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CORRELATION BETWEEN MYOCARDIAL BLOOD FLOW AND TISSUE SUCCINATE DURING ACUTE ISCHEMIA

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ABSTRACT

This study was performed to clarify the relationship between degree plus duration of myocardial ishemia and tissue succinate or lactate contents, and to assess whether their accumulation might be a metabolic marker of ischemic myocardium. Regional myocardial blood flow (MBF) was measured using the hydrogen gas clearance method in anesthetized open-chest dogs subjected to 10 min or 60 min of myocardial ischemia by coronary ligation. The contents of succinic acid and lactic acid in myocardium corresponding to locations of MBF measurement were quantitatively analyzed by gas chromatography-mass spectrometry. In severely ischemic areas with MBF less than 20 ml/min/100g, significant increases in both succinic and lactic acids were observed 10 min after coronary occlusion. Sixty min of ischemia induced significant increase in the myocardial succinic acid content not only in severely but also in moderately ischemic areas. In contrast, lactic acid was significantly increased only in the severely ischemic area 60 min after coronary occlusion. These results indicate a good correlation between degree plus duration of myocardial ischemia and tissue succinic acid content, suggesting that its accumulation in myocardium may be a reliable and sensitive metabolic marker of coronary ligation ischemia.

Key Words: Myocardial ischemia, Succinate, Lactate, Myocardial blood flow, Gas chromatography-mass spectrometry

INTRODUCTION

It is well known that ischemia induces myocardial damage, which is accompanied by a variety of metabolic alterations in myocardial tissue.[1–3] The majority of in vivo studies to investigate the effects of ischemia on myocardial metabolism have been carried out using experimental animals with coronary artery ligation.[4,5] Since this experimental model produces variable reduction in myocardial blood flow (MBF) in ischemic areas,[6] only limited information has previously been available concerning the relationships between severity of myocardial ischemia, degree of decrease in MBF, and the degree of metabolic alterations.

The introduction of flow meters to detect regional MBF[6–8] has, however, made it possible to quantify absolute levels of MBF and to correlate the severity of ischemia with electrophysiological or biochemical changes in the myocardium under ischemic conditions. We previously described a close relationship between a decrease in MBF and the severity of myocardial mitochondrial dysfunction or ultrastructural alterations.[9,10]

While the accumulation of lactate has been considered to be a reliable and early marker of ischemic changes in myocardial metabolism,[1,11,12] alterations in amino acid metabolism have also been reported to exist in mammalian oxygen-deprived heart.[13,14] Augmented myocardial

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alanine release and glutamate uptake, and the stimulation of succinate production have been demonstrated in patients with ischemic heart disease,[15] and in ischemic rabbit hearts,[13] respectively. Therefore, the present study was designed to investigate the relationship between MBF and tissue succinate or lactate contents in coronary ligated dogs and to assess whether the accumulation of succinate or lactate could be used as a metabolic marker to assess the severity and duration of ischemia.

MATERIALS AND METHODS

Animal preparation

This study was conducted on 30 adult mongrel dogs of either sex ranging in weight between 8 to 14 kg. The animals were anesthetized with sodium pentobarbital (50 mg/kg) given intraperitoneally. In each case, the trachea was intubated and artificial ventilation was instituted with a Harvard ventilator using room air. Maintenance of normal pH and blood gas values was obtained by adjusting tidal volume with regular checking of blood samples taken from the cannulated right femoral artery. Thoracotomy was performed through the fourth left intercostal space, and the heart was exposed following pericardiotomy.

Experimental protocol

The left anterior descending coronary artery (LAD) of each dog was ligated at a point just distal to the first diagonal branch (Fig. 1). Dogs were divided into two groups, subjected to 10 min or 60 min of myocardial ischemia by LAD ligation using a silk suture. After 10 or 60 min of LAD occlusion, specimens of cardiac muscles corresponding to MBF measurement sites were collected for determination of tissue succinate and lactate.

MBF measurements

MBF was measured by polarographic recording of hydrogen desaturation with platinum electrodes as described by Aukland et al.[7] Six wire-type platinum electrodes 0.08 mm in diameter were introduced into the heart muscle and fixed in position at a depth of 4–5 mm from the epicardial surface (midmyocardium): two electrodes in the ischemic area; two in the potentially border area; and the other two in the nonischemic area, as schematically indicated in Fig. 1. The electrodes were connected to a tissue rheometer (UH Meter PHG 201, Unique Medical Co., Ltd., Tokyo) and the hydrogen gas method was followed. Inhaled gas was changed from room air to a gas mixture containing about 8% hydrogen. After 3 min, the gas was switched back to room air, and the hydrogen in the myocardium was washed out. The MBF was calculated by the formula based on Kety's approach to blood-tissue exchange of inert gases.[16]

Sample preparation

Myocardial specimens were collected in about one sec, using a biopsy drill with a diameter of 5 mm,[17] and rapidly frozen in liquid nitrogen. After removing one third of the epicardial and endocardial portions of the myocardium, the samples were thoroughly homogenized in 0.5 ml of physiological saline solution in the presence of stable analogues of succinate and lactate (20 μg of succinic acid-d_6, 10 μg of sodium D, L-lactate-1,2,3-$^{13}C_3$, respectively), using a Potter homogenizer. Homogenized samples were deproteinized by addition of 3 ml of ethanol, and then the collected supernatant was evaporated at 40°C for 5 min to eliminate the ethanol by a rotary evaporator. After addition of 3 ml of distilled water, the sample solution was adjusted to pH 1.0 by 3N HCl. Organic acid fractions were obtained by repeated extraction (twice) with 3 ml of

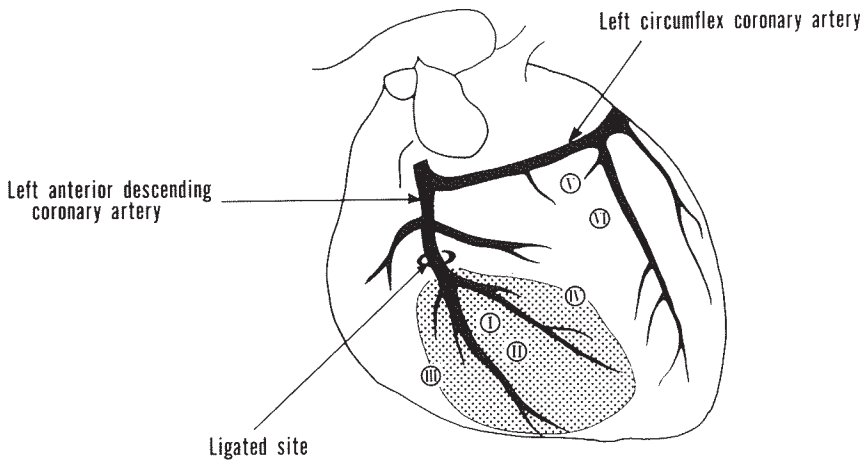

Fig. 1. Schematic diagram of the main coronary arteries in the canine heart. The ligated site is indicated, immediately distal to the first diagonal branch. The dotted area represents the region of ischemia. Platinum electrodes were inserted into the midmyocardium at the sites labeled I to VI (I and II, ischemic areas; III and IV, border areas; V and VI, nonischemic areas), and regional myocardial blood flow measured.

ethyl acetate. Organic solvent extracts (6 ml) were dried with anhydrous sodium sulphate (2g) and then evaporated to dryness at room temperature for 30 min under a stream of nitrogen.

The dried extracts were trimethylsilylated by adding 0.2 ml of N, O-bis (trimethylsilyl) trifluoroacetamide (Tokyo Kasei Co., Tokyo) to the residue; the mixtures were then heated to 60°C for 1 h in glass tubes. Aliquots of solution were subjected to gas chromatography-mass spectrometry analysis.[18,19]

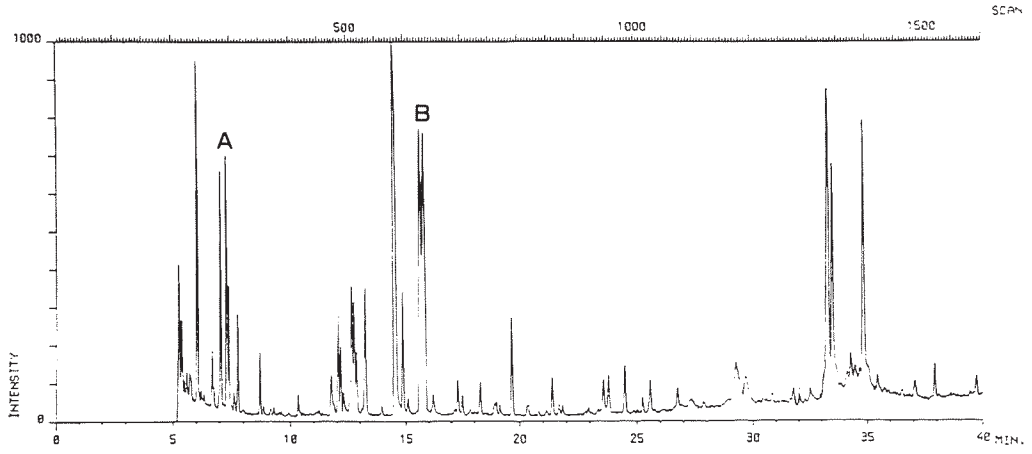

Fig. 2. Gas chromatogram of organic acids from canine myocardium after 60 min of ischemia. A mixture of homogenized myocardium and stable analogues of succinic acid and lactic acid was analyzed. The peaks were identified as follows: A, lactic acid and its analogue (sodium D,L-lactate-1,2,3-$^{13}C_3$); B, succinic acid and its analogue (succinic acid-d_6).

Gas chromatography-mass spectrometry

A Hewlett-Packard 5710A gas chromatograph (MA, USA) was used in combination with a JMS D-300 double-focusing mass spectrometer (JEOL, Tokyo). The data were stored and processed with a JMA 2000 data system (JEOL, Tokyo). The gas chromatograph was equipped with an OV-101 open-tubular glass capillary column (30 m×0.25 mm I.D.) and a splitless injector. Injection temperature was 250°C and the column temperature was programmed to change from 70°C to 260°C at 3°C/min. Electron-impact ionization (EI) mass spectra were recorded under the following conditions: ionizing energy, 70 eV; ionizing current, 300 μA; ion source temperature, 200°C; and accelerating voltage, 3 kV.[19,20]

Fig. 2 demonstrates a gas chromatogram of typical organic acid profiles in canine myocardium from the ischemic area after 60 min of LAD ligation. Each component of the profile was identified by EI mass spectra. The EI mass spectrum obtained from peak B (shown in Fig. 2) is demonstrated in Fig. 3 as an example, and the molecular ions at m/Z 247 and 251 were succinic acid and succinic acid-d_6, respectively. Quantitative determinations were performed using the peak height ratio between succinic acid and its stable analogue obtained from mass chromatograms. In this way lactic acid was identified and quantitatively determined.

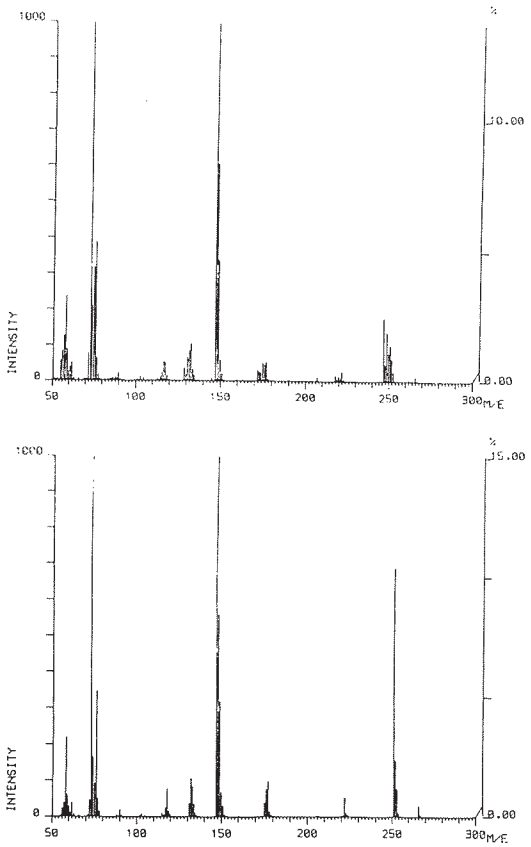

Fig. 3. Electron-impact ionization mass spectra of succinic acid (top) and succinic acid-d_6 (bottom), obtained from peak B in Fig. 2.

Statistical analysis

The results presented are mean±SE values. Statistical analysis was carried out by analysis of variance with Scheffe's test for multiple comparisons. The probability was considered significant if less than 0.05.

RESULTS

Fig. 4 and Fig. 5 show the effects of ischemia on the contents of succinic acid and lactic acid in the canine myocardium, in relation to the severity and duration of ischemia. In the assessment of change in the contents of succinic and lactic acids, the myocardium was divided into four regions according to MBF values. The MBF of the middle layer of the left ventricular wall in sham-operated animals was 103±22 ml/min/100g (units omitted below), and taking the mean-2SD of this value as the lower limit of normal, values of 60 and above were regarded as non-ischemic areas. The severely ischemic area was the region of the myocardium with MBF values of less than 20, the moderately ischemic area had MBF 20–40, and the mildly ischemic area MBF 40–60.

As shown in Fig. 4, 10 min of ischemia induced a significant increase in the content of succinic acid only in the severely ischemic area (1.87±0.42 μmol/g wet weight, units omitted below) as compared with the nonischemic area (0.45±0.14). Myocardial lactic acid content was also markedly higher in the severely ischemic area, with a value of 18.7±2.8, than in the non-ischemic area (7.0±0.6) (Fig. 5). After 60 min of coronary occlusion, myocardial succinic acid content was significantly increased not only in the severely but also in the moderately ischemic area. In contrast, only the severely ischemic area was found to have a significantly higher myocardial lactic acid content than the nonischemic area.

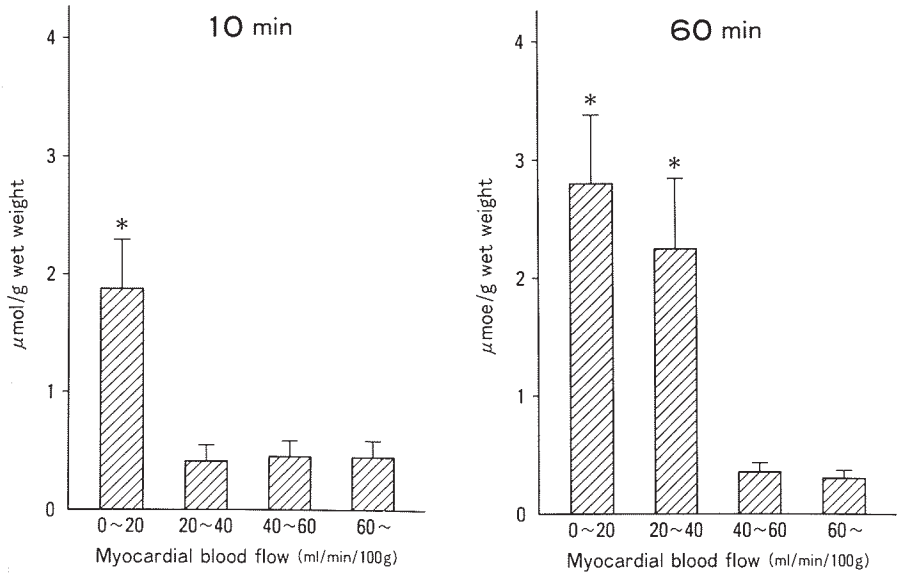

Fig. 4. Histograms showing the relationship between myocardial blood flow (MBF) and tissue succinic acid content after 10 min (left) and 60 min (right) of ischemia. Values are means±SE. *$p<0.01$ vs nonischemic area with MBF more than 60 ml/min/100g.

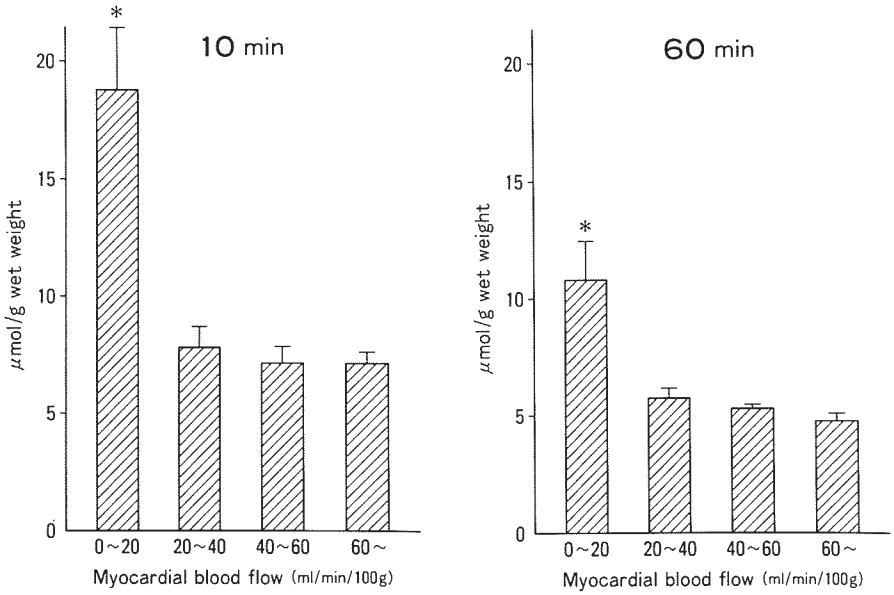

Fig. 5. Histograms showing the relationship between myocardial blood flow (MBF) and tissue lactic acid content after 10 min (left) and 60 min (right) of ischemia. Values are means±SE.
*$p<0.01$ vs nonischemic area with MBF more than 60 ml/min/100g.

DISCUSSION

The present investigation, designed to evaluate the effects of coronary-ligated ischemia on MBF and tissue succinate or lactate accumulation, revealed myocardial succinate content to be a more sensitive parameter than lactate content.

Since gas chromatography-mass spectrometry is a highly sensitive method of analysis which allows simultaneous measurement of mixtures containing a great number of compounds, it is often used to detect trace amounts of substances in the body. Thus, it was considered the most suitable method for separating and quantitatively determining organic acids in the 30 to 60 mg samples of myocardium, corresponding to MBF measurement sites, available in our experimental system.

Occlusion of a major coronary artery results in rapid conversion to anaerobic metabolism with consequent loss of high energy phosphates from the ischemic myocardium.[1,5,10,21] Synthesis of high energy phosphates continues in the ischemic tissue but at a much reduced rate compared with control aerobic conditions, because anaerobic glycolysis is the only source of new high energy phosphates, which are thus derived from the breakdown of glycogen to lactate.[1,3,5,22] Accumulation of lactate may therefore be considered as a metabolic marker of myocardial ischemic changes.

Traditionally, amino acids have not been thought of as active metabolites in either normal or ischemic myocardium, although specific examples such as aspartate have been known to be important for the function of certain intracellular shuttle mechanisms. This passive role of amino acids in cardiac metabolism has been questioned, however, and in vitro studies of anoxic rabbit myocardium corroborated an augmented glutamate utilization with alanine production.[13,15,23,24]

In a study of perfused anaerobic rat hearts, Penny and Cascarano also observed that some metabolites in the tricarboxylic acid cycle became accumulated. Succinate was markedly increased after perfusion with oxaloacetate +α-oxoglutarate or fumarate + malate + glutamate, and suggested that these metabolites substantially contribute to energy production from extraglycolytic sources.[25] Moreover, Sanborn et al. observed, using radioactive compounds, that the conversion of aspartate and glutamate to succinate was augmented during anoxia in the rabbit heart.[14] Taegtmeyer showed a reduced succinate production by hypoxic papillary muscles of rabbit heart after administration of the aminotransferase inhibitor, aminooxyacetate. These observations strongly indicate the likely existence of the pathways hypothesized by Hochachka et al., for anaerobic muscular energy production.[26]

The important finding in this investigation was that only in severely ischemic areas, the content of lactic acid markedly increased 60 min after the onset of ischemia. In contrast, at this time point, significant increases in the content of succinic acid were also observed in the moderately ischemic area. Although numerous high energy phosphates derived from anaerobic glycolysis are believed to be utilized in the ischemic myocardium,[1,5] non-glycolytic energy production through phosphorylation in the tricarboxylic acid cycle may be considered to play a role in the maintenance of cell function under these conditions.[24,27] This consideration and the results of our present study suggest that augmented succinate production from breakdown of amino acids occurred soon after (10 min) the onset of severe ischemia concomitantly with augmented lactate production from breakdown of glucose, and that the amino acid breakdown developed more markedly than glucose breakdown in moderately ischemic myocardium 60 min after the onset of ischemia. Our results thus suggest that accumulation of succinate in myocardium may be a reliable and sensitive metabolic marker of myocardial ischemia.

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