patchy labeling on the peripheral sarcolemma appears to be in areas where there was cell to cell contact. Bar, 5 μm.

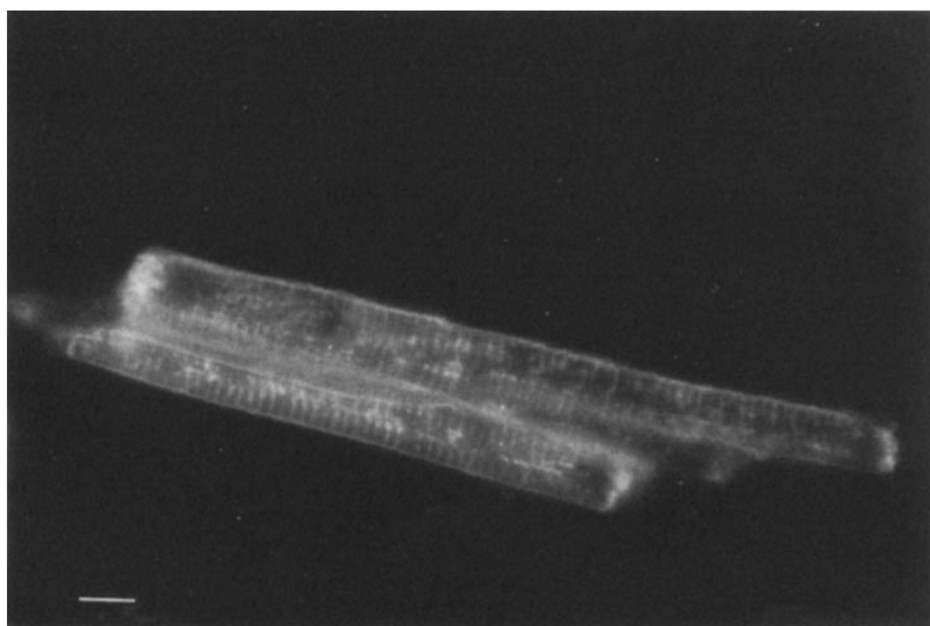


Figure 5. Photomicrograph of a guinea pig myocyte exposed to WGA-FITC. Fluorescent staining clearly labels the peripheral sarcolemma and the T tubules. Bar, 10 μm.

Figure 6. Immunogold labeling of the Na^+-Ca^{2+} exchange protein in ultra-thin cryosections of the rat papillary muscle. Labeling of a portion of the T tubular membrane with 10-nm gold particles is seen. TT, lumen of T tubule. Bar, 0.2 μm.

labeling of the cell membrane is present as discrete, very bright foci at the openings of the transverse tubules. The regular spacing between the intensely labeled foci in cells in both Figs. 3 and 4 is 2.0 μm. In addition to intense fluorescent labeling of the transverse tubular membrane and patchy labeling of the peripheral sarcolemma, 50% of the cells exhibited bright fluorescence at the ends of the cells or in steplike areas in the longitudinal region of the sarcolemma (see Fig. 4 *B*). These areas had presumably been sites where the membrane formed intercalated discs in the intact tissue.

WGA-FITC-labeled Myocytes

WGA does not have a preferential distribution in the peripheral or the T-tubular portion of the myocardial sarcolemma (26). This enabled a comparison between WGA-FITC-labeled isolated myocytes and the immunofluorescence seen in isolated myocytes exposed to the mAb to the Na^+-C^{2+} exchanger. Fig. 5 shows a WGA-FITC-labeled myocyte. The labeled cells have a clear fluorescent line around the periphery of the fiber in addition to T-tubular labeling. In myocytes labeled for the exchanger, this uninterrupted line of fluorescence in the periphery of the fiber was not seen.

Immunodetection by EM

The Na^+-Ca^{2+} exchange protein is only 0.1–0.2% of the total protein in the myocardial sarcolemma (4). The reactivity of the exchanger to antibodies was decreased by even low concentrations of glutaraldehyde. Immunocytochemical techniques that resulted in minimum handling of the myocardial cell gave the best labeling.

Post-embedding labeling, on sections from freeze-dried rat papillary muscles that were embedded at low temperature in Lowicryl K_4M, had almost no label.

Immunolabeling performed on ultra-thin cryosections of sucrose-infused ultra-rapidly frozen rat papillary muscle produced labeling that was consistent but sparse. Fig. 6 shows that typical labeling pattern that was seen. Immunogold, when present, was found predominantly on the circle of membrane forming the T tubules (Fig. 6). Labeling of the cell surface was again sporadic, with only a few gold particles along the peripheral sarcolemma.

The highest density of immunolabeling was seen after label-fracture. Here whole isolated myocytes were exposed to the antibodies (either mAb or polyclonal), then ultra-rapidly frozen and fractured. Minimal handling of the isolated cells took place. This technique allowed the visualization of immunogold, attached to external epitopes of the exchanger, over extensive areas of cell membrane. After fracturing, the gold-labeled outer half of the membrane remains attached to the Pt/C replica (see Fig. 7). The inner membrane half (P face) and the remainder of the cell falls away. The gold labeling is seen superimposed on the image of a freeze-fractured E face of the membrane. The result is the simultaneous observation of the gold label and the replica of the E face of the sarcolemma in one single coincident image (Fig. 8). The gold particles do not cast shadows since they are located between the supporting glass surface and the cell surface. As can be seen from Fig. 7, the fracture plane runs through the sarcolemmal bilayer and only allows visualization of the opening portion of the T-tubule sarcolemma. On the E face of the sarcolemma, replicas of T-tubules membrane, are displayed as broken off stumps projecting above their origin at the peripheral sarcolemma (see Figs. 7 and 8). In all replicas examined from over 50 cells, the gold label appeared to cluster on membrane forming the beginning of the T-tubules. This is clearly seen in Fig. 8. In contrast to the clustering of gold labeling at the membrane forming the T-tubular openings, gold was sparsely distributed over the peripheral region of the sarcolemma.

Discussion

There has been strong evidence for the existence of a Na^+-Ca^{2+} exchange mechanism in the surface membrane of cardiac muscle for over 20 yr (20). The exchanger plays a significant role in excitation-contraction coupling in cardiac muscle even through the exact details are still under investigation. Bridge et al. (3) recently showed the ability of the exchange to extrude Ca^{2+} from myocytes to produce relaxation. This demonstrates the exchanger can function as the major efflux pathway for Ca^{2+}.

The exchanger protein has been isolated, and the molecular cloning, expression, and amino acid sequencing of the protein has been achieved (13). These studies indicate that

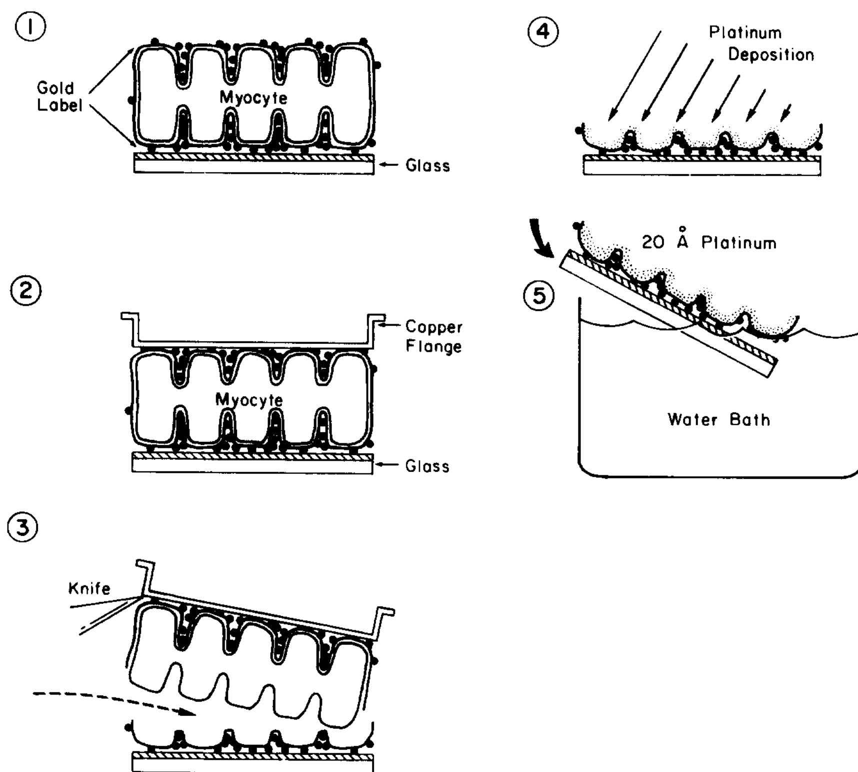


Figure 7. Diagram to illustrate the steps involved in label-fracture technique. (*1*) Cell has been labeled with Mab against the exchange protein followed by IgG-gold label. (*2*) Copper flange is placed on surface of the cell creating a sandwich which is rapidly frozen in liquid propane. (*3*) Frozen sandwich is fractured. The outer half of the SL attached to the Alcian blue glass remains adhered while the rest of the cell is lost. (*4*) Replica is formed from 20 A° platinum deposition. (*5*) Platinum replica with outer half of membrane and its bound gold label remains as a unit which is floated off the glass onto the water surface to be picked up on an electron microscopic grid.

the exchanger is an integral membrane protein. However, the distribution of the exchanger in the cardiac cell membrane has not previously been described. This is the first report on the distribution of the exchanger in cardiac myocytes using immunocytochemical labeling. The consistent and intense labeling of the transverse tubules with conventional and confocal immunofluorescent microscopy in both isolated and intact myocytes indicates a dense distribution of exchanger sites in the T tubules. There is an overall less intense labeling of the peripheral sarcolemma possibly indicating fewer exchanger sites in the periphery. Areas of intense labeling that did occur in the peripheral sarcolemma may correspond to areas where the cells were in close contact, possibly the intercalated disc area, including areas adjacent to gap junctions. In contrast, isolated guinea pig myocytes labeled with WGA-FITC, which binds to *N*-acetyl-D-glucosamine and sialic acid (2, 14) in the glycocalyx of the sarcolemma, both along the periphery of the cell and in the T tubules, produces uniform fluorescence along the entire cell surface (Fig. 5). Thus, it appears that the fluorescent labeling seen with antibodies to the Na^+-Ca^{2+} exchanger is not the result of the geometry of the T tubules but reflects the distribution of the exchanger. It is interesting that guinea pig atrial cells, which essentially lack a transverse tubular system, but do exhibit Na^+-Ca^{2+} exchanger activity, exhibit distinct and uniform immunofluorescence labeling along the surface sarcolemma (data not shown).

There may be some degree of species variability in the density of the exchanger sites between peripheral sarcolemma and the T tubules. Preliminary studies with isolated rabbit ventricular cells show more uniform immunofluorescence in the peripheral sarcolemma than seen in rat guinea pig cardiac myocytes. Further quantitative studies will need to resolve this issue.

Studies with immunoelectron microscopy showed a similar distribution of the Na^+-Ca^{2+} exchange protein as that seen at the light microscope level. Ultra-thin cryosections produce cross-sections of the T tubular membrane throughout the myocyte. Labeling was sparse such that not all T tubular profiles were labeled. As a result, quantitation of sites was not possible. However, when present, the label was found on the membrane profile of the tubules with occasional label on the cell surface.

Label-fracture of the isolated myocytes resulted in a high labeling efficiency and allowed visualization of immunogold label over large areas of sarcolemma. The disadvantage of label-fracture is that only a short segment of the T tubule membrane is visualized and the interior of the T tubules is not seen (see Fig. 7). In more than 50 cells, the gold labeling was most dense on the stumps of membrane originating from transverse tubules (Fig. 8) suggesting a greater density of sites at this location. An issue of concern is the possible movement of labeled components after fracture during thawing and washing. However, in previous label-fracture studies there has been no evidence that this occurs. Clear domain structure has been identified with this labeling technique that would not have occurred if there was post-fracture movement of gold label (5, 9, 17). The nonhomogeneous distribution of the exchanger, with domains of heaviest labeling in the T tubules was different from the label-fracture pattern we saw with other cell surface antigens (e.g., collagen IV; Frank, J. S., unpublished observation) and makes displacement after fracturing unlikely.

In the present studies, we are labeling a transmembrane protein that most likely partitions with the inner half of the membrane after fracturing. The fact that we had discrete sites of extracellular labeling indicates that the label remained in position even though it may have been freed from some

Figure 8. Label-fractured electron micrograph of the E face of the guinea pig myocyte. Clusters of gold particles are seen on the stumps of two T tubules. A few gold particles are seen in peripheral sarcolemma as well. Bar, 0.2 μm.

portion of the exchanger. A better understanding of the partitioning properties of the exchanger with fracturing is needed. It is possible that antibodies may alter the way the protein partitions in the membrane with freeze-fracture.

The apparent high density of Na^+-Ca^{2+} exchanger sites seen in this study in the T tubular membrane of ventricular myocytes may have profound significance in cardiac excitation-contraction coupling. Most of the junctional SR is found in association with T tubular membrane. The Ca^{2+} release channel is a component of the feet located in the junctional space between the T tubules and the SR (8, 22). It is of great interest that the Na^+-Ca^{2+} exchanger, as the main efflux route for Ca^{2+}, would be located in sarcolemma closest to the release of Ca^{2+}. This juxtaposition of transporters may influence the temporal and spatial distribution of Ca^{2+} transients. In addition, our observations provide ultrastructural support for the recent proposal by Leblanc and Hume (10) that a small amount of Ca^{2+} influx via Na^+-Ca^{2+} exchange can induce SR Ca^{2+} release. In this case, Ca^{2+} entering the cell across the T tubular membrane would be optimally located to open SR release channels.

In future studies it will be important to determine if the exchanger is randomly distributed in the T tubules or if it is localized to areas where the T tubular membrane forms junctions with the SR.

We thank R. Goodenough and Dr. J. Tormey for their assistance with the cryosectioning. We thank M. Kreman for his technical help with the label-fracture. We are also grateful to Michelle Wilhite for the poly (vinyl alcohol).

This work was supported by National Institutes of Health grants HL 28791-10, HL 27821, EY 02422, the Laubisch Fund, and the Medical Research Council of Canada.

Received for publication 18 June 1991 and in revised form 24 January 1992.

References

1. Barzilai, A., R. Spanier, and H. Rahamimoff. 1984. Isolation, purification and reconstitution of the Na^+ gradient-dependent Ca^{2+} transporter (Na^+-Ca^{2+} exchange) from brain synaptic plasma membranes. *Proc. Natl. Acad. Sci. USA.* 81:6521–6525.
2. Bhavanandan, V. P., and A. W. Kathi. 1979. The interaction of Wheat Germ agglutinin with sialoglycoproteins. *J. Biol. Chem.* 254:4000–4008.
3. Bridge, J. H. B., J. R. Smolley, and K. W. Spitzer. 1990. The relationship between charge movements associated with I_{Ca} and I_{Na-Ca} in cardiac myocytes. *Science (Wash. DC).* 248:376–378.
4. Cheon, J., and J. P. Reeves. 1988. Site density of the sodium-calcium exchange carrier in reconstituted vesicles from bovine cardiac sarcolemma. *J. Biol. Chem.* 263:2309–2315.
5. Chevelier, J., P. Pinto da Silva, P. Repoihe, R. Gobin, X. Y. Wang, J. Grossetete, and J. Bourget. 1985. Structural and cytochemical differentiation of membrane elements of the apical membrane of amphibian bladder epithelial cells. A label-fracture study. *Biol. Cell.* 55:181.
6. Cook, N. J., and U. B. Kaupp. 1988. Solubilization, purification and reconstitution of the sodium-calcium exchanger from bovine retinal rod outer

segments. *J. Biol. Chem.* 263:11382–11388.

7. Hilgemann, D. W., D. A. Nicoll, K. D. Philipson. 1991. Charge movements during Na^+ translocation by native and cloned cardiac Na^+-Ca^{2+} exchanger. *Nature (Lond.).* 352:715–718.
8. Inui, M., A. Saito, and S. Fleischer. 1987. Isolation of the ryanodine receptor from cardiac sarcoplasmic reticulum and identity with the feet structures. *J. Biol. Chem.* 262:15637–15642.
9. Kan, F. W. K., and P. Pinto da Silva. 1986. High resolution mapping of surface glycoconjugates in sperm cells: a label fracture study. *J. Histochem. Cytochem.* 35:1069.
10. Leblanc, N., and J. R. Hume. 1990. Sodium current-induced release of Ca from cardiac sarcoplasmic reticulum. *Science (Wash. DC).* 248:273–412.
11. MacKenzie, D., and R. S. Molday. 1982. Organization of rhodopsin and a high molecular weight glycoprotein in rod photoreceptor disc membranes using monoclonal antibodies. *J. Biol. Chem.* 257:7100–7105.
12. Mitri, R., and M. Morad. 1985. A uniform enzymatic method for dissection of myocytes from hearts and stomachs of vertebrates. *Am. J. Physiol.* 249:H1056–H1060.
13. Nicoll, D. A., S. Longoni, and K. D. Philipson. 1990. Molecular cloning and functional expression of the cardiac sarcolemmal Na^+-Ca^{2+} exchanger. *Science (Wash. DC).* 250:562–565.
14. Peters, B. P., S. Ebisu, I. J. Goldstein, and M. Flashner. 1979. Interaction of Wheat Germ agglutinin with sialic acid. *Biochemistry.* 18:5505–5511.
15. Philipson, K. D. 1990. The cardiac Na^+-Ca^{2+} exchanger. *In* Calcium and the Heart. G. A. Langer, editor. Raven Press, New York. 85–108.
16. Philipson, K. D., S. Longoni, and R. Ward. 1988. Purification of the cardiac Na^+-Ca^{2+} exchange protein. *Biochim Biophys. Acta.* 945:298–306.
17. Pinto da Silva, P., and F. W. K. Kan 1984. Label-fracture: a method for high resolution labeling of cell surfaces. *J. Cell Biol.* 99:1156.
18. Reeves, J. P., and K. D. Philipson. 1989. Sodium-calcium exchange activity in plasma membrane vesicles. *In* Sodium-Calcium Exchanger. T. J. A. Allen, D. Noble, and H. Reuter, editors. Oxford University Press, Oxford. 27-53.
19. Reid, D. M., Friedel, U., Molday, R. S., and Cook, N. J. Identification of the Na-Ca exchanger as the major ricin-binding glycoprotein of bovine rod outer segments and its localization to the plasma membrane. 1990. *Biochemistry.* 29:1601–1607.
20. Reuter, and Seitz. 1968. The dependence of calcium efflux from cardiac muscle on temperature & external ion composition. *J. Physiol.* 195:451–470.
21. Roth, J. 1986. Post-embedding cytochemistry with gold-labelled reagents. *J. Microsc.* 143:125–137.
22. Smith, J. S., T. Imagawa, K. P. Campbell, and R. Coronado. 1988. Purified ryanodine receptor from rabbit skeletal muscle is the calcium release channel of sarcoplasmic reticulum. *J. Gen. Physiol.* 92:1–26.
23. Stegemann, M., Meyer, R., Haas, H. G., and M. Robert-Nicoud. 1990. The cell surface of isolated cardiac myocytes. A light microscope study with use of fluorochrome-coupled lectins. *J. Mol. Cell Cardiolo.* 22:787–803.
24. Tokuyasu, K. T. 1989. Use of poly (vinylpyrsolidone) and poly (vinyl alcohol) for cryoultramicrotomy. *Histochem. J.* 21:163–171.
25. Vemuri, R., S. Longoni, and K. D. Philipson. 1989. Ouabain treatment of cardiac cells induces enhanced Na^+-Ca^{2+} exchange activity. *Am. J. Physiol.* 256:C1273–1276.
26. Zampighi, G. A., J. E. Hall, G. R. Ehring, and S. A. Simm. 1989. The structural organization and protein composition of lens fiber junctions. *J. Cell Biol.* 108:2255–2275.