ALTERATIONS IN MEMBRANE Na^+-Ca^{2+} EXCHANGE IN THE AGING MYOCARDIUM

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ABSTRACT

Heart failure is a common event in the elderly. Although it is generally believed that age-induced biochemical alterations of the myocardium are directly involved in the heart failure usually encountered in the elderly, several gaps still exist in our understanding of the mechanisms involved in the contractile failure of the aged myocardium. Virtually nothing is known, for example, about the integrity of the sarcolemmal (SL) Na^+-Ca^{2+} exchange mechanism in the aging heart. In this study we have examined the status of Na^+-Ca^{2+}-exchange in SL membranes from the aging myocardium. Male Sprague Dawley rats were used and were divided into 3 groups: young (4-6 months old); middle aged (14-17 months) and old age (24-27 months). Purified SL membranes were isolated from ventricular tissues. Ca^{2+}-influx of Na^+-loaded vesicles from old hearts was depressed relative to the middle-aged group, which in turn was lower than the Ca^{2+} accumulated by vesicles from young hearts. These changes were observed at different concentrations of Ca^{2+} and at different times of incubation. The results suggest that Ca^{2+} transport by SL Na^+-Ca^{2+} exchange mechanism is attenuated in the aging myocardium and might therefore be involved in age-induced heart failure.

INTRODUCTION

Heart failure is a common event in the elderly (1). In search of a specific biochemical lesion to account for the depressed performance of the aged myocardium, a variety of biochemical defects has been seen in different experimental models. Although some of these findings appear to be conflicting and contradictory (1-3), it is generally believed that age-induced biochemical alterations of the myocardium are directly involved in the heart failure commonly encountered in the elderly (4-6). Despite these observations several gaps still exist in our understanding of the mechanisms involved in the contractile failure of the aged myocardium. Virtually nothing is known, for example, about the status of the sarcolemmal Na^+-Ca^{2+}-exchange mechanism in the aging heart even though its presence has been demonstrated in that subcellular organelle (7-10). In this study, therefore, we have examined the activity of the Na^+-Ca^{2+}- exchange system in sarcolemmal membranes from the aging myocardium. It has been suggested that the Na^+-Ca^{2+}-exchanger transports Ca^{2+} into the cell as well as extruding it from the cell during the cardiac contraction-relaxation cycle. Thus, it appears to be involved in cardiac contractility.

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RESULTS

Effect of Aging on Ca^{2+}-Influx (Uptake)

As shown in Figs. 1 and 2, cardiac sarcolemmal vesicles accumulated Ca^{2+} when an outwardly directed Na^+ gradient was generated across the vesicular membranes. In these experiments the vesicles were loaded internally with either Na^+ or K^+ and then diluted in a solution containing 160mM KCl and $^{45}CaCl_2$. However, when the Ca^{2+} accumulating capability of the cardiac membrane vesicles from the three groups of rats was compared at various times of incubation (Fig. 1), it was observed that Ca^{2+} influx of Na^+-loaded vesicles from old hearts was depressed relative to the middle-aged group, which in turn was lower than the Ca^{2+} accumulated by vesicles from young hearts.

The data in Fig. 2 depict the dependence on Ca^{2+} concentration of Na^+-induced Ca^{2+} transport by the sarcolemmal vesicles. Ca^{2+} uptake under these conditions also appeared to be influenced by age since the Ca^{2+} accumulated by cardiac vesicles from old hearts was depressed relative to that of the middle-aged group. In fact, the influence of Ca^{2+} on Ca^{2+} influx by the Na^+-Ca^{2+} exchange system was highest in heart sarcolemmal vesicles from the young animals.

Passive Ca^{2+} uptake (measured in the absence of a transmembrane Na^+ gradient) did not differ between the three groups of animals at any incubation time studied (Table 1), thus suggesting a similarity in permeability of the membrane vesicles used in this study. Therefore, these results seem to suggest that sarcolemmal Ca^{2+} transport by the Na^+-Ca^{2+} exchanger is affected by age. The attenuated activity of this Ca^{2+} transporter does not appear to be due to a change in its affinity for Ca^{2+} since the Michalis constant [K_m (Ca^{2+})] was similar for the three groups of sarcolemmal vesicles. It was 49.23 ± 2.54 μM for young hearts and 53.23 ± 3.75 μM and 48.13 ± 2.30 μM for middle-aged and old hearts respectively. In addition, it should also be pointed out that the decrease in the Na^+-Ca^{2+} exchange activity in the aging myocardium did not appear to have been the

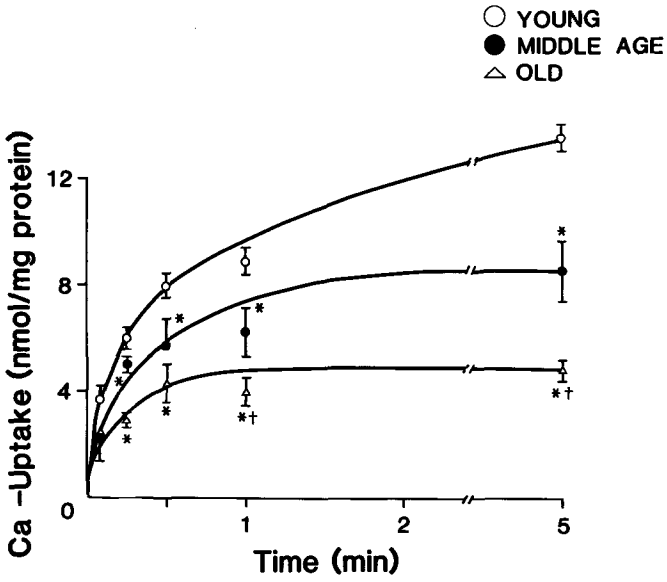

Fig. 1. Time course of Na^+-dependent Ca^{2+}-uptake in sarcolemmal vesicles from young, middle-aged and old hearts. Assay was conducted in the presence of 40uM $^{45}CaCl_2$ at 370°C (see Methods for details). All samples were run in duplicates. Numbers of experiments were: young = 6 middle age = 4, and old = 3. Each result is the mean ± S.E. of the number of experiments indicated.

*Significantly different from young ($p<0.05$).
†Significantly different from middle-aged ($p<0.05$).

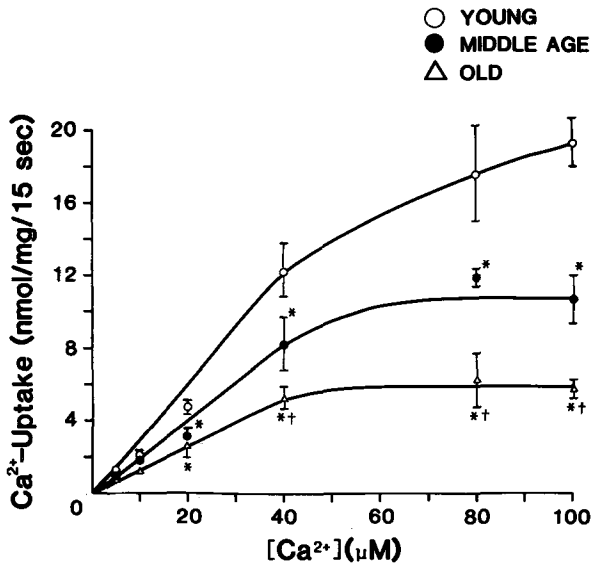

Fig. 2. Na^+dependent Ca^{2+} uptake in the presence of different Ca^{2+} concentrations in sarcolemmal vesicles from young, middle-aged and old hearts. Vesicles were loaded passively with Na^+ and K^+(see Methods for further details), then suspended in 160mM KCl for studying Ca^{2+} uptake during 15 seconds as a function of different concentrations of Ca^{2+}. Each data point was determined in duplicate. Numbers of experiments were: young = 6; middle age = 4, and old = 3. Each result is the mean ± S.E. of the number of experiments indicated.

*Significantly different from young ($p<0.05$)
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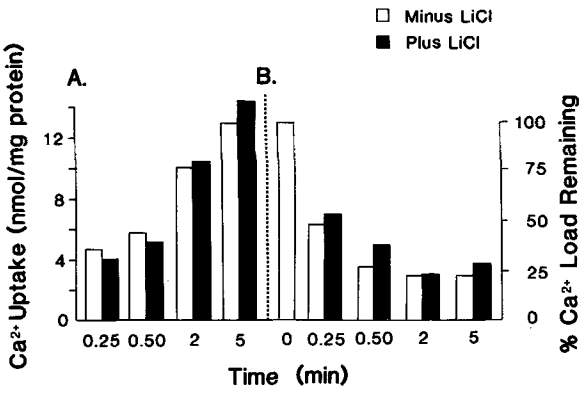

Fig. 3.
A. Uptake of Ca^{2+} by cardiac sarcolemmal vesicles from young rats in the presence of 160mM KCl (▭) or 160mM LiCl, (■) as described in Methods.
B. Efflux of Ca^{2+} from cardiac sarcolemmal vesicles. After 5 min of Ca^{2+} uptake (see section A: T = O in the figure) the vesicles were diluted with either 110mM KCl, 50mM NaCl (▭) or 110mM LiCl, 50mM NaCl (■) and harvested by filtration at the indicated times as described in Methods. Both dilution media contained 1.0mM EGTA and 20mM imidazole, pH 7.4. All assays were conducted in the presence of 40uM $^{45}CaCl_2$ at 37°C. Ca^{2+} transported by the Na^+-Ca^{2+} exchange system (expressed as percent Ca^{2+} load remaining) was determined after correcting for Na^+ independent (passive) efflux assessed by diluting the Ca^{2+} loaded vesicles with equal volumes of a solution containing 20mM imidazole, 1.0mM EGTA pH 7.4 and either 160mM KCl or 160mM LiCl.

Similar results were obtained for the middle-aged and old animals.

Results represent the mean of a typical experiment done in duplicate.

result of a difference in the passive loading of Na^+ because the leakiness of the vesicles appeared to be similar in the three groups of hearts. Further, since it has been suggested that the Na^+-Ca^{2+} exchanger has symmetrical properties (1), it can be ssumed that Ca^{2+} efflux by this Ca^{2+} transporter would also be depressed in the aged heart. In fact, we observed that Na^+-dependent Ca^{2+} efflux via the Na^+-Ca^{2+} exchange process of these sarcolemmal vesicles was also depressed in the aging heart (data not shown).

Miscellaneous Assays

In a separate set of experiments, the effect of Li^+ on Ca^{2+} transport by the Na^+-Ca^{2+} exchange system in these vesicular preparations was investigated. The results of a typical experiment are presented in Fig. 3. Although Li^+ has been reported to inhibit Ca^{2+} influx and enhance Ca^{2+} efflux of mitochondrial Na^+-Ca^{2+} exchange mechanism (10), Li^+ neither blocked Ca^{2+} uptake nor stimulated Ca^{2+} release by the Na^+-Ca^{2+} exchanger in these sarcolemmal preparations. These observations suggest that mitochondrial membrane did not appear to have contributed in a major way to

Table 1. Sarcolemmal passive Ca^{2+} uptake and protein yield.

	Duration of Incubation						Protein Yield (mg/g tissue) (wet weight)
Group	5 sec	15 sec	30 sec	1 min	2 min	5 min	
Young (n = 6)	3.8 ± 0.6	3.6 ± 0.7	3.3 ± 0.9	4.0 ± 0.7	4.1 ± 0.8	4.3 ± 0.6	0.12 ± 0.004
Middle Aged (n = 4)	4.3 ± 1.0	4.3 ± 1.1	3.8 ± 0.7	4.2 ± 0.8	4.1 ± 1.0	4.7 ± 0.5	0.12 ± 0.008
Old (n = 3)	3.3 ± 0.1	3.6 ± 0.5	3.7 ± 0.6	4.1 ± 0.8	3.0 ± 1.1	4.6 ± 0.2	0.11 ± 0.006

Each result is the mean ± S.E. of the number of experiments indicated in parentheses. Activities are expressed in nmol/mg protein.

Table 2. Ouabain-sensitive Na^+-K^+ ATPase activity in homogenates and sarcolemmal (SL) enriched vesicles from young, middle-aged and old rat hearts.

Group	Homogenate	SL-Enriched Vesicles	Fold Purification
Young (n = 3)	0.65	14.20	21.85
Middle-aged (n = 2)	0.31	6.90	21.56
Old (n = 2)	0.20	4.10	20.50

Values are averages of the number of experiments indicated in parentheses. Activities are expressed in μ moles pi/mg protein/hr.

the results reported here. Further, the preparations used in this study appeared to be highly enriched with sarcolemmal vesicles as indicated by their ouabain-sensitive Na^+-K^+ ATPase activity. A comparison of the activity of this enzyme in homogenates and purified sarcolemmal vesicles revealed that its activity was increased about 22-fold in young; 22-fold in middle-aged and 21-fold in old cardiac vesicles respectively (Table 2). These data seem to suggest that sarcolemma of high purity was obtained from hearts of the different groups of animals employed in this investigation. Further, it must be pointed out that the ouabain-sensitive Na^+K^+ ATPase activity observed in vesicles from the young animals is similar to that reported elsewhere (14, 15). The attenuated Na^+-K^+ ATPase activity observed in the middle-aged and old heart sarcolemmal vesicles may be a reflection of the effect of aging on this enzyme system. In fact, the present results confirm previous reports by Baskin *et al.* (16) and Katano *et al.* (17). It should also be pointed out that since no ATP was present in the assay medium, any Ca^{2+} transport by contaminating sarcoplasmic reticulum would not be via the ATP-dependent Ca^{2+} transport normally measured in sarcoplasmic reticulum (15).

DISCUSSION

Ca^{2+} transport in and out of the cell is believed to play a strategic role in the cardiac contraction-relaxation cycle. In this regard it appears that if Ca^{2+} entry and removal from the cell is altered, then there may be a concomitant alteration in myocardial performance. In support of this hypothesis, sarcolemmal Ca^{2+} transport has been reported to be depressed (18) and Ca^{2+} levels were observed to be elevated (19) in the failing myocardium of diabetics. In these hearts the Na^+Ca^{2+} exchange system was defective (18). Its activity was also depressed in the ischemic rabbit heart (15). It is well known that ischemic damage to myocardial cells is accompanied by an increase in intracellular Ca^{2+} (20, 21) as well as attenuated cardiac contractility (20, 22). Similar observations were made in the aging myocardium. Baskin *et al.* (23) reported, for example, that intracellular Ca^{2+} is elevated in the myocardium of aging rats, and Froehlich *et al.* (5) as well as Capasso *et al.* (1) further observed that cardiac performance was depressed in old rats. An attenuated sarcoplasmic reticular activity is believed to be involved in these abnormalities of the aging heart. As shown in our study, aging also appears to affect cardiac sarcolemmal Ca^{2+} transport and, as such, could also be a contributing factor in the intracellular Ca^{2+} derangements and altered myocardial contractility of the elderly.

The results presented here have shown that the activity of the Na^+-Ca^{2+} exchange system is depressed in the aging myocardium. The exact role of this Ca^{2+} transporter is still unclear, but it is believed to be involved in both contraction and relaxation (24, 25, 27). In this regard, the consequences of alterations in the activity of the Na^+-Ca^{2+} exchange system are difficult to interpret. It should be noted, however, that the properties of the Na^+-Ca^{2+} exchange process in heart sarcolemma appears to be symmetrical (15); therefore, regardless of the sidedness of vesicles in these membrane preparations, the changes observed in this Ca^{2+} transporting system would alter Ca^{2+} transport in or out of the cell and therefore could influence cellular activity.

In addition to a defective sarcoplasmic reticular activity, this study suggests additional mechanisms which could contribute to the elevated intracellular Ca^{2+} levels encountered in the aging myocardium. Since the Na^+-pump (Na^+-K^+ ATPase) is depressed in the aging hearts, it appears that this inhibition could lead to an elevation of sarcoplasmic Na^+ which in turn could increase Ca^{2+} influx via the Na^+-Ca^+ exchange mechanism. Since Na^+-Ca^{2+} exchange is depressed in the aging heart, it appears that the impact of an increased internal Na^+ on Ca^{2+} accumulation would be reduced, but, nevertheless, will still be expected to cause a net increase of

intracellular Ca^{2+} especially if Na^{+}-Ca^{2+} exchange were more important for efflux than influx (15).

The mechanism(s) by which the aging process affects Na^{+}-Ca^{2+} exchange activity is a matter of speculation. Philipson *et al.* (26) have shown, for example, that the activity of this Ca^{2+} transporter could be regulated by the sarcolemmal phospholipid content. In this regard, it appears that an alteration in the phospholipid microenvironment could be involved in the observed depression of the sarcolemmal Na^{+}-Ca^{2+} exchange mechanism of the aging myocardium. In fact, it should be pointed out that phosphatidylcholine and phosphatidylethanolamine composition have been reported to be altered in sarcolemma from aging hearts (28). In addition, since cellular lipid levels have been reported to be increased in old hearts (3), it is also possible that other membrane lipids (cholesterol, for example) may be involved in this attenuated Na^{+}-Ca^{2+} exchange process of the aged myocardium. Whatever the mechanism(s), these results suggest that the Na^{+}-Ca^{2+} exchange system is depressed in the aging heart and therefore might be involved in the reduction in cardiac contractility and the elevated myocardial Ca^{2+} levels commonly encountered in the elderly.

EXPERIMENTAL PROCEDURES

Animal Model

Male Sprague-Dawley rats were used in this study. They were divided into three groups: (1) young (4-6 months old; (2) middle-aged (14-17 months old) and (3) old (24-27 months old). Animals were sacrificed by decapitation. Hearts were quickly excised. Atria and any large vessels were carefully trimmed and the remaining ventricular tissue was used for experiments.

Preparation of Cardiac Sarcolemmal Membrane Vesicles

Sarcolemmal vesicles were isolated from ventricular tissue essentially as outlined by Kuwayama and Kanazawa (11). Briefly, weighed ventricular tissue was diced and suspended in 6 volumes (vol) of a medium containing 250mM mannitol, 70mM Tris adjusted to pH 7.4 with H_2SO_4 (mannitol buffer). The suspension was homogenized with a polytron PT-10 (4x20 sec; setting 4), then centrifuged at 1,900 x g for 20 min. The supernatant was saved and the pellet was resuspended in 6 vol of mannitol buffer. The above procedures (the homogenization of the resuspended pellet with polytron, the centrifugation of homogenate, the resuspension of pellet with 6 vol mannitol buffer and saving of supernatant) were repeated under the same conditions four more times. The supernatants were pooled, then centrifuged at 70,000 x g for 25 min. The pellet was resuspended in 8 ml of mannitol buffer and homogenized with a glass teflon homogenizer, layered over 25 mL of 0.64 M sucrose, 20mM imidazole HCl (pH 7.4), then centrifuged at 70,000 x g for 90 min. The membranes at the sucrose/mannitol interface were harvested and washed with 6 vol of a solution containing 20mM Imidazole-HCl (pH 7.4) and either 160mM NaCl (NaCl buffer) or 160 mM KCl (KCl-buffer). The suspensions were centrifuged at 69,400 x g for 25 min. and the final pellets were resuspended in NaCl and KCl buffer. All isolation steps were carried out at 0-5 °C, and assay procedures were initiated immediately after completion of the isolation protocol. Protein was determined by the method of Lowry *et al.* (12). Sarcolemmal membrane fractions were isolated from pools of 6 to 8 hearts (young) and 3 to 4 hearts (middle-aged and old) rats.

Measurement of $Na^{+}Ca^{2+}$ Exchange

Vesicles suspended in the NaCl and KCl buffers were incubated at 37 °C for 45 min. (13). This allowed NaCl and KCl to enter the vesicles by passive diffusion. Aliquots (25ul) of the NaCl- and KCl-loaded vesicles were added to a series of tubes containing an incubation mixture (at 37 °C) consisting of 160mM KCl, 20mM imidazole, pH 7.4, plus desired concentrations of $^{45}CaCl_2$ in a final volume of 500ul to induce Na^{+}-Ca^{2+} exchange (Ca^{2+} influx). The reaction was terminated by filtering the aliquots of incubation mixture through millipore filters (0.45um pore size) then washing with 5 mL of buffer containing 160mM KCl and 1mM $LaCl_3$. The quantity of $^{45}Ca^{2+}$ in the filtered vesicles was measured by liquid scintillation. Ca^{2+} uptake in the absence of a sodium gradient was termed "passive Ca^{2+} uptake" (13). Each data point was determined by subtracting the Ca^{2+} accumulated by KCl-loaded vesicles from that of the NaCl-loaded vesicles (13).

Measurement of Ouabain-Sensitive Na^{+}-K^{+} ATPase Activity

Ouabain-sensitive $Na^{+}K^{+}$ ATPase activity was assayed in a medium containing 50mM Tris-HCl (pH 7.4), 100mM NaCl, 10mM KCl, 4mM $MgCl_2$, 1mM EDTA, 5mM NaN_3 and 4mM ATP at 37 °C for 10 min. The activity was calculated as the difference between ATP hydrolysis measured as inorganic phosphate released with and without 2mM ouabain present (14).

Statistical Analysis

Data are expressed as means ± standard error (S.E.) of the mean. Statistical significance was determined by a one-way analysis of variance followed by Newmann-Keul's test with $p<0.05$ chosen as the criterion of significance.

REFERENCES

1. Capasso, J.M., Malhotra, A., Remily, R.M., Scheuer, J., and Sonnenblick, E.H.: Effect of age on mechanical and electrical performance of rat myocardium. *Am. J. Physiol.*,a 245: H72-H81, 1983.

2. Herbener, G.H.: A morphometric study of age-dependent changes in mitochondrial populations of mouse liver and heart. *J. Gerontol.*, 31: 8-12, 1976.

3. Sachs, H.G., Colgan, J.H., and Lazarus, M.J.: Ultrastructure of the aging myocardium: A morphometric approach. *Amer. J. Anat.*, 150: 63-71, 1977.

4. Alpert, N.R., Gale, H.H., and Taylor, N.: The effect of age on contractile protein ATPase activity and the velocity of shortening, in Factors Influencing Myocardial Contractility, edited by Tanz, R.D., Kavaler, F., and Roberts, J., New York, Academic Press, 1967, pp. 127-133.

5. Froehlich, J.P., Lakatta, E.G., Beard, E., Spurgeon, H.A., Weisfeldt, M.L., and Gerstenblith, G.: Studies of sarcoplasmic reticulum function and contraction duration in young and aged rat myocardium. *J. Mol. Cell. Cardiol.*, 10: 427-438, 1978.

6. Heller, L.J., and Whitehorn, W.V.: Age-associated alterations in myocardial contractile properties. *Amer. J. Physiol.*, 222: 1613-1619, 1972.

7. Bers, D.M., Philipson, K.D., and Nishimoto, A.Y.: Sodium-calcium exchange and sidedness of isolated cardiac sarcolemmal vesicles. *Biochim. et Biophys. Acta,* 601: 358-371, 1980.

8. Heyliger, C.E., Takeo, S., and Dhalla, N.S.: Alterations in sarcolemmal Na^+-Ca^{2+} *exchange in hypertrophied heart. Can. J. Cardiol.,* 1: 328-339, 1985.

9. Pitts, B.J.R.: Stoichiometry of sodium-calcium exchange in cardiac sarcolemmal vesicles. *J. Biol. Chem.,* 254: 6232-6275, 1979.

10. Reeves, J.R., and Sutko, J.L.: Sodium-calcium ion exchange in cardiac membrane vesicles. *Proc. Natl. Acad. Sci.* (U.S.A.), 76: 590-594, 1978.

11. Kuwayama, H., and Kanazawa, T.: Purification of cardiac sarcolemmal vesicles: high sodium pump content and ATP-dependent calmodulin-activated calcium uptake. *J. Biochem.,* 91: 1419-1426, 1982.

12. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J.: Protein measurement with the folin phenol reagent. *J. Biol. Chem.,* 193: 265-275, 1951,

13. Daley, M.J., Elz, J.S., and Nayler, W.G.: Sacrolemmal enzymes and Na^+-Ca^{2+} exchange in hypoxic, ischemic, and reperfused rat hearts. *Amer. J. Physiol.,* 247: H237-H243, 1984.

14. Pierce, G.N., and Dhalla, N.S.: Sarcolemmal Na^+-Ca^{2+}-ATPase activity in diabetic rat heart. *Amer. J. Physiol.,* 245: C241-C247, 1983.

15. Bersohn, M.M., Philipson, K.D., and Fukushima, J.Y.: Sodium-calcium exchange and sarcolemmal enzymes in ischemic rabbit hearts. *Amer. J. Physiol.,* 242: C288-C295, 1982.

16. Baskin, S.I., Roberts, J., and DeSousa, B.N.: Na^+, K^+-ATPase and age-dependent digitalis toxicity. *Pharmacol.,* 19: 132, 1977.

17. Katano, Y., Akera, A., Temma, K., and Kennedy, R.H.: Enhanced ouabain sensitivity of the heart and myocardial sodium pump in aged rats. *Eur. J. Pharmacol.,* 105: 95-103, 1984.

18. Makino, N., Dhalla, K.S., Elimban, V., and Dhalla, N.S.: Heart sarcolemmal Na^+-Ca^{2+} exchange and Ca^{2+} pump activities in chronic diabetes. *Fed. Proc.,* 44: 830, 1985.

19. Bhimji, S., Godin, D.V., and McNeill, J.H.: Biochemical and functional changes in hearts from rabbits with diabetes. *Diabetologia,* 28: 452-457, 1985.

20. Henry, P.D., Sauchleib, R., Davis, J., Weiss, E.S., and Sobel, B.E.: Myocardial contracture and accumulation of mitochondrial calcium in ischemic rabbit heart. *Amer. J. Physiol.,* 233: H677-H684, 1977.

21. Shen, A.C., and Jennings, R.B.: Myocardial calcium and magnesium in acute ischemic injury. *Amer. J. Pathol.,* 67: 417-433, 1972.

22. McLaurin, L.P., Rolette, E.L., and Grossman, W.: Impaired left ventricular relaxation during pacing-induced ischemia. *Amer. J. Cardiol.,* 32: 751-757, 1973.

23. Baskin, S.I., Uricchion, F.J., and Kendrick, Z.V.: The effect of age on the regional distribution of four cations in the rat heart. *Age,* 2: 64-67, 1979.

24. Mullins, L.J.: The generation of electric currents in cardiac fibers by Na/Ca exchange. *Amer. J. Physiol.,* 236: C103-C110, 1979.

25. Reuter, H., and Seitz, N.: The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. *J. Physiol.,* 195: 451-470, 1968.

26. Philipson, K.D., Frank, J.S., and Nishimoto, A.Y.: Effects of phospholipase C on the Na^{+}-Ca^{2+} *permeability of cardiac sarcolemmal vesicles. J. Biol. Chem.,* 258: 5905-5910, 1983.

27. Langer, G.A., Frank, J.S., and Brady, A.J.: The myocardium, in International Review of Physiology, Vol. 9, edited by Guyton, A.C., and Cowley, A.W., Baltimore, MD, University Park Press, 1976, pp. 191-237.

28. Amad, A.B., and Clay, S.W.: Age-dependent alterations in lipids and function of rat heart sarcolemma. *Mech. Aging Develop.,* 19: 333-342, 1982.