Carnitine Distribution in Subepicardial and Subendocardial Regions in Normal and Ischemic Dog Hearts

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SUMMARY

In order to evaluate the role of carnitine on fatty acid metabolism in subepicardial (Epi) and subendocardial (Endo) regions in ischemic heart, tissue levels of carnitine, free fatty acids (FFA), and adenosine triphosphate (ATP) were determined in ischemic, non-ischemic and border areas in dog hearts with acute regional ischemia. Acute regional ischemia was induced by ligation of the left anterior descending coronary artery for 15 min.

In normal hearts, tissue carnitine levels were lower in Endo than in Epi. On the other hand, FFA levels were higher in Endo than in Epi. In ATP levels, no significant differences were observed between Endo and Epi. In acute regional ischemia, tissue carnitine levels decreased not only in ischemic and border areas but also in nonischemic area. And the levels were lower in Endo than in Epi in all areas. Tissue ATP levels were also lower in Endo than in Epi in all areas. From nonischemic area toward the center of ischemic area, the difference in ATP levels between Endo and Epi became more prominent. Tissue FFA levels increased in ischemic and border areas, while no significant differences were observed between Endo and Epi.

These results confirmed that Endo was metabolically more anaerobic than Epi even in normal heart and it became more prominent in the heart with acute regional ischemia.

Additional Indexing Words:

Acute regional ischemia Ischemic area Nonischemic area Border area Free fatty acids Adenosine triphosphate

A great attention has been focuced on the accumulation of fatty acyl derivatives, as one of the causes of the cellular damage in ischemic myocardium.[1)-3)]

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Carnitine, a water-soluble naturally occurring amino acid, is essential for fatty acyl derivatives to penetrate across the inner mitochondrial membrane[4),5)] and to be transported to the sites of oxidation in the mitochondria. It is synthesized from lysine in the liver and transported to the heart[6)] where free fatty acids (FFA) are used as a major metabolic fuel.[7),8)] A reduction in tissue levels of carnitine has been demonstrated in ischemic myocardium[9)-12)] and negative correlation has been observed between tissue levels of carnitine and adenosine triphosphate (ATP) in ischemic dog hearts.[12)]

In acute regional ischemia, border area between ischemic and surrounding nonischemic areas has been reported to represent reversibly damaged tissue.[13)] Metabolic and regional flow studies have also demonstrated that the subendocardium tended to become ischemic more easily than subepicardium, when coronary blood flow was reduced.[14)] From these observations, it is important to know metabolic events in border area and subepicardial tissue in ischemic area, because those tissues can be potentially salvaged by medical therapy.

The purpose of this study was to determine the tissue levels of carnitine, FFA, and ATP in subepicardial and subendocardial regions of ischemic, nonischemic, and border areas in the dog hearts with acute regional ischemia and to evaluate the role of carnitine in fatty acid metabolism in those tissues.

Materials and Methods

Thirty-one mongrel dogs weighing 8 to 15 Kg were used as experimental animals and divided into 2 major groups: 21 normal and 10 experimental infarction dogs.

Anesthesia was induced with intravenous sodium pentobarbital (30 mg/Kg body weight) and ventilation was maintained by means of a Harvard animal respirator with room air. A left thoracotomy was performed through the 4th intercostal space. The pericardium was opened and the heart was exposed. In 10 experimental infarction dogs, a short length of the left anterior descending branch of the coronary artery was dissected free from surrounding tissue and a silk thread was placed around it. After 15 min of coronary occlusion, beating hearts were removed from the animals and transmural tissue (1 Gm) representing ischemic area (supplied by the ligated artery), nonischemic area (supplied by left circumflex artery) and border area (between ischemic and surrounding nonischemic areas) were rapidly excised. Briefly each transmural tissue was divided into subendocardial (inner half) and subepicardial (outer half) layers and frozen with Wollenberger clamp cooled to the temperature of liquid nitrogen. These procedures, from removal of the heart until freezing the tissues, were made within 30 sec. The frozen and pressed samples were cracked into fragments on a block of dry ice and stored at $-70°C$. Extraction and analysis were made on these frozen tissues within 3 days.

Twenty-one normal dogs were divided into 2 minor groups. In 11 dogs, transmural tissues were obtained from ventricles and atria. And carnitine levels were determined in these transmural tissues. In 10 dogs, transmural tissues from the left ventricles were divided into subepicardial and subendocardial layers. Carnitine, FFA, and ATP levels in these tissues were used as the controls of that in ischemic hearts.

Free carnitine was determined enzymatically using carnitine acetyl transferase (CAT) by the method of Marquis and Fritz.[15] One Gm of the frozen tissue was homogenized in 4 ml of cold 7.0% (wt/vol) perchloric acid with a Polytron homogenizer and centrifuged at 12,000 g for 10 min. The supernatant (2.0 ml), after addition of 5 μl of 0.1 M pottasium sulphate buffer, was ajusted to pH 6.5—7.0 with 1N KOH and maintained at 0—4°C for 30 min. After additional centrifugation at 12,000 g for 10 min, the supernatant was used for the determination of free carnitine. The basic reaction mixture contained, in a volume of 1.0 ml, 200 μ mole Tris-HCl buffer at pH 7.8, 0.2 μ mole 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), 0.3 μ mole acetyl-CoA and 2.5 μ mole EDTA. L-carnitine standards (10 to 80 n mole) were included with each assay. Reactions were initiated by addition of 1.0 ml of basic reaction mixture to a test tube containing 1.0 ml of the sample. Before and 5 min after the addition of 10 μl CAT solution (1 mg protein/ml, pH 7.5), absorption of DTNB with sulfhydril was measured at 412 nm. Carnitine concerntration was calculated from the absorbancy changes before and after the addition of CAT.

ATP was determined by enzyme assay using hexokinase and glucose-6-phosphate dehydrogenase[16] and FFA was determined by the method of Itaya and Ui.[17] Values were expressed for wet tissue weight as mean±SD.

Statistical analysis was made by paired or non-paired Student's t test, as appropriate.

Results

Tissue levels of carnitine, ATP, and FFA in normal heart:

Tissue levels of carnitine in ventricles and atria were shown in Table I. Carnitine levels were significantly higher in ventricles than in atria and the levels in left sided heart were lower than that in right sided. Tissue levels of various metabolites in subepicardial and subendocardial regions were shown

Table I. Tissue Levels of Carnitine in Normal Dog Hearts

	Carnitine (n mole/Gm)	
Right ventricle	1,072±515	$p<0.01$
Left ventricle	942±466	
Right atrium	793±315	$p<0.05$
Left atrium	712±219	

Values were expressed per Gm wet tissue weight and represented as mean± SD. Statistical analysis was made by paired t test.

in Table II. Carnitine levels were significantly lower in subendocardial region than in subepicardial region. On the other hand, FFA levels were significantly higher in subendocardial region than in subepicardial region. No significant differences were observed in ATP levels, between subepicardial and subendocardial regions.

Changes in tissue levels of carnitine, ATP, and FFA following coronary artery occlusion:

Tissue levels of carnitine, ATP, and FFA after 15 min of coronary artery occlusion were shown in Figs. 1, 2, and 3.

Carnitine levels significantly decreased not only in ischemic area but also in nonischemic area, as compared with normal controls. Carnitine levels in border area represented the intermediate value between the levels in ischemic and nonischemic areas. In all areas, carnitine levels were significantly lower in subendocardial regions than in subepicardial regions (Fig. 1).

Table II. Tissue Levels of Carnitine, ATP and FFA in Left Ventricular Muscle in Normal Dog Hearts

	Carnitine (n mole/Gm) n=10	ATP (μ mole/Gm) n=10	FFA (μEq/Gm) n=6
Subendocardium	1,028±428	4.41±0.65	3.04±0.61
Subepicardium	1,106±464	4.78±0.64	2.51±0.49
	p<0.01	N. S.	p<0.05

Values were expressed per Gm wet tissue weight and represented as mean±SD. Statistical analysis was made by paired t test.

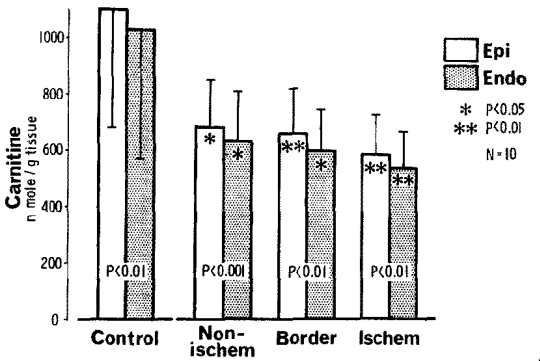

Fig. 1. Effects of acute regional ischemia on tissue levels of carnitine. Values were expressed as mean±SD. Asterisks represent the significance of change with respect to the respective controls (by non-paired t test). P values represent the difference between subepicardial and subendocardial regions (by paired t test). Abbreviations: Non-ischem=nonischemic area; Border=border area; Ischem=ischemic area; Epi=subepicardial region; Endo=subendocardial region.

Tissue levels of ATP decreased in ischemic and border areas, as compared with normal controls and that in nonischemic areas. And the levels in border area represented the intermediate value between the levels in ischemic and nonischemic areas. Compared with carnitine levels, reduction in ATP content in subendocardial regions was more prominent than that in subepicardial regions (Fig. 2).

Tissue levels of FFA increased in ischemic and border areas, as compared with normal controls and that in nonischemic areas. However, no significant differences were observed in the levels in subepicardial and subendocardial regions (Fig. 3).

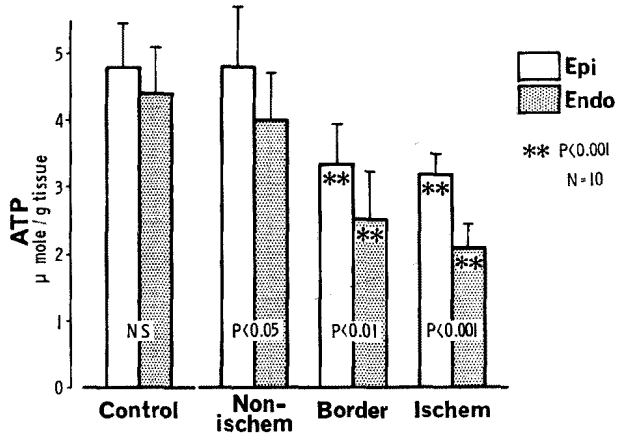

Fig. 2. Effects of acute regional ischemia on tissue levels of ATP. Values are expressed as mean±SD. Asterisks and abbreviations are identical to Fig. 1.

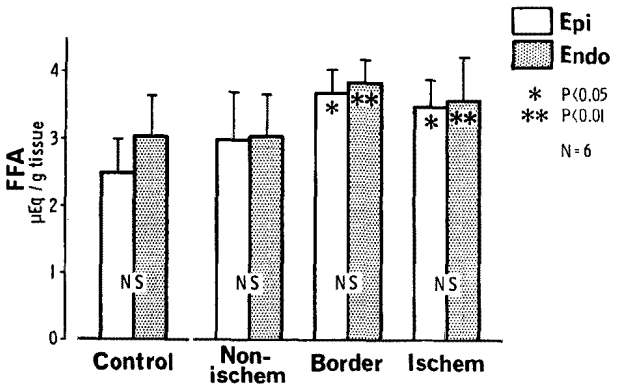

Fig. 3. Effects of acute regional ischemia on tissue levels of FFA. Values are expressed as mean±SD. Asterisks and abbreviations are identical to Fig. 1.

Discussion

In ischemic myocardium, the amount of oxygen to support oxidative phosphorylation is reduced and results in reduced ATP production. At the same time, accumulated NADH in electron transport chain, by inhibiting β-oxidation of fatty acids, increases long chain acyl coenzyme A (CoA) and long chain acyl carnitine.[10),11),18)-20)] In proportion to the increase in long chain acyl carnitine, free carnitine decreases.[20)] Because free carnitine is necessary for acyl CoA to be converted to acyl carnitine,[4)] the reduction in free carnitine exaggerates the accumulation of long chain acyl CoA. Because high levels of long chain acyl CoA inhibit adenine nucleotide translocase activity,[21)-23)] removal of ATP from mitochondrial matrix to cytosol is reduced. As the results, ATP production in ischemic myocardium is impaired both by reduced supply of oxygen and the accumulation of long chain acyl CoA.[12)] Administration of exogenous carnitine in this situation has been reported to reduce the accumulation of long chain acyl CoA and lead to increased ATP production.[20),24),25)]

Adverse effects of high plasma FFA in acute myocardial infarction have been reported in men and animals.[26)-28)] And several explanations have been proposed as the mechanisms, such as nonspecific detergent action on biomembranes[29)] and uncoupling of oxidative phosphorylation in the mitochondria.[30)-32)] However, these results could not be reproduced by other investigators.[33),34)] These discrepant results can be explained by the proposition that any deleterious effects of FFA on the ischemic myocardium are due not to the absolute levels of FFA itself but to the accumulated fatty acyl derivatives subsequent to impaired oxidation of FFA.[1)] In other words, tissue carnitine concentrations are supposed to be an important factor that protect against the adverse effects of FFA as well as ischemic myocardial damage.[35)]

Moving inward from surrounding nonischemic area to ischemic area, severe and progressive derangement of metabolism, flow and electrophysiologic characteristics have been demonstrated.[13),36)] In this study as well, tissue levels of carnitine and ATP in border area represented the intermediate value between the levels in ischemic and nonischemic areas.

Reduction in carnitine levels in nonischemic area suggests that so called " nonischemic area " may be metabolically not normal but tend to be ischemic. Similar changes have been observed in the levels of long chain acyl carnitine and long chain acyl CoA and adenine nucleotide translocase activity.[11),20),22)] In nonischemic area, reduction in carnitine levels is supposed to have covered the reduction in ATP and the accumulation of FFA. In ischemic area, on the other hand, metabolic derangement was too severe to be

covered by the reduction in carnitine and followed by the reduction in ATP and the accumulation of FFA.

It is well known that, even in normal heart, there is significant gradient in pO_2 and regional blood flow from epicardium to endocardium.[37] Following coronary artery occlusion, pO_2 and regional blood flow declines more rapidly in subendocardial region than in subepicardial region.[14),37] Tissue levels of carnitine were lower in subendocardial region than in subepicardial region not only in ischemic and border areas but also in nonischemic area. The same finding was also observed in normal ventricular muscle. Moving inward from nonischemic area to ischemic area, the difference in ATP levels between subepicardial and subendocardial regions became more prominent. These results confirmed that subendocardial region was metabolically more anaerobic than subepicardial region even in normal heart and it became more prominent in ischemic heart.

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