Stimulation of Sodium-Calcium Exchange by Cholesterol Incorporation into Isolated Cardiac Sarcolemmal Vesicles*

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Modification of the cholesterol content of highly purified cardiac sarcolemma from dog ventricles was accomplished by incubation with phosphatidylcholine liposomes containing various amounts of cholesterol. The degree of cholesterol enrichment could be varied by changing the liposomal cholesterol/phospholipid ratio or varying the liposome-membrane incubation time. Na⁺-Ca²⁺ exchange measured in cholesterol-enriched sarcolemmal vesicles was increased up to 48% over control values. The stimulation of Na⁺-Ca²⁺ exchange was associated with an increased affinity of the exchanger for Ca²⁺ \((K_m = 17 \mu M)\) compared with \(K_m = 22 \mu M\) for control preparations. Na⁺-Ca²⁺ exchange measured in cholesterol-depleted membrane preparations was decreased by 15%. This depressed activity was associated with a decreased affinity of the exchanger for Ca²⁺ \((K_m = 27 \mu M)\). These changes were not due to either a change in membrane permeability to Ca²⁺ or an increase in the amount of Ca²⁺ bound to sarcolemmal vesicles. The stimulating effect of cholesterol enrichment was specific to the Na⁺-Ca²⁺ exchange process since sarcolemmal Ca²⁺-Mg²⁺ ATPase activity was depressed 40% by cholesterol enrichment. Further, K⁺-p-nitrophenylphosphatase and Na⁺-K⁺ ATPase were inhibited by cholesterol enrichment. It has been proposed that Na⁺-Ca²⁺ exchange is important in the regulation of intracellular Ca²⁺ concentration and, thus, force generation in the heart (1). However, the effect of cholesterol on cardiac sarcolemmal Na⁺-Ca²⁺ exchange is presently unknown. In view of (i) the capacity of cholesterol to alter the function of other sarcolemmal ion transport enzymes and (ii) the known dependence of the model content of isolated membranes from a variety of tissues has been shown to cause alterations in the activities of Na⁺-K⁺ ATPase (8-11) and Ca²⁺-Mg²⁺ ATPase (12, 13). Thus, precedents exist for the interaction of cholesterol with cardiac sarcolemmal enzymes.

Several observations support the possibility that membrane cholesterol may influence Na⁺-Ca²⁺ exchange. Cholesterol has a known capacity to alter the physical properties of a membrane (14). Cholesterol has also been shown to directly interact with membrane-bounded proteins (15, 16). Thus, if the Na⁺-Ca²⁺ exchange protein is sensitive to the membrane lipid environment, cholesterol may be important in regulating its function. Studies using doxyl group or amphiphile incorporation (17, 18), phospholipase (19), or saponin treatment (20) of the cardiac sarcolemmal membrane to alter its physical characteristics and composition have demonstrated a significant dependence of the Na⁺-Ca²⁺ exchange mechanism upon the membrane lipid environment. Thus, in view of (i) the capacity of cholesterol to alter the function of other sarcolemmal ion transport enzymes and (ii) the known dependence of the Na⁺-Ca²⁺ exchanger on the membrane lipid environment, it was likely that cholesterol may modify Na⁺-Ca²⁺ exchange. However, the effect of cholesterol on cardiac sarcolemmal Na⁺-Ca²⁺ exchange is presently unknown. In view of the proposed role for Na⁺-Ca²⁺ exchange in cardiac function, cholesterol modification of its activity has potential significance.

Our results demonstrated that incorporation of cholesterol into isolated cardiac sarcolemmal vesicles resulted in a significant stimulation of Na⁺-Ca²⁺ exchange. This stimulation was specific to Na⁺-Ca²⁺ exchange as the activities of both Ca²⁺-Mg²⁺ ATPase and Na⁺-K⁺ ATPase were inhibited by cholesterol enrichment. Oxidation of membrane cholesterol in situ resulted in a complete abolition of Na⁺-Ca²⁺ exchange. These data suggest that cholesterol plays an important role in the modulation of Na⁺-Ca²⁺ exchange activity.

EXPERIMENTAL PROCEDURES

Materials—All chemicals and reagents were purchased from Sigma. The alamethicin was kindly donated by R. L. Keene, The Upjohn Co. Deoxyribonuclease (DNase) was purchased from Cooper Biomedical Inc., Malvern, PA.

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**Vesicular Preparations**—Phosphatidylcholine and phosphatidylcholine-cholesterol liposomes were prepared using a modified method of Papahadjopoulos (21). Since it has been shown that the addition of 1 mol % of α-tocopherol protects against lipid peroxidation (22), a chloroform solution of phosphatidylcholine and mixtures of phosphatidylcholine with cholesterol was added, and the mixture was allowed to swell for 10 h at 37°C in the presence of 0.03 mol of α-tocopherol/100 mol of phospholipid were dried in the dark under a stream of nitrogen gas. All traces of chloroform were removed by further drying under vacuum for 12 h at room temperature. Buffer A (140 mM NaCl, 20 mM MOPS, pH 7.4) or buffer B (140 mM KCl, 20 mM MOPS, pH 7.4), 0.2 mol/mol of phospholipid, was added, and the mixture was subjected to ultracentrifugation at 41000 g for 10 min. ATPase activity measured in a medium containing 41000 g for 10 min. ATPase activity measured was rapidly diluted into a medium containing 41000 g for 10 min. ATPase activity measured the presence of 0.1 mM KCl, 1 mM MgCl2, 5 μM CaCl2, 0.3 μCi of 4CaCl2, 20 mM MOPS, pH 7.4, 37°C. The reaction was stopped by the addition of Na2EDTA and the final pellet resuspended in the appropriate medium (buffer A or B).

To ensure that fusion, adsorption, or entrapment of the liposomes with sarcolemma did not confound our results, sarcolemmal vesicles were treated as described with cholesterol-phosphatidylcholine liposomes prepared in the presence of 25°C. Cholesterol enrichment was performed by incubation of sarcolemmal vesicles with cholesterol-rich liposomes at 4°C overnight (16-18 h). Cholesterol-depleted sarcolemmal vesicles were incubated with a similar amount of both phosphatidylcholine liposomes. The liposome-sarcolemma suspensions were spun for 45 min in a Beckman TL-100 ultracentrifuge to remove large vesicles and undispersed lipid (23). The supernatant contained liposomes with cholesterol/phospholipid in proportions of 9:1 to a maximum of 5:1, which is near the upper limit reported for incorporation of cholesterol into phospholipid vesicles (24). The liposomes were used immediately. In the present study, liposomal cholesterol/phospholipid ratios indicate the initial proportion of cholesterol to phosphatidylcholine before incorporation of the sterol into phospholipid vesicles.

Sarcolemmal vesicles were prepared from canine left ventricles as described in detail previously (25). Cholesterol enrichment was performed by incubation of sarcolemmal vesicles with cholesterol-rich liposomes at 4°C overnight (16-18 h). Cholesterol-depleted sarcolemmal vesicles were incubated with a similar amount of both phosphatidylcholine liposomes. The liposome-sarcolemma suspensions were spun for 20 min at 104,000 × g in a Beckman TL-100 ultracentrifuge. The pellets were washed once and the final pellet resuspended in the appropriate medium (buffer A or B).

**Assay Procedures**—K+-dependent p-nitrophenylphosphate phosphatase activity was measured in 50 mM Tris, 5 mM MgCl2, 1 mM EGTA, 5 mM p-nitrophenylphosphate, and 20 mM KCl, pH 7.4, 37°C. The K+-dependent phosphatase activity measured in the same reaction medium without KCl was subtracted. The reaction volume was 1 ml and contained about 8 μg of sarcolemmal protein. The reaction was quenched after 7 min with 2 ml of 1 N NaOH, and the absorbance at 410 nm was used to determine the amount of p-nitrophenol liberated as Na-K adenosine triphosphatase (Na-K-ATPase) activity was assayed in a medium containing 50 mM Tris, 120 mM NaCl, 3.5 mM MgCl2, 1 mM EGTA, 5 mM Na3citrate, 100 mM KCl, 3 mM ATP, pH 7.0, at 37°C. The reaction volume was 1 ml, and about 8 μg of protein was used. The reaction time was 10 min, ATPase activity measured in a KCl-free mixture was subtracted. ATPase activity in the absence of K+ was the same as ATPase activity in the presence of K+ plus 2.5 μM digitoxigenin. Maximally stimulated ATPase activity was measured in the presence of 12.5 μg of alamethicin/ml. Inorganic phosphate liberated was measured by the method of Fiske and SubbaRow (26).

Na+-Ca2+ exchange was measured as the rate of Na+-dependent Ca2+ uptake as described elsewhere (27). Briefly, 0.005 ml of Na+-loaded (buffer A) sarcolemmal vesicles (1.2-1.5 mg of protein/ml) was rapidly diluted into 0.245 ml of Ca2+-free medium containing 0.03 mol of α-tocopherol and various Ca2+ concentrations. After the appropriate reaction time, the Ca2+ uptake was stopped by the addition of 0.03 ml of 140 mM KCl, 1 mM LaCl3 via a rapid quenching device, as described (27). Vesicles were retained on Sarstori cellulose nitrate filters (pore size = 0.45 μm) and washed with two 5 ml aliquots of 140 mM KCl, 0.1 mM LaCl3. Values were corrected for passive (Na+-independent) Ca2+ uptake and for bound Ca2+ by subtraction of blank values obtained using Ca2+ uptake medium which contained 140 mM NaCl instead of KCl. Previous studies on the time course of Na+-dependent Ca2+ uptake have shown the exchange to exhibit linearity to about 5 s (28).

**RESULTS**

**Modification of Sterol Content of Sarcolemmal Vesicles**—Cardiac sarcolemmal vesicles were incubated at 4°C for various periods of time (0-18 h) in the presence of phosphatidylcholine or cholesterol/phosphatidylcholine liposomes (cholesterol/phospholipid ratio of 2:1 mol/mol). Portions of the mixtures were removed at various times and centrifuged at 140,000 × g for 20 min to separate the treated sarcolemmal vesicles from the liposomal vesicles. Previous studies (34) have shown that one centrifugal step is adequate to cleanly separate liposomes from microsomal vesicles. In order to be absolutely sure of this separation, two centrifugal steps were employed in the present study. To further ensure that any recovered sterol associated with the enriched sarcolemma represented incorporation into the membrane and not entrapment of the liposomes, sarcolemmal vesicles were cholesterol-enriched using [14C]cholesterol containing liposomes. Less than 0.5% of the initial [14C]cholesterol was associated with sarcolemmal vesicles incubated with either a liposome-free [14C]cholesterol medium or a suspension of liposomes containing [14C]cholesterol.

Fig. 1 shows a typical sterol modulation experiment. After a 16-18 h incubation with pure phosphatidylcholine vesicles, a 17% depletion in sarcolemmal membrane cholesterol content was observed. The depletion of cholesterol was time-dependent but relatively slow. Incubation of sarcolemma with liposomes rich in cholesterol (2:1 mol/mol cholesterol/phos-
The enrichment of membrane vesicles was performed by incubation of sarcolemma with 2:1 cholesterol/phospholipid liposomes at 4 °C. Cholesterol-depleted membrane preparations were obtained by incubation of sarcolemmal vesicles with cholesterol-free phospholipid liposomes at 4 °C. See "Experimental Procedures" for details. These data represent the results of a typical experiment.

Fig. 1. Time dependence of cholesterol incorporation and depletion in sarcolemmal membrane vesicles. Cholesterol enrichment of membrane vesicles was performed by incubation of sarcolemma with 2:1 cholesterol/phospholipid liposomes at 4 °C. Cholesterol-depleted membrane preparations were obtained by incubation of sarcolemmal vesicles with cholesterol-free phospholipid liposomes at 4 °C. See "Experimental Procedures" for details. These data represent the results of a typical experiment.

Fig. 2. Effect of liposomal cholesterol/phospholipid ratio on cholesterol content of sarcolemma. Sarcolemmal vesicles were incubated at 4 °C for 16-18 h with liposomes prepared with various cholesterol/phospholipid ratios. Ratios indicated represent the mol fraction of initial lipid composition before sonication. See "Experimental Procedures" for details. Results are expressed as the average ± S.E. percent increase in cholesterol content over controls (control cholesterol content = 0.49 ± 0.06 μmol/mg of protein). (n = 5-7 except for bar at 1.5:1.0 cholesterol/phospholipid which is the mean of two experiments.) *p < 0.05 versus control values.

In a further attempt to control the amount of cholesterol transferred, sarcolemmal vesicles were incubated with liposomes prepared with increasing proportions of cholesterol to phospholipid. As shown in Fig. 2, maximal cholesterol enrichment was accomplished using liposomes prepared with an initial cholesterol/phospholipid ratio of 2:1. Less cholesterol transfer occurred when liposomes of lower cholesterol/phospholipid ratios were used.

Effects of Cholesterol on Ion Transport Activity—The effects of altering membrane cholesterol content on Na⁺-Ca²⁺ exchange were monitored during its linear phase (1.5 s). Enrichment of sarcolemmal membranes with cholesterol resulted in a stimulation in Na⁺-Ca²⁺ exchange activity (Fig. 3). With an increase in cholesterol of 64% above control, there is a corresponding 30% increase in Na⁺-Ca²⁺ exchange activity. Cholesterol depletion resulted in a 15% decrease in exchange activity. Modification of sarcolemmal cholesterol content with liposomes of intermediate cholesterol content resulted in intermediate effects on Na⁺-Ca²⁺ exchange.

Fig. 3. Effect of liposomal cholesterol/phospholipid ratio on Na⁺/Ca²⁺ exchange. Sarcolemmal vesicles were incubated at 4 °C for 16-18 h with liposomes prepared with various cholesterol/phospholipid ratios. Ratios indicated represent the mol fraction of initial lipid composition before sonication. The Na⁺-dependent Ca²⁺ uptake reaction proceeded for 1.5 s at [Ca²⁺] = 10 μM. Control Na⁺/Ca²⁺ exchange was 2.3 ± 0.5 nmol of Ca²⁺ accumulated/mg/s. See "Experimental Procedures" for details. Values represent means ± S.E. percent of control (n = 5). *p < 0.05 versus control values.

The dependence of the initial rate of Na⁺-dependent Ca²⁺ uptake on Ca²⁺ concentrations in control, cholesterol-depleted, and cholesterol-enriched sarcolemmal membrane preparations was examined as a function of assay reaction time (Fig. 4). Similar to the results presented in Fig. 3, enrichment of membrane vesicles with cholesterol resulted in a 25% increase in the initial rate of exchange (1.5 s). At longer incubation times, the cholesterol-enriched membrane vesicles exhibited a 48% greater Ca²⁺ accumulating ability than control vesicles. No significant differences from control were observed in cholesterol-depleted vesicle preparations.

Fig. 5 presents the results from several experiments relating Na⁺-Ca²⁺ exchange to the sarcolemmal membrane cholesterol content. The exchange shows a positive correlation with cholesterol content (r = 0.7). The dependence of the initial rate of Na⁺-dependent Ca²⁺ uptake on Ca²⁺ concentrations in control, cholesterol-depleted, and cholesterol-enriched membranes is shown as a Lineweaver-Burk plot in Fig. 6. The Km for the control preparations was found to be 22 μM which is similar to that...
Cholesterol Effects on Sodium-Calcium Exchange

Since the observed alteration in the Ca\textsuperscript{2+} accumulating abilities of the isolated sarcolemmal vesicles may be due to changes in the Ca\textsuperscript{2+} permeability of these vesicles, it was important to examine passive Ca\textsuperscript{2+} efflux from control, cholesterol-enriched, and cholesterol-depleted membrane preparations after loading the vesicles with \textsuperscript{45}Ca\textsuperscript{2+}. No differences were observed in passive membrane permeability of treated vesicles compared with control preparations (Fig. 8).

It has been suggested that sarcolemmal bound calcium may be involved in the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange process (38). Therefore, it was imperative that the effect of modification of membrane cholesterol content on energy-independent Ca\textsuperscript{2+} binding to sarcolemmal vesicles be investigated. As shown in Table I, no significant differences were observed in the amount of Ca\textsuperscript{2+} bound at any Ca\textsuperscript{2+} concentration examined.

Table II shows the effect of cholesterol enrichment and depletion on Na\textsuperscript{+}-K\textsuperscript{+}-ATPase and K\textsuperscript{+}-p-nitrophenophosphatase activities of control, cholesterol-enriched, and cholesterol-depleted membrane preparations. A small reduction in the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in cholesterol-depleted membrane preparations was observed. The same qualitative response was observed in cholesterol-enriched membranes although the reduction in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was not as marked. This inhibitory trend persisted in the presence or absence of alamethicin.

reported by others (35–37). Cholesterol enrichment of sarcolemmal vesicles increased the apparent affinity of the exchange mechanism for Ca\textsuperscript{2+} ($K_m = 17$ \textmu M), while cholesterol depletion had the opposite effect ($K_m = 27$ \textmu M). The $V_{max}$ of the exchange mechanism for control, cholesterol-depleted, and cholesterol-enriched membrane vesicles was 7.9, 7.4, and 8.0 nmol/mg/s, respectively.

For comparative purposes, the effect of cholesterol enrichment on the sarcolemmal Ca\textsuperscript{2+} pump (Ca\textsuperscript{2+}-Mg\textsuperscript{2+}-ATPase) is shown in Fig. 7. The Ca\textsuperscript{2+} accumulating ability of the Ca\textsuperscript{2+} pump is inhibited 40% by cholesterol enrichment at all incubation times.

TABLE I

<table>
<thead>
<tr>
<th>Calcium concentration</th>
<th>Calcium bound</th>
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<tbody>
<tr>
<td>Control membranes</td>
<td>Cholesterol-depleted membranes</td>
</tr>
<tr>
<td>mm</td>
<td>nmol/mg protein</td>
</tr>
<tr>
<td>0.05</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td>0.50</td>
<td>38.7 ± 2.0</td>
</tr>
<tr>
<td>1.00</td>
<td>65.4 ± 2.1</td>
</tr>
<tr>
<td>2.50</td>
<td>132.2 ± 4.2</td>
</tr>
</tbody>
</table>

Fig. 5. Na\textsuperscript{+}-dependent Ca\textsuperscript{2+} influx rate as a function of membrane cholesterol/phospholipid ratio. Sarcolemmal membrane vesicles were incubated overnight with liposomes of various cholesterol/phospholipid ratios. See "Experimental Procedures" for details. Ca\textsuperscript{2+} uptake occurred for 1.5 s at [Ca\textsuperscript{2+}] = 10 \textmu M.

Fig. 6. Lineweaver-Burk plot of the Ca\textsuperscript{2+} dependence of Na\textsuperscript{+}-dependent Ca\textsuperscript{2+} influx in control, cholesterol-depleted, and cholesterol-enriched membrane preparations. Time for Ca\textsuperscript{2+} uptake was 1.5 s (n = 3).

Fig. 7. ATP-dependent Ca\textsuperscript{2+} uptake in control and cholesterol-enriched sarcolemma vesicles. See "Experimental Procedures" for details (n = 4). * $p < 0.05$ versus control values.

Fig. 8. Time dependence of passive Ca\textsuperscript{2+} efflux. Control, cholesterol-depleted, and cholesterol-enriched membrane vesicles were first loaded with Ca\textsuperscript{2+} by Na\textsuperscript{+}-dependent Ca\textsuperscript{2+} influx for 2 min in 0.25 ml of uptake medium. After 2 min, 0.25 ml of EGTA solution was added, and Ca\textsuperscript{2+} efflux was allowed to proceed for various times. See "Experimental Procedures" for details. The data are presented as the percent of the initial Ca\textsuperscript{2+} load remaining in the membrane vesicles. The initial Ca\textsuperscript{2+} loads were: control, 32 ± 3 nmol/mg protein; cholesterol-depleted, 34 ± 1 nmol/mg protein; cholesterol-enriched, 37 ± 1 nmol/mg protein (n = 3).

TABLE I

Effect of cholesterol depletion and enrichment on energy-independent Ca\textsuperscript{2+} binding to isolated sarcolemmal membranes

Cholesterol depletion involved overnight incubation of sarcolemmal vesicles with cholesterol-free phospholipid liposomes, and cholesterol enrichment was performed by incubating membrane vesicles with 2:1 cholesterol/phospholipid liposomes. See "Experimental Procedures" for Ca\textsuperscript{2+} binding protocol. Values are expressed as means ± S.E. (n = 6).
Cholesterol Effects on Sodium-Calcium Exchange

**TABLE II**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Control membranes</th>
<th>Cholesterol-depleted membranes</th>
<th>Cholesterol-enriched membranes</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺-pNPPase activity</td>
<td>23.0 ± 1.9</td>
<td>22.4 ± 3.5</td>
<td>22.5 ± 1.3</td>
</tr>
<tr>
<td>% of control</td>
<td>97.8 ± 14.0</td>
<td>98.8 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Na⁺⁺-K⁺⁺-ATPase activity</td>
<td>79.1 ± 2.3</td>
<td>58.3 ± 2.3*</td>
<td>65.3 ± 3.1*</td>
</tr>
<tr>
<td>% of control</td>
<td>73.8 ± 2.7</td>
<td>82.4 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Na⁺⁺-K⁺⁺-ATPase activity + alamethicin</td>
<td>213.8 ± 9.5</td>
<td>189.6 ± 12.4</td>
<td>199.0 ± 8.8</td>
</tr>
<tr>
<td>% of control</td>
<td>84.5 ± 4.6</td>
<td>92.6 ± 3.8</td>
<td></td>
</tr>
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</table>

*p < 0.05 versus control values.

**DISCUSSION**

Incubation of cholesterol-rich liposomes with cardiac sarcolemmal vesicles resulted in a net transfer of cholesterol from liposomes to membrane vesicles. The amount of cholesterol transfer could be controlled by varying the vesicle-liposome incubation time or by altering the liposomal cholesterol/phospholipid ratio. Several previous studies have shown that cholesterol moves between vesicles by transfer through the aqueous phase and not via fusion between the donor and acceptor vesicles (39–41). In this study, experiments involving the incubation of sarcolemma with [¹⁴C]sucrose containing liposomes and phospholipid profiles of the control and cholesterol-enriched membrane preparations (data not presented) indicated that fusion between donor liposomes and sarcolemmal vesicles was not a significant problem. These observations suggest that neither adsorption of liposomes to membrane nor co-sedimentation of liposomes confounded our results.

The present results support an important role for membrane cholesterol in sarcolemmal Na⁺⁺-Ca⁺⁺ exchange. Cholesterol enrichment of sarcolemmal vesicles resulted in a significant increase in the initial rate of Na⁺⁺-Ca⁺⁺ exchange (Fig.

**FIG. 10. Effect of cholesterol oxidase and H₂O₂ treatment on Na⁺⁺-Ca⁺⁺ exchange.** A, Na⁺⁺-dependent Ca⁺⁺ influx in untreated membrane vesicles. B, exchange activity in cholesterol oxidase (4 units/mg protein)-treated sarcolemma. C, exchange activity in cholesterol oxidase (2 units/mg protein)-treated sarcolemma. D, membrane treated with 50 μmol of H₂O₂/mg of protein. E, membrane treated with 50 μmol of H₂O₂/mg of protein in the presence of 300 units of catalase/mg of protein. F, membrane treated with cholesterol oxidase in the presence of 300 units of catalase/mg of protein. See “Experimental Procedures” for details. Data are the average of two experiments.

**FIG. 9. Illustration of two-dimensional thin layer chromatographic plates of cholesterol oxidase-treated and control sarcolemmal vesicles.** A, cholesterol oxidase-treated sarcolemma; B, control sarcolemma. a, origin; b, unidentified oxidation product; c, native cholesterol; d, cholest-4-en-3-one; e, unidentified oxidation product; f, solvent front materials.

To obtain further information regarding the nature of the cholesterol-modulating effect on Na⁺⁺-Ca⁺⁺ exchange, the effects of cholesterol modification in situ were examined. Cardiac sarcolemmal vesicles were treated with cholesterol oxidase which converts cholesterol to several oxidized derivatives, one of which has been identified by us to be cholest-4-en-3-one, as shown in Fig. 9. The results of a typical oxidation experiment are shown in Fig. 10. On treatment with cholesterol oxidase, Na⁺⁺-Ca⁺⁺ exchange was virtually eliminated. Since 1 mol of H₂O₂ is generated per mol of cholesterol oxidized, it was imperative to distinguish the effects of membrane peroxidation from the direct effects of cholesterol modification. Oxidation of all membrane cholesterol with cholesterol oxidase would yield 0.5 μmol of H₂O₂/mg of sarcolemmal protein. As shown in Fig. 9, no change in Na⁺⁺-Ca⁺⁺ exchange activity was observed on treatment of sarcolemmal membrane with 0.05 μmol of H₂O₂/mg of protein. With increased H₂O₂ (50 μmol/mg of protein), a 30% reduction in enzyme activity was observed. This decrease in Na⁺⁺-Ca⁺⁺ exchange activity caused by 50 μmol of H₂O₂/mg of protein was prevented by the inclusion of catalase (300 units/mg of protein) in the reaction mixture. Catalase, however, did not protect the membrane vesicles from the inhibitory effects of cholesterol oxidase on the Na⁺⁺-Ca⁺⁺ exchanger.
In view of the well recognized association of cholesterol with cardiovascular disease, it is reasonable to hypothesize that cholesterol may directly alter myocardial viability through its effects on sarcolemmal ion transport. Since Na+-K+-ATPase is inhibited with cholesterol enrichment, intracellular Na+ concentrations may increase in the intact cell, favoring a gain of Ca2+ through elevated Na+-Ca2+ exchange. Coupled with depressed Ca2+ pumping ability, intracellular Ca2+ overload may develop. In this regard, cholesterol accumulation in the myocardium has been associated with altered Ca2+ homeostasis and cardiac dysfunction (7, 45).

REFERENCES