CALCIUM TRANSPORT IN ISOPROTERENOL-INDUCED MYOCARDIAL NECROSIS

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ABSTRACT

Calcium transport by cardiac subcellular particles in isoproterenol-induced myocardial necrosis in rats and rabbits was investigated. The ability of mitochondria to bind and accumulate calcium in the necrotic heart was similar to that of the control. Calcium binding, but not calcium uptake, by heavy microsomes of the necrotic heart was found to be depressed. This change in calcium was apparent over a wide pH range as well as at various concentrations of calcium in the incubation medium. The decrease in calcium binding by heavy microsomes was not due to the efflux of calcium from the microsomal vesicles and was seen within one hour of the subcutaneous injection of isoproterenol. At an increased animal room ambient temperature, not only was mortality due to isoproterenol injection increased, but also a decrease in the calcium uptake by heavy microsomes of the surviving animals was observed. These results suggest an alteration in the regulatory mechanisms responsible for the control of intracellular calcium in the necrotic heart.

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I. STATEMENT OF THE PROBLEM

Although isoproterenol-induced necrosis has been suggested to be due to a massive influx of calcium into the myocardial cell (1), no information concerning the abilities of the subcellular particles, fragments of sarcoplasmic reticulum (heavy microsomes) and mitochondria, to transport calcium in the necrotic heart is available in the literature. Furthermore, calcium transport by the subcellular particles in various other models of heart failure has been shown to be defective (2 - 14). It was therefore the purpose of this study to investigate changes in the abilities of both mitochondrial and heavy microsomal fractions of the necrotic heart to bind and accumulate calcium under different experimental conditions. Myocardial necrosis was induced in rats and rabbits by injecting high doses of isoproterenol. Preliminary results reported in this study were presented before the Canadian Federation of Biological Societies (15).

II. REVIEW OF LITERATURE

A. Calcium Accumulation by Subcellular Particles of Heart

The importance of calcium in heart function has now been well recognized and several excellent reviews on this subject are available in the literature (16 - 19). Various investigators have presented evidence that suggests that variations in the amount of intracellular calcium ion released by the sarcoplasmic reticulum during excitation-contraction coupling may be the mechanism responsible for the regulation of myocardial contractility (20 - 22). The current concept of excitation-contraction coupling implies that the entry of calcium into the myocardial cell and the release of calcium from the sarcolemmal superficial sites in response to depolarization, result in myocardial contraction by activating the contractile apparatus. In the heart, sarcoplasmic reticulum and mitochondria are considered to bring about relaxation due to their abilities to sequester calcium by energy dependent mechanisms (2, 10, 23 - 34). Thus the ability of subcellular particles to regulate intracellular calcium constitutes an important factor for determining the status of contraction-relaxation cycle of the heart.

The sarcoplasmic reticulum is believed to occupy a central position in the regulation of intracellular calcium in the heart while the participation of mitochondria in the processes of excitation-contraction and relaxation is generally questioned on the basis of lack of continuity of these structures with the cell membrane and thus our inability to explain calcium release from these particles. Although the rate of calcium uptake by mitochondria is less

than that by the sarcoplasmic reticulum it should be pointed out that mitochondria are present in abundance in the myocardium. Furthermore, Haugaard et al. (28) have suggested that the transport of calcium in mitochondria is not a one way street and that the relative concentrations of ATP, Pi and Mg^{++} are the important factors in determining which way the calcium ion will move. Changes in the intracellular concentrations of various ions such as H^+, Na^+ and K^+ during depolarization may be conceived to release calcium from both sarcoplasmic reticulum and mitochondria. Similarly, a small amount of Ca^{++} influx during depolarization may be considered to bring about calcium release from subcellular particles of the heart. It has been suggested that calcium release from the heart mitochondria may be mediated by chemical stimulus rather than electrical current (26).

Over the past 5 years several investigators have attempted to show an abnormality of the subcellular components to transport calcium in heart failure induced by different procedures. For example, Gertz et al. (7) have reported that the calcium pumping ability of heavy microsomes was markedly impaired in spontaneously failing dog heart-lung preparation. Both mitochondrial and heavy microsomal fractions of the substrate-depleted heart, which failed to generate contractility, have been observed to bind as well as accumulate calcium to a lesser extent in comparison to the control preparations (2, 3). A defect in the ability of heavy microsomes to transport calcium in ischemic dog heart has also been shown (8, 9). Calcium transport by heavy

microsomes, but not by mitochondria, has also been reported to be reduced in the isolated hypoxic rat heart (35). A decrease in calcium uptake by the microsomes was also demonstrated in failing human hearts (5, 10) as well as in the failing calf heart due to chronic pulmonary hypertension (12). Heart failure in genetically myopathic hamsters (BIO 14.6) has also been observed to be associated with depression in calcium binding and uptake by the mitochondria and microsomes (4, 6, 13, 14). A thorough survey of the literature revealed no information concerning the abilities of the subcellular components of the necrotic heart to bind and accumulate calcium.

B. Catecholamine-Induced Myocardial Necrosis

Catecholamines have been suggested to play a primary role in the pathogenesis of heart failure (36). Isoproterenol and other catecholamines, in a wide range of doses, have also been reported to produce massive cardiac necrosis (37 - 39). Although norepinephrine stores in the isoproterenol-induced necrotic heart are reduced (40 - 42), similar to other types of failing hearts (43 - 49), the significance of this observation is far from clear. It has been suggested that accumulation of metabolites formed during deamination of endogenous catecholamines is a cause of isoproterenol-induced myocardial necrosis (50).

The myocardial damage to the ultrastructure of mitochondria, sarcoplasmic reticulum and myofibrils by isoproterenol was found to have a focal distribution (51, 52). Loss of high energy phosphate compounds has also

been reported in the hearts of animals treated with isoproterenol (53 - 55) and propranolol has been found to prevent this change (56). Isoproterenol-induced necrosis has been described as closely resembling the lesion observed in patients with myocardial infarction as well as that in experimental cardiac infarct produced by coronary artery ligation (57, 58). An increase in cardiac lactate and a decrease in pyruvate and glycogen concentrations indicate a shift from aerobic to anaerobic metabolism after isoproterenol treatment (53).

Although the exact mechanisms by which isoproterenol induces necrosis are not known, several possibilities have been suggested from time to time. Raab (37) has postulated that catecholamines create an hypoxic imbalance between the oxygen supply and oxygen demand in the heart. The peripheral decrease in blood pressure due to the vasodilatory effects of isoproterenol has been suggested to reduce coronary perfusion pressure and thus cause the myocardium to become hypoxic (57). Since isoproterenol dilates arteriolar smooth muscle, it opens up precapillary shunts causing the blood to bypass capillary circulation thereby decreasing perfusion of the myocardial cells (59). The alteration in cell permeability to various fatty acids and subsequent limitation of the available supply of an essential substrate has also been proposed as another mechanism of cardiac lesion produced by isoproterenol (60, 61). The influx of free fatty acids into the cell due to catecholamines would probably uncouple mitochondria (62) and thus may

lower the energy state of myocardium and result in necrosis.

The myocardial necrosis induced by isoproterenol has been shown to be associated with substantial changes in the ionic content of the heart (63 - 65), and in the opinion of some investigators, is a result of ischemia which occurs both from the hemodynamic changes and increased cardiac work (38, 66 - 68). According to Fleckenstein (1) isoproterenol-induced necrosis is caused by calcium overload in the myocardium since various agents, prenylamine, verapamil, compound D600 and compound Bay a 1040, which specifically block calcium influx, also prevent catecholamine-induced necrosis. Whether or not the electrolyte shift and myocardial ischemia are the mechanisms of primary importance for catecholamine-induced myocardial necrosis remains to be investigated. However, in view of the dramatic elevation of intracellular calcium in the isoproterenol-induced necrotic heart, as well as the important role of calcium in myocardial metabolism, structure and function, the possibility of calcium participation in genesis of myocardial necrosis seems quite probable.

III. METHODS

A. Induction of Necrosis

In one series of experiments, albino rabbits (1.5 to 2 Kg) were injected subcutaneously twice with 2 mg/Kg isoproterenol* in normal saline over a period of 48 hours. Isoproterenol in doses above 5 mg/Kg was found to be lethal in rabbits. These animals were fed regular rabbit pellets (Victor Fox Foods, Ltd., Winnipeg, Manitoba) ad lib and kept at an ambient room temperature of 70 to 72°F unless otherwise indicated in the text. In another series of experiments, albino rats (300 to 350 g) were injected subcutaneously twice with 80 mg/Kg isoproterenol in normal saline over a period of 48 hours unless indicated in the text. This dose of isoproterenol is commonly employed in the induction of myocardial necrosis in rats and is well below the LD_{50} (69). These animals were fed ad lib on rat pellets and kept at an ambient room temperature of 70 to 72°F. Control rabbits and rats were injected with an equivalent volume of normal saline. The rabbits were sacrificed by cervical dislocation while the rats were sacrificed by decapitation and the hearts were rapidly removed and placed in ice cold sucrose solution. The atria were dissected out and the ventricles trimmed of fat and connective tissue. The presence of myocardial necrosis similar to that observed by Rona (39) due to the administration of isoproterenol was established by light microscopy.

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* DL-N-isopropylarterenol B grade - Calbiochemicals, Los Angeles 63, California; Isoproterenol-HCl, U.S.P. - Winthrop Laboratories, New York, New York 10016

B. Isolation of Mitochondria

After thorough washing in 0.25 M sucrose, 20 mM Tris-HCl, pH 7.0, 1 mM EDTA solution, the ventricle tissue was weighed, and minced with scissors. The tissue was homogenized in 10 volumes of media containing 0.18 M KCl, 10 mM EDTA, 20 mM Tris-HCl, pH 7.4, 0.5% fatty acid free bovine albumin, in a Waring blendor for 2 x 10 seconds with a one minute interval. The homogenate was filtered through 4 layers of gauze and centrifuged at 1,000 x g for 15 minutes to remove cell debris. The mitochondria were isolated from the supernatant by centrifuging at 10,000 x g for 30 minutes. After washing once in 50 mM KCl, 20 mM Tris-HCl solution at pH 6.8, the mitochondrial pellet was suspended in the same solution at a protein concentration of approximately 1 to 2 mg/ml. The above method is similar to that of Sordahl and Schwartz (70).

C. Isolation of Sarcoplasmic Reticulum

The washed tissue was minced with scissors and placed in 10 volumes of 15 mM Tris-HCl, pH 6.8, 10 mM $NaHCO_3$ and 5 mM Na-azide solution. The homogenate was prepared in a Waring blendor operated at medium speed for two periods of 10 seconds separated by 1 minute. After filtering through 4 layers of gauze, the homogenate was centrifuged at 1,000 x g for 10 minutes and then at 10,000 x g for 30 minutes to remove cell debris and mitochondria, both pellets being discarded. The resulting supernatant was centrifuged at 40,000 x g for 1 hour to remove the fragmented sarcoplasmic

reticulum as the heavy microsomal pellet. After washing in 0.6 M KCl, 20 mM Tris-HCl, pH 6.8 the fraction was suspended at a final protein concentration of 0.5 to 1.0 mg/ml. This method is similar to that of Harigaya and Schwartz (10).

D. Measurement of Calcium Transport

a) Calcium Binding: Calcium binding by mitochondria and microsomes was measured in a medium consisting of 100 mM KCl, 10 mM $MgCl_2$, 20 mM Tris-HCl, pH 6.8, 0.1 mM $^{45}CaCl_2$, 4 mM Na-ATP in a total volume of 1 or 2 ml. The mitochondrial protein concentration in the incubation medium was 0.3 to 0.4 mg/ml whereas the microsomal fraction concentration was 0.1 to 0.2 mg/ml. The mitochondrial or microsomal suspension was pre-incubated for 2 minutes at 25°C and for a further 2 minute period in the presence of ATP. The reaction was started by the addition of $^{45}CaCl_2$ (New England Nuclear, Dorval, Quebec) and stopped by millipore filtration (Millipore Corp., pore size 0.45 μ). The amount of ^{45}Ca in 0.1 ml of the filtrate was analyzed in 10 ml of Bray's solution (71) in a Packard Tri Carb scintillation spectrometer.

b) Calcium Uptake by Microsomes: Calcium uptake by microsomes was measured by the method described for binding except that 5 mM K-oxalate was added to the incubation medium and 0.05 to 0.1 mg microsomal protein/ml at a temperature of 37°C was employed.

c) Calcium Uptake by Mitochondria: Calcium uptake by mitochondria was determined at 37°C in the presence of 5 mM inorganic phosphate (Pi) and 5 mM sodium succinate at a mitochondrial protein concentration of 0.1 to 0.2 mg/ml.

d) Calcium Release: Calcium release from loaded microsomes in the absence of K-oxalate was studied according to the method of Pretorius et al. (32). Microsomes were loaded with ^{45}Ca by incubating in the medium described for calcium binding. At 5 minutes, exactly 0.5 ml medium was removed from the incubation tube, filtered and replaced with 0.5 ml of 100 mM Tris-EDTA, pH 6.8. Serial samples were then taken and analyzed for ^{45}Ca by the above mentioned methods.

All of the above procedures were carried out in a cold room at 0 to 4°C. Either a Sorvall RC2-B or an International B20-A refrigerated centrifuge was used. The protein concentration was determined according to the method of Lowry et al. (72).

IV. RESULTS

Calcium binding and uptake by the control and necrotic rabbit heart mitochondria were determined at different intervals of incubation and the results are described in Table I. No significant ($P > 0.05$) changes in calcium binding or uptake by the necrotic heart mitochondria were observed. It can be seen from Table II that sodium azide, a well known inhibitor of mitochondrial calcium transport, depressed calcium binding by the mitochondria from the control and necrotic hearts to a similar degree. On the other hand, azide did not affect calcium binding by the microsomal fractions of the control and necrotic hearts, although calcium binding by heavy microsomes of the necrotic heart was significantly ($P < 0.01$) decreased in comparison to the control (Table II).

The time-course of calcium binding and uptake by the heavy microsomal fractions from the control and necrotic rabbit hearts is shown in Figure 1. Calcium binding was depressed without significant ($P > 0.05$) changes in calcium uptake by the microsomes of the necrotic heart when the animals were maintained at normal room temperature. This defect in calcium binding by heavy microsomes was found over a wide pH range (Figure 2) as well as at various concentrations of calcium in the incubation medium (Figure 3). The calcium binding by the microsomal fractions of the apex, right ventricle and left ventricle of the necrotic heart was also lower than the respective control values ($P < 0.05$) where the calcium uptake in various areas of the necrotic heart was similar to those in the control heart (Table III).

TABLE I

Ca^{++} Accumulation by the Mitochondrial Fraction of Control and Necrotic Rabbit Hearts*

Time of Incubation (min)	Ca^{++} Accumulation (nmoles Ca^{++}/mg protein)**			
	Binding		Uptake	
	Control	Necrotic	Control	Necrotic
2	19.2 ± 4.9	25.0 ± 4.4	152 ± 36.7	122 ± 28.1
5	34.8 ± 4.2	37.6 ± 5.6	202 ± 36.6	180 ± 40.1
10	59.8 ± 8.4	63.2 ± 7.4	288 ± 34.3	262 ± 41.9

*Necrosis was induced by two subcutaneous injections of isoproterenol (2 mg/Kg), administered 24 hours apart.

**Each value is a mean ± S.E. of 5 to 6 experiments.

TABLE II

Influence of Sodium Azide on Calcium Binding by Subcellular Fractions from Control and Necrotic Rabbit Hearts*

	Calcium Binding (nmoles/mg protein/10 min)**			
	Mitochondria		Heavy Microsomes	
	Without Azide	With 5 mM Azide	Without Azide	With 5 mM Azide
Control	71.6 ± 8.0	10.3 ± 1.4	36.3 ± 4.5	37.5 ± 4.6
Necrotic	66.9 ± 8.4	8.4 ± 1.1	20.4 ± 1.4	21.9 ± 3.8

*Myocardial necrosis was induced by two subcutaneous injections of isoproterenol (2 mg/Kg) administered 24 hours apart.

**Each value is a mean ± S.E. of 4 to 6 experiments.

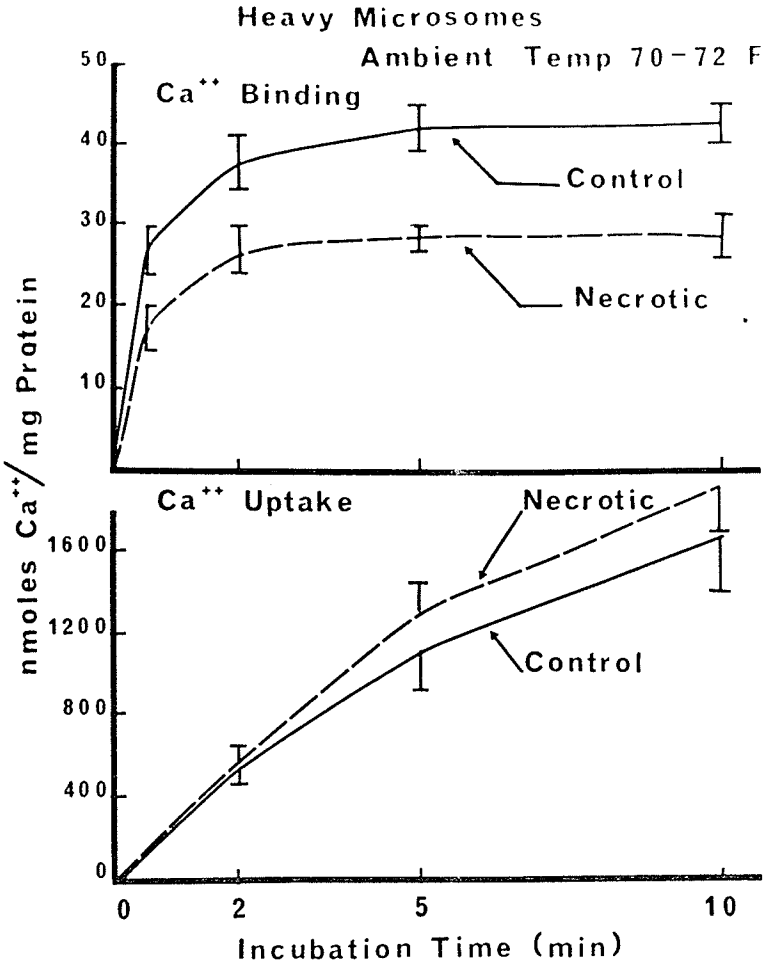

FIGURE 1 Time-course of calcium binding (upper panel) and calcium uptake (lower panel) by the control and isoproterenol-induced necrotic rabbit hearts. Necrotic hearts were obtained from rabbits which received two injections of isoproterenol (2 mg/Kg) 24 hours apart. Each value is a mean ± S.E. of 10 to 14 experiments.

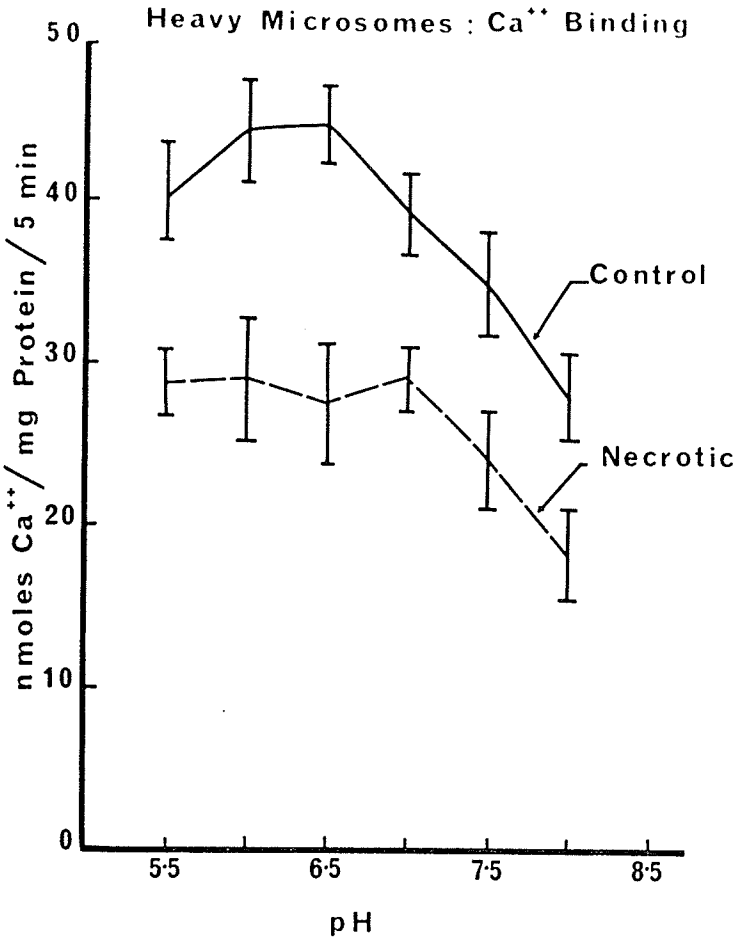

FIGURE 2 Calcium binding by the heavy microsomal fractions of the control and isoproterenol-induced necrotic rabbit hearts at different pH values of the incubation medium. The necrosis was induced as described under Figure 1. Each value is a mean ± S.E. of 4 to 6 experiments. The calcium binding by control microsomes was higher ($P < 0.05$) than that by the necrotic preparations.

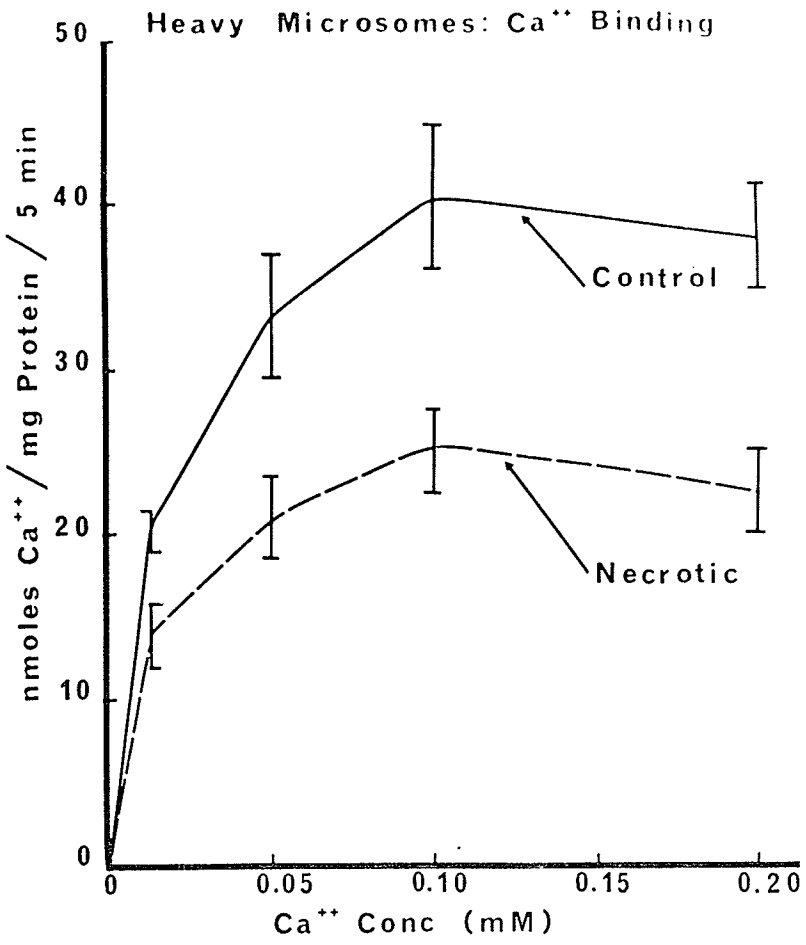

FIGURE 3 Calcium binding by the heavy microsomal fractions from control and isoproterenol-induced necrotic rabbit hearts at different concentrations of calcium in the incubation medium. The necrosis was induced as described under Figure 1. Each value is a mean ± S.E. of 4 to 6 experiments. The calcium binding by control microsomes was higher ($P < 0.05$) than that by the necrotic preparations.

TABLE III

Calcium Binding and Uptake by the Heavy Microsomes from Various Areas of Control and Necrotic Rabbit Hearts

Area	Calcium Accumulation (nmoles/mg protein)*	
	Control	Necrotic
A. Binding		
Apex	25.1 ± 1.8	17.2 ± 2.0
Right ventricle	25.8 ± 1.1	19.9 ± 1.7
Left ventricle	30.1 ± 1.5	21.2 ± 1.6
B. Calcium Uptake		
Apex	219.8 ± 31	246.2 ± 43
Right ventricle	206.9 ± 23	283.0 ± 51
Left ventricle	456.9 ± 67	462.8 ± 40

*Each value is a mean ± S.E. of 3 to 4 experiments. Myocardial necrosis was induced as described under Figure 1.

TABLE IV

Mortality, Heart Wt/Body Wt Ratio and Yields of Subcellular Particles from Control and Necrotic Hearts of Rabbits kept at Different Ambient Temperatures*

	Control	Necrotic	
		70 - 72° F	82 - 84° F
Mortality %	6 (35)	15 (26)	46 (13)
Heart wt/body wt ratio x 10^3	2.23 ± 0.05 (33)	2.70 ± 0.11 (22)	2.94 ± 0.12 (7)
Mitochondrial yield (mg protein/g heart)	1.90 ± 0.37 (8)	1.95 ± 0.23 (8)	2.05 ± 0.56 (6)
Heavy microsomal yield (mg protein/ g heart)	0.62 ± 0.08 (14)	0.75 ± 0.07 (14)	0.66 ± 0.16 (7)

*The results are a mean ± S.E. of the number of animals shown in brackets. Myocardial necrosis was induced by the method described under Figure 1.

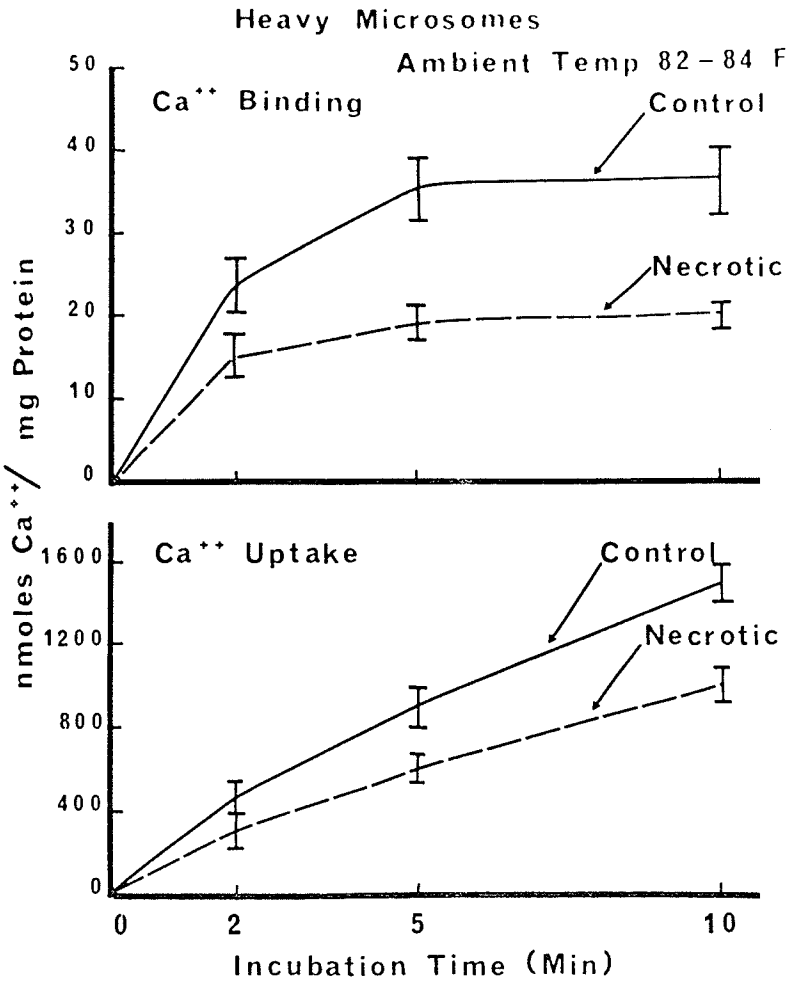

FIGURE 4 Time-course of calcium binding (upper panel) and calcium uptake (lower panel) by the heavy microsomal fractions of the hearts from control and necrotic rabbits kept at an ambient temperature of 82 to 84°F. The necrosis was induced as described under Figure 1. Each value is a mean ± S.E. of 5 to 6 experiments.

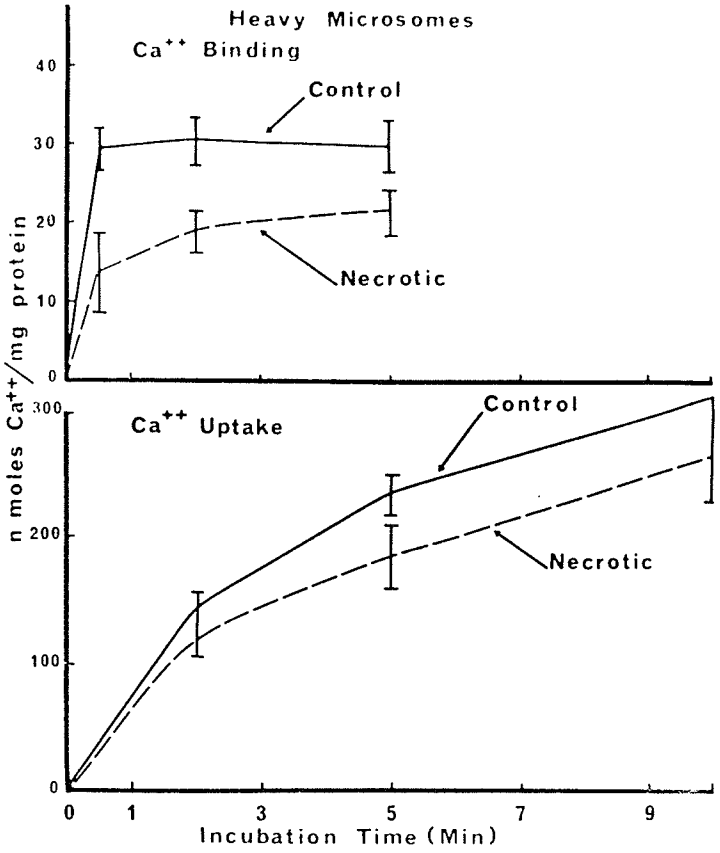

FIGURE 5 Calcium binding (upper panel) and calcium uptake (lower panel) by control and necrotic rat heart microsomes. Myocardial necrosis in rats was induced by injecting isoproterenol (80 mg/Kg, twice over a period of 48 hours). Each value is a mean ± S.E. of 5 to 10 experiments.

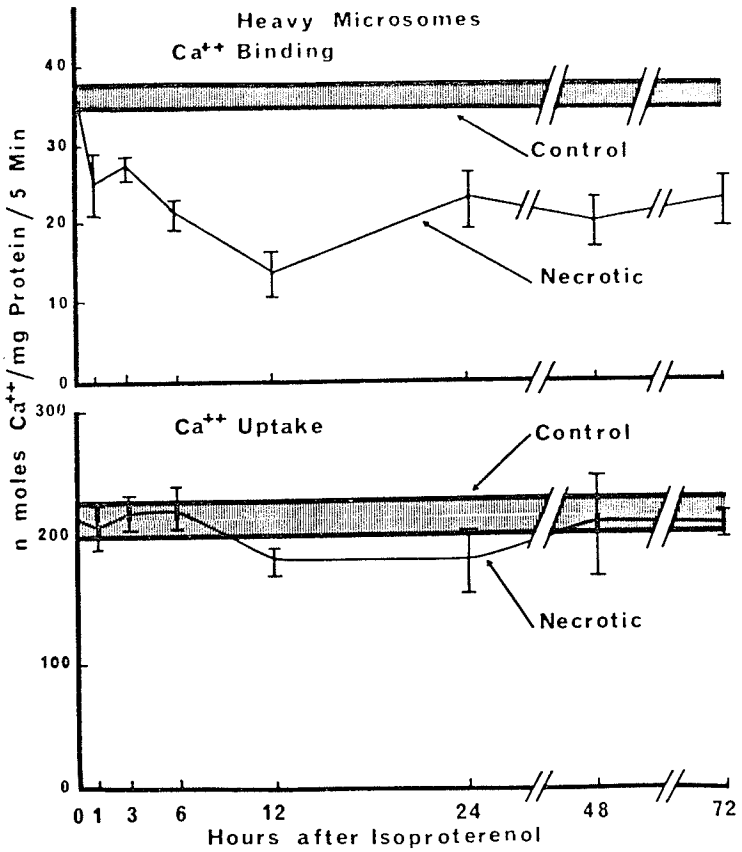

FIGURE 6 Calcium binding (upper panel) and calcium uptake (lower panel) by the heavy microsomes of the control and necrotic rat hearts obtained at different intervals after the subcutaneous injection of a single dose of 80 mg/Kg isoproterenol. Each value is a mean ± S.E. of 3 experiments.

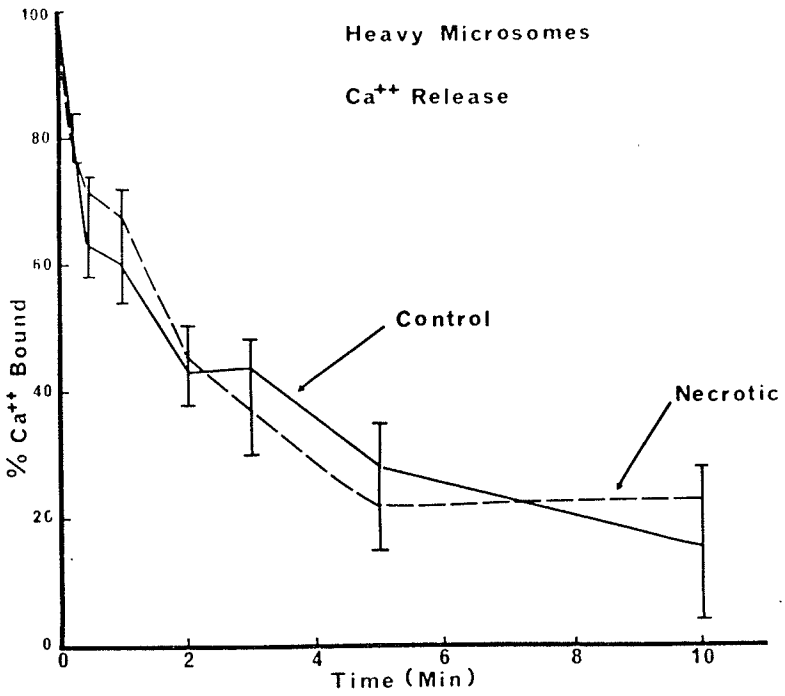

FIGURE 7 Calcium release from control and necrotic rat heart microsomal fractions. Myocardial necrosis was induced as described under Figure 5. The amounts of calcium bound by the control and necrotic microsomes were 31 ± 1.6 and 18 ± 1.8 nmoles Ca^{++}/mg protein respectively at the start of calcium release experiment (0 min values). Each value is a mean ± S.E. of 3 to 4 experiments.

When isoproterenol was administered to rabbits housed at an ambient temperature of 82 to 84°F, mortality was markedly increased (Table IV). This is in agreement with the observations reported by other workers in rats (73). The increment in heart wt/body wt ratio in surviving animals kept at 82 to 84°F was not different from those at 70 to 72°F ($P > 0.05$). Isoproterenol has also been shown to increase heart wt/body wt ratio in rats (53). The yields of mitochondrial and microsomal proteins from the necrotic hearts were essentially similar to those from the control hearts (Table IV). It was interesting to note that heavy microsomes from the isoproterenol-treated rabbits kept at 82 to 84°F showed a decrease ($P < 0.05$) not only in calcium binding but also in calcium uptake (Figure 4). The mitochondria obtained from hearts of necrotic rabbits kept at 82 to 84°F bound calcium similar to those of the control under identical conditions (data not shown).

The transport of calcium by the heavy microsomal preparation from the necrotic rat heart was also studied and the results are shown in Figure 5. Calcium binding, but not calcium uptake, by the necrotic rat heart microsomes was decreased significantly ($P < 0.05$). In one series of experiments rats were given a single dose of isoproterenol (80 mg/Kg) and the hearts removed at various intervals. It was found that the microsomal calcium binding, but not calcium uptake, in these hearts was reduced ($P < 0.05$) at different intervals (between 1 to 72 hours) after the injection (Figure 6).

Although the absolute amount of calcium released from the necrotic heart microsomes was less than the control preparation, the patterns of calcium release from both preparations were similar (Figure 7).

V. DISCUSSION

In this study it has been demonstrated that calcium binding and uptake by necrotic heart mitochondria are unaltered, while calcium binding by the heavy microsomal fraction is decreased without changes in calcium uptake. These findings are similar to those observed in the early phases of myocardial hypoxia and ischemia (35, 9). Since isoproterenol has been suggested to cause tissue hypoxia (66, 38), it is possible that the mechanism for the observed decrease in calcium binding by the heavy microsomes of the necrotic heart are similar to those for the hypoxic heart. Although we observed a decrease in calcium binding within one hour after the injection of isoproterenol, the present study was not designed to provide information concerning the cause-effect relationship between changes in calcium binding and myocardial hypoxia in isoproterenol treated rats.

The observed decrease in calcium binding by the heavy microsomal fraction of the necrotic heart does not appear to be due to contaminating inert protein since calcium uptake by these particles was unaltered and the yields of these particles were not significantly different from control values. Mitochondrial contamination in the necrotic heart microsomes also seems unlikely as sodium azide, a well known inhibitor of calcium transport, had no effect on calcium binding. It is difficult to explain this defect in calcium binding by necrotic heart microsomes on the basis of ATP insufficiency (53, 1), increased intracellular free fatty acids (60) and lysosomal enzyme degradation (74) since mitochondria from the same tissue showed no such alteration. It

is, however, possible that mitochondrial membranes are more resistant to the damaging influence of the above factors than heavy microsomal membranes.

The defect in calcium binding by heavy microsomes of the necrotic heart was evident both in rats and rabbits and was apparent over a wide pH range and at various concentrations of calcium in the incubation medium. This change was not due to increased calcium efflux since the pattern of calcium release from the loaded microsomal vesicles was similar in control and necrotic hearts. Since sarcoplasmic reticulum has been reported to swell in necrotic hearts (74, 75), the observed decrease in calcium binding may be the result of structural damage. It is also possible that decreased calcium binding may be due to conformational changes or changes in the phospholipid-protein composition of the microsomal membranes of the necrotic heart. Such mechanisms have been postulated previously for other types of failing hearts (3, 6).

Calcium uptake, in the presence of oxalate, by heavy microsomes has been shown to be unaltered in the necrotic heart. However, at higher ambient temperatures, which have been shown to increase the toxicity of isoproterenol (73) we were able to demonstrate a significant decrease in calcium uptake by heavy microsomes from necrotic hearts. This indicates the possibility that calcium binding is more sensitive to necrosis than calcium uptake, which is affected only in severe myocardial necrosis. These results

are in agreement with the findings of others (3, 9) who showed that calcium binding was decreased soon after the onset of substrate free perfusion in the rat heart and ischemia in the dog, and that calcium uptake was affected only after prolonged treatment. The greater sensitivity of calcium binding to pathological changes supports the contention of some investigators who maintain that calcium binding by subcellular fractions is a more physiological measure of calcium transport than calcium uptake in the presence of oxalate (29).

Depression of the ability of heavy microsomes to bind calcium reflects an alteration in the control of intracellular calcium which may result in an increase in the concentration of free calcium in the cytoplasm of the necrotic heart. In addition, Fleckenstein (1) has demonstrated an increase in calcium influx into the isoproterenol-induced necrotic heart. Thus the decreased ability of subcellular mechanisms to regulate intracellular calcium under conditions of massive influx of calcium support the contention concerning the role of calcium in the pathogenesis of myocardial necrosis.

VI. CONCLUSIONS

In this study the abilities of mitochondrial and microsomal fractions of the control and isoproterenol-induced necrotic hearts to transport calcium were examined. The following conclusions were drawn:

1) The ability of mitochondria to bind and accumulate calcium was normal in the necrotic heart.

2) The ability of necrotic heart heavy microsomes to bind calcium was decreased without any apparent defect in calcium uptake.

3) Maintaining the animals at a higher ambient room temperature increased not only the mortality due to isoproterenol, but also calcium uptake by necrotic heart heavy microsomes of the surviving animals.

4) The results described in this study suggest a defect in the intracellular regulation of calcium in the isoproterenol-induced necrotic heart. It is concluded that such damage in the presence of increased cellular calcium, may contribute to the pathogenesis of necrosis.

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