CHARACTERISATION OF THE CANINE CARDIAC SARCOLEMMA IN EXPERIMENTAL MYOCARDIAL ISCHEMIA.

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ABSTRACT

The cardiac sarcolemma was characterized in 13 normal and 11 ischemic dog hearts by enzyme analysis and compositional assays. Significant decreases in the activities of the sodium-potassium and calcium pumps and structural compositional disturbances were observed in ischemia. High concentrations of oleic acid, a fatty acid and palmitoyl carnitine, a fatty acid intermediate caused inhibition of the enzyme pump activities of the normal sarcolemma. Thus, ischemia results in the functional impairment of the sarcolemma. Accumulation of fatty acid and fatty acid intermediates, occurring in myocardial ischemia, could be an underlying mechanism.

KEY WORDS: Myocardial ischaemia, canine cardiac sarcolemma.

INTRODUCTION

The sarcolemma is a functional ionic barrier responsible for regulating ionic fluxes across the cell. These transmembrane fluxes control vital cellular functions like myocardial contraction-relaxation.

Although a small amount of calcium is essential for cardiac muscle concentration, recent studies have indicated that an excessive amount of calcium in the cytoplasm may induce myocardial cell damage under pathological conditions (1). Abnormal handling of calcium at the sarcolemma in myocardial ischemia has been reported by different investigators (2,3). Myocardial ischemia is responsible for disturbances in lipid metabolism. An accumulation of fatty acid and fatty acid intermediates has been reported to impair sarcolemmal function (4). However, the participation of such alterations in ischemic injury has yet to be clarified and the mechanisms underlying the transition from ischemia to infarction, yet to be confirmed. This paper describes some experiments which seem to provide evidence that myocardial ischemia results in defective sarcolemmal function.

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MATERIALS AND METHODS

Healthy mongrel dogs were used for experiments. The normal hearts were isolated by a left thoractomy by the procedure of Chien et al (5). Myocardial Ischemia was induced by ligating the left anterior descending coronary artery by the procedure of Chien et al (5). Ischemia was maintained for a period of 30 minutes. The heart was thereafter detached and the ischemic region was delineated on the basis of its cyanosed appearance. This portion was excised off and treated as the ischemic heart.

The myocardial sarcolemma from the normal and ischemic hearts were isolated by the procedure of Jones et al (6), with modifications by Colvin et al (7). The sarcolemma was characterized by estimating the activity of two marker enzymes and composition of lipids.

Marker enzyme analysis

The sarcolemma markers sodium, potassium adenosine triphosphatase (Na^+/K^+ ATPase) and calcium adenosine triphosphatase (Ca^{2+} ATPase) [also a marker for sarcoplasmic reticulum] and the effect of ouabain on Na^+/K^+ ATPase were assayed by the procedure of Colvin et al (7). Cytochrome c oxidase, the mitochondrial marker was assayed by the proce-

ture of Sottocasa et al (8).

Angiotensin converting enzyme, the endothelial marker, was assayed by the method of Holmquist et al (9).

Assay of lipids

The sarcolemmal lipids including phospholipids were extracted by the procedure of Colvin et al (7). Phospholipids of the sarcolemma were quantitated as the amount of the membrane phosphorus content and estimated by the method of Chen et al (10). The sarcolemmal phospholipids were characterized by thin layer chromatography (TLC). Cholesterol and triglycerides were assayed from extracted sarcolemmal lipids by the methods of Kersch et al (11) and Trinder (12). The sarcolemmal fatty acids were characterised by gas chromatography (GC) by a modified method of Tibbits et al (14). The extracted lipids were digested with a 2:1 mixture of 85% sulphuric acid: 70% perchloric acid and derivatized with boron trifluoride in methanol. The derivatized sample was then loaded onto a 10% diethylene glycol succinate column. The fatty acid composition was calculated by an integrator linked to the Gas Chromatograph system (Netel Chromatographs Ltd.). Calculations were based upon dividing the integrated area of each peak by the total area of all identified peaks on the chromatogram, and the amount of each fatty acids was expressed as the percentage of total fatty acids. In general, the area of total identified peaks accounted for 90% of the total integrated area of each run, after the solvent peak. Identification of each fatty acid peak was based

on the retention times of fatty acid standards.

Effects of a fatty acid and fatty acid intermediate on Na⁺/K⁺ ATPase and Ca²⁺ ATPase of the sarcolemma.

The in vitro effects of oleic acid, and palmitoyl carnitine, on the activities of Na⁺/K⁺ ATPase and Ca²⁺ (7) were studied by incubating varying concentrations of the above with normal sarcolemmal membrane preparation.

Data analysis

All data comparing 2 or more groups were represented by mean $\pm$ S.D., values and were subjected to statistical analysis. The student's 't'-test was used to evaluate the significance of the data with $P < 0.05$ as the level of significance.

RESULTS

We have isolated and characterised the sarcolemma from 13 normal and 11 ischemic dog hearts.

CHARACTERISATION OF THE SARCOLEMMMA

Marker enzyme assay

The activities of the marker enzymes in the normal and ischemic membranes are given in table 1. The sarcolemma markers show appreciable activity, whereas the mitochondrial and endothelial markers show relatively low activities. 0.1 mM ouabain completely inhibited Na⁺/K⁺ ATPase activity.

Table 1: Marker enzyme analysis of the cardiac sarcolemma.

Enzymes	Marker	Specific Activities of normal sarcolemma [n=13]	Specific Activities of ischemic sarcolemma [n=11]
*Na ⁺ /K ⁺ ATPase	Plasma Membrane	1.039 $\pm$ 0.149 uMPi/mg protein/min	0.374 $\pm$ 0.04 uMPi/mg protein/min
Ca ²⁺ ATPase	Plasma membrane & Sarcoplasmic Reticulum	1.283 $\pm$ 0.15 uMPi/mg protein/min	0.408 $\pm$ 0.05 uMPi/mg protein/min
Cytochrome c oxidase	Inner Mitochondrial Membrane	0.048 $\pm$ 0.006 uMPi/mg protein/min	0.031 $\pm$ 0.005 uMPi/mg protein/min
Angiotensin converting enzyme	Endothelial Membrane	1.165 $\pm$ 0.09 mU/mg protein	1.168 $\pm$ 0.07 mU/mg protein

*0.1 mm ouabain completely inhibited the activity of Na⁺/K⁺ ATPase.

Figure 1. Comparison of Na⁺/K⁺-ATPase and Ca²⁺-ATPase in normal (n=13), ischemic control (n=11) and ischemic membranes (n=11). Statistical significance * (P<0.05).

Figure 2. Comparison of lipids in normal (n=6) and ischemic membranes (n=6). PL=Total phospholipids; CHOL=Cholesterol; TRIG=Triglycerides; PC=Phosphatidylcholine; PE=Phosphatidylethanolamine; SPH=Sphingomyelin; PS=Phosphatidylserine; LPC=Lysophosphatidylcholine. Statistical significance * (P<0.05).

Figure 1 shows the comparison of the activities of the sodium-potassium and calcium pumps in the normal, ischemic control (the non-ischemic portion of the ischemic heart) and ischemic membranes. There is a significant decrease of both pumps (p<0.05) in ischemia.

Changes in lipid composition

Figure 2 compares the lipid components in the Indian Journal of Clinical Biochemistry, 1997, 12 (1), 49-54

normal and ischemic membranes. Phospholipids, the major lipid component of the sarcolemma, decrease significantly (P<0.05) in ischemia, whereas cholesterol and triglyceride concentrations do not change. Among the phospholipids, phosphatidylcholine and phosphatidylethanolamine, the two major phospholipids decreased significantly (P<0.05) in ischemia, while the concentration of other phospholipids did not change.

Figure 3. Comparison of fatty acid concentrations in normal (n=3) and ischemic membranes (n=3). C₁₀=Capric acid, C₁₂=Lauric acid, C₁₄=Myristic acid, C₁₆=Palmitic acid, C₁₈₍₁₎=Palmitoleic acid, C₁₈=Stearic acid, C₁₈₍₂₎=Oleic acid, C₁₈₍₃₎=Linolenic acid. Statistical significance * (P<0.05).

Figure 3 compares the fatty acid composition in the normal and ischemic membranes. The saturated fatty acids lauric, myristic and stearic, and the unsaturated fatty acid palmitoleic acid decrease significantly (P<0.05) in ischemia, whereas palmitic acid and oleic acid, the most abundant saturated and unsaturated fatty acid and linolenic acid, an unsaturated fatty acid increased significantly (P<0.05) in ischemia. Thus there is a significant (P<0.05) per cent increase in fatty acid unsaturation in the normal (56.18 ± 5.03) and ischemic membranes (65.03 ± 4.73).

Effects of oleic acid and palmitoyl carnitine on the enzyme pumps of the normal sarcolemma

Increasing concentrations of oleic acid inhibited the activities of both enzyme pumps in the normal sarcolemma with total inhibition by 0.2 mM oleic acid

Figure 4. Effect of Oleic Acid on Na⁺/K⁺-ATPase and Ca²⁺-ATPase activities. The data presented is representative of three separate experiments in each case.

Figure 5. Effect of Palmitoyl Carnitine on Na⁺/K⁺-ATPase and Ca²⁺-ATPase activities. The data presented is representative of three separate experiments in each case.

(Fig. 4). Likewise, the increasing concentrations of palmitoyl carnitine inhibited the activities of both the enzyme pumps. However, 0.2 mM palmitoyl carnitine could completely inhibit the activity of Ca²⁺ ATPase but only partially inhibited the activity of Na⁺/K⁺ ATPase (Fig. 5)

DISCUSSION

The results of marker enzyme analysis indicate that the sarcolemma fraction is a fairly purified preparation, as the levels of mitochondrial and endothelial contaminations are low. However, we do not rule out any sarcoplasmic reticulum contamination.

The dog heart is a widely used model (5,15) as the ischemic pattern evolved in dogs corresponds quite well with that of man. The sarcolemmal enzyme pumps are impaired in ischemia as is evident from our studies. There have been few reports on the decrease in the activities of sarcolemmal Na⁺/K⁺ ATPase and Ca²⁺ ATPase in ischemia (16-19). A reduction in the Na⁺/K⁺ ATPase activity presumably prevents the myocytes from re-establishing transmembrane electrolyte concentration gradients, which could block subsequent repolarisation, excitation and contraction. Many mechanisms may be responsible for a reduction of Na⁺/K⁺ ATPase activity with ischemia such as decreased levels of ATP, altered lipid metabolism, or free radical damage. An additional factor which might adversely affect the sodium pump function during ischemia may be an increase in free intracellular calcium. It has been demonstrated that calcium may compete with sodium at the inner plasma membrane and disrupt the cycle of the enzyme.

In addition, the biological activity of intracellular proteins such as calmodulin or protein kinase C may also secondarily affect pump function during ischemia. A reduction in Ca²⁺ ATPase activity would be responsible for a reduced calcium ion exit from the cell. This would lead to an increase in calcium ion accumulation, which has already taken place probably due to impairment of the sarcoplasmic reticulum calcium pump and a probable impairment to the sodium-calcium exchanger (2). The mechanisms responsible for the reduction of Ca²⁺ ATPase activity may be decreased level of ATP, alterations in metabolism of lipids (4) or free radical damage (21). Lipid metabolism is known to be altered in ischemia (4). Very few studies have shown that the sarcolemmal structural composition is affected (15, 26, 27). Our results indicate that the lipid composition is affected. Our observation of a reduced phospholipid content after 30 minutes of experimental ischemia could be explained as a initiation of membrane breakdown and loss of permeability. Although there has been a report on the cholesterol loss from myocardial membranes in the ischemic state (26), we have not found a similar change. There are no reports to date about the status of sarcolemmal membrane triglycerides in ischemia. Decreased amounts of the major phospholipids phosphatidylcholine and phosphatidylethanolamine observed in ischemia could mean a derangement of the membrane structure. A similar finding has been reported earlier (15). Variations in the fatty acid composition with a significant increase in percentage of

unsaturation in ischemia observed by us indicate that the membrane fluidity, which is controlled by the fatty acid composition and cholesterol content, is affected. Hence permeability would be altered, thus allowing for a surge of extra cellular ions, which in turn would disturb the vital cellular functions. There is a report, however, showing increase in percentage saturation of the sarcolemmal fatty acid chains of phosphatidylcholine and phosphatidylethanolamine in ischemia (27).

In order to correlate a decrease in the enzyme pump activities with a possible lipid alteration, we simulated an *in vitro* condition resembling ischemia where there is an accumulation of free fatty acids and intermediates. Our results agree with previous reports on inhibition of the enzyme pump by fatty acids and their intermediates (19, 28, 29). This suggests that the accumulation of free fatty acid and free fatty acid intermediates could be a probable mechanism which brings about impairment of both pumps in ischemia.

Intracellular accumulation of long chain fatty acids, fatty acyl carnitine and fatty acyl CoA could result due to [a] inhibition of the citric acid cycle and

[b] inhibition of fatty acid oxidation (4). The lipids that accumulate are amphiphiles which can induce major changes in the membrane function by insertion of free amphiphiles molecules into the bilayer of the membrane.

We conclude from this study that ischemia is responsible for the functional impairment of both the sodium and calcium pumps of the sarcolemma and alterations in the sarcolemmal lipid composition. This could lead to disturbances in regulation of ionic fluxes, especially calcium ions and bring about calcium overload. Fatty acid and fatty acid intermediate accumulation could be responsible for the above impairment. Irreversible damage to the sarcolemma would lead to cell death or myocardial infarction.

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