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An assessment of anaerobic metabolism during ischemia and reperfusion in isolated guinea pig heart

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The effects of total ischemia and subsequent reperfusion on the formation of anaerobic metabolism products and their release into myocardial effluent were studied in isolated guinea pig hearts. During 30-min ischemia myocardial ATP and phosphocreatine decreased to 34 and 15% of the initial levels, respectively; this was accompanied by alanine formation and approximately stoichiometric glutamate loss. The increase in malate in ischemic myocardium corresponded to the anaplerotic flux aspartate → oxaloacetate → malate; the succinate production being commensurable to α-ketoglutarate formation in the alanine aminotransferase reaction. The release of lactate, alanine, succinate, creatine and pyruvate trace amounts into the myocardial effluent was observed during an early phase of the reperfusion using ^{1}H-NMR. The rates of metabolite release reduced as follows: lactate ≫ alanine > succinate > creatine. By the 30th min of the reperfusion the decrease in these metabolites tissue contents was accompanied by the recovery of ATP and phosphocreatine levels up to 65 and 90% of the initial ones, respectively. The data obtained demonstrate that the formation and the release of succinate, alanine and creatine from the heart as well as of lactate may indicate profound disturbances in energy metabolism.

Introduction

Severe myocardial ischemia is known to be associated with a depletion of tissue high-energy phosphates and glycogen [1,2]. The liberation of breakdown products of energy-providing substrate, purine nucleotides, oxypurines, phosphate and lactate from ischemic myocardium has been well documented by many investigators [3–5]. It has been elucidated in the recent years that the onset of ischemia also led to accumulation of alanine and certain tricarboxylic acid cycle intermediates, citrate, malate and succinate in the cardiac tissue [6–9]. The data are available on the partial loss of creatine from ischemic myocardium as a result of ultrastructural changes of sarcolemma [10]. The indicated compounds can possibly be regarded as potential markers of myocardial ischemia. Myocardial net release of alanine and citrate in patients with chronic ischemic heart disease testifies to this possibility [11,12]. However, the metabolic rearrangements resulting in the formation of these compounds are not properly studied yet [6]. The contemporary literature is practically deprived of the information on their release from ischemic myocardium. The present study was undertaken to examine the alterations in metabolism of the main tricarboxylic acid cycle

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intermediates, related amino acids and creatine occurring under total myocardial ischemia. ^{1}H-NMR and enzymatic methods were applied to determine which of these compounds diffuse from the heart during subsequent reperfusion and to compare their yield with the lactate production.

Materials and Methods

Reagents. The enzymes were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. and Boehringer-Mannheim, F.R.G. ATP and phosphocreatine were obtained from Fluka, Switzerland. Other chemicals were purchased from Merck, Darmstadt, F.R.G. The solutions were prepared using deionized water (Milli Ro4 - Milli-Q, U.S.A.)

Heart perfusion. The experiments were performed on the isolated hearts of male guinea pigs (300–400 g). The hearts were perfused at 37 °C with oxygenated (95% O_2/5% CO_2) Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 2.5 mM Ca^{2+} and 5.5 mM glucose without recirculation. During the first 10 min the perfusion was carried out according to Langendorff [13] and during the next 20 min – according to Neely [14]. The hearts were allowed to beat spontaneously with the rate of 222 ± 4/min. After 30 min preliminary perfusion (steady state) the aortic output was 32 ± 3 ml/min per g wet tissue, the heart work index expressed as the product of the minute volume times the mean aortic pressure, was 2795 $\pm$ 206 ml/min per mm Hg. The hearts were subsequently arrested by 3 min retrograde perfusion with Krebs-Henseleit buffer containing 18 mM K^+ and subjected to total normothermal ischemia during the next 27 min. After the period of ischemic arrest 15 min open reperfusion was performed according to Langendorff and during further 15 min according to Neely. By the end of the reperfusion the aortic output recovered to 16 ± 3 ml/min per g wet tissue and the heart work index to 1480 ± 238 ml/min per mm Hg, the heart rate was 212 ± 6/min.

Preparation of tissue extracts and perfusates. By the end of the preperfusion, total ischemia or reperfusion the hearts were quickly frozen with a Wollenberger clamps precooled with liquid nitrogen. Perchloric acid extraction of tissues was performed as described elsewhere [15]. The myocardial effluents were collected in the ice-cooled tubes during the 30th min of preperfusion, and during the 1st, 2nd, 5th, 10th and 30th min of the reperfusion. For NMR measurements 2 ml of the effluents were freeze-dried and dissolved in 1 ml ^2H_2O (99%). The rest of perfusate was treated by cold 70% perchloric acid (0.1 ml/ml of perfusate) and centrifuged at $2000 \times g$ for 15 min at 4 °C. The supernatant was adjusted to pH 7.4 with 5 M K_2CO_3 and iced for 20 min. The $KClO_4$ precipitate was removed by centrifugation at 4 °C. Tissue dry wts. were determined by weighing a portion of washed pellets from the extraction with perchloric acid after they were dried overnight at 110 °C [15].

Analytical methods. The concentrations of the metabolites were assayed by enzymatic methods. ATP was measured as described by Lamprecht and Trantschold [16]. Phosphocreatine was determined in the same cuvette by subsequent addition of ADP and creatine kinase. ADP and creatine were measured by method of Bernt et al. [17] in the same assay. Glutamate, alanine, aspartate and lactate were determined essentially according to standard procedures [18–21]. The above-mentioned metabolites were measured in a Yanako UV-2000 spectrophotometer. An Aminco SPF-500 fluorometer was used for determination of the tricarboxylic acid cycle intermediates according to Williamson and Corkey [22].

NMR measurements. The spectra of the myocardial effluents were registrated in standard 5 mm ampoules using a Bruker WM-500 NMR spectrometer at 27 °C. The resolution in proton spectra at 500 MHz allowed for measurements of signals for all studied metabolites at concentration not less than 0.02 mM. To stabilize the resonance conditions the residual H_2O signal was presaturated for 2 s before data acquisition. The ^{1}H-NMR spectrum was acquired within 10 min. The resonance identifications were verificated by the addition of the corresponding compounds into the ampoule with the investigated sample.

Results

NMR and enzymatic studies of myocardial effluents

Fig. 1 illustrates ^{1}H-NMR spectra of the cardiac effluents obtained during the reperfusion after 30 min total ischemia. The spectrum of the pre-

ischemic coronary perfusate is presented for the sake of comparison (Fig. 1a). A high intensity of lactate, alanine, succinate and creatine signals as well as a low intensity of pyruvate one were observed in the spectra of the effluents corresponding to the first 2 min of the reperfusion. The intensity of the signals of the indicated compounds was significantly reduced by the 5th min of the reperfusion. Lactate resonances were only identified in the spectra of the perfusates obtained after 10 min of the reperfusion.

The release of these compounds from the post-ischemic myocardium was evaluated quantitatively by the enzymatic methods. During the first 2 min of the reperfusion the lactate leakage by an order exceeded the one for the other metabolites (Fig. 2a and b). As the duration of the reperfusion increased, the lactate release drastically reduced and by the 10th min of the reperfusion it was equal to the steady-state value (4.45 ± 0.51 μmol/g dry wt. per min). The rates of the rest metabolites release decreased as follows: alanine > succinate > creatine. According to NMR data only traces of these metabolites were detected in the myocardial effluents by the 10th min of the reperfusion. Creatine efflux ceased after the first 5 min of the reperfusion.

Cellular energy state of ischemic and reperfused heart

30 min total ischemia led to a depletion of 21.66 μmol of phosphocreatine per g dry wt. and resulted in practically equimolar increase in the content of creatine (22.16 μmol/g dry wt., Table I). As the result the total creatine content in the ischemic heart remained equal to the initial one. By the end of the reperfusion the phosphocreatine tissue content as well as the phosphocreatine/

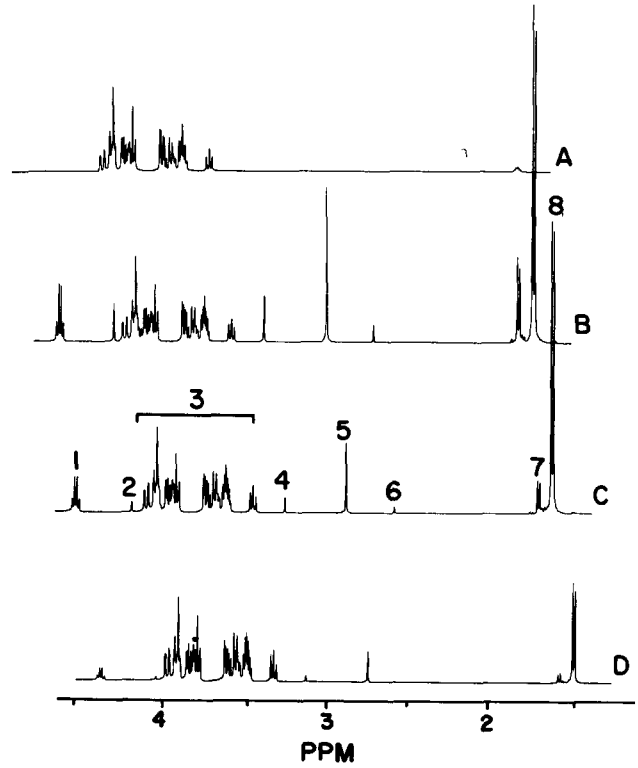

Fig. 1. 500 MHz ^{1}H-spectra of myocardial effluents of isolated perfused guinea-pig heart: A, 30 min of preperfusion (steady state); B, C, D, 1st, 2nd and 5th min of reperfusion after 30 min total ischemia, respectively. Resonance identifications: 1, lactate CH; 2, creatine CH_2; 3, glucose; 4, creatine CH_3; 5, succinate; 6, pyruvate; 7, alanine CH_3; 8, lactate CH_3.

TABLE I

EFFECTS OF TOTAL ISCHEMIA AND REPERFUSION ON TISSUE CONTENTS OF CREATINE AND HIGH-ENERGY PHOSPHATES IN ISOLATED GUINEA-PIG HEARTS

The hearts were perfused with bicarbonate Krebs-Henseleit buffer containing 5.5 mM glucose and 2.5 mM Ca^{2+} as described in Materials and methods. Metabolite concentrations are expressed in μmol/g dry wt. Data are the mean ± S.E. of 6–10 experiments. Cr, creatine; PCr, phosphocreatine; ΣCr, total creatine (Cr + PCr). Statistical treatment of the data presented in Tables I–III was performed using Scheffé multiple comparison method.

	Cr	PCr	ΣCr	PCr/Cr	ATP	ADP	ATP/ADP
Steady state	17.87 ± 1.37	25.42 ± 1.46	43.34 ± 2.10	1.42 ± 0.16	21.85 ± 1.12	4.12 ± 0.51	5.30 ± 0.31
Ischemia	40.03 ± 3.18 [a]	3.76 ± 0.54 [b]	43.82 ± 3.06	0.09 ± 0.02 [a]	7.39 ± 0.81 [b]	4.86 ± 0.49	1.52 ± 0.18 [a]
Reperfusion	16.90 ± 2.31 [c]	22.62 ± 2.75 [c]	39.50 ± 3.21	1.35 ± 0.19 [c]	14.26 ± 0.93 [a,c]	4.24 ± 0.42	3.36 ± 0.28 [a,c]

[a] $P < 0.05$, from the steady state.
[b] $P < 0.01$, from the steady state.
[c] $P < 0.05$, from ischemia.

creatine ratio recovered to the preischemic values despite the slight decrease in the total creatine occurring due to the partial creatine loss (about 3.55 μmol/g dry wt.). In contrast to phosphocreatine, ATP content recovered to 65% of the initial value, the ATP/ADP ratio being significantly decreased in the reperfused hearts as compared to the preischemic one (Table I). After the reperfusion the heart work index was reduced to 53% of the steady-state value (see Materials and Methods). Probably the loss of adenine nucleotides leading to a low restoration of ATP may be the reason for poor functional recovery during the reperfusion. However, these data indicate that the synthesis of phosphocreatine occurred rapidly even in energy-depleted post-ischemic myocardium. This possibility is related both to preservation of the inner mitochondrial membrane during the reperfusion [4] and to reversible damage of the sarcolemma which prevented the creatine kinase and creatine leakage into the perfusate [10]. Preservation of intracellular pool of creatine may be essential for phosphocreatine formation in the creatine kinase reaction, because K_m value of creatine is high [17].

Influence of ischemia and reperfusion on the end products of glycolysis, glutamate and aspartate

The increase in myocardial alanine during ischemia by 8.63 μmol/g dry wt. corresponded to the decrease in the glutamate tissue content by 8.33 μmol/g dry wt. Ischemia did not affect glutamine formation significantly. It was shown

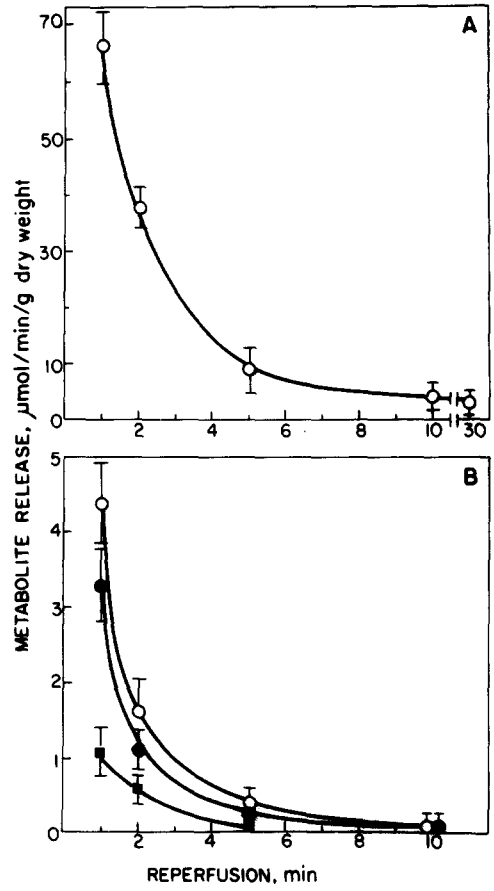

Fig. 2. The release of the anaerobic metabolism products into myocardial effluent during reperfusion of isolated guinea pig heart after 30 min total ischemia. (A) ○, lactate; (B) ○, alanine; ●, succinate; ■, creatine. Vertical bars denote S.E. of mean for 6–8 experiments.

TABLE II

CHANGES IN TISSUE CONTENTS OF SOME AMINO ACIDS AND LACTATE IN ISOLATED GUINEA-PIG HEARTS UNDER EFFECTS OF TOTAL ISCHEMIA AND SUBSEQUENT REPERFUSION

Perfusion conditions and the analytical procedures were as described in Materials and Methods. Values are the mean ± S.E. (in μmol/g dry wt.) of 6–8 experiments.

	Alanine	Glutamate	Aspartate	Lactate
Steady state	2.47 ± 0.44	16.94 ± 1.06	2.19 ± 0.28	3.37 ± 0.58
Ischemia	11.10 ± 1.24 [a]	8.61 ± 1.12 [a]	1.00 ± 0.19 [a]	142.70 ± 3.98 [b]
Reperfusion	3.18 ± 0.61	10.03 ± 1.28 [a]	1.18 ± 0.45	4.03 ± 0.81

[a] $P < 0.05$, from the steady state.
[b] $P < 0.01$, from the steady state.

previously that alanine is, like lactate, the end product of glycolysis and its carbon skeleton is formed from pyruvate, glutamic acid being a source of the amino group [23]. By the end of the reperfusion the alanine tissue content reduced to the initial one. The total alanine release during the reperfusion (approxim. 8.35 μmol/g dry wt., Fig. 2b) plus its content in the heart after the reperfusion (3.18 μmol/g dry wt.) were equal to its tissue content at the end of the ischemic period (Table II). These data indicate that alanine formed during ischemia is completely released into the myocardial effluent under the reperfusion. During the normothermal ischemic cardiac arrest the lactate production exceeded the alanine formation 17 times (Table II). By the 10th min of the reperfusion the lactate tissue content decreased to the initial value mainly due to washout of its bulk amount. This fact suggests that the cardiac muscle, damaged by severe, prolonged ischemia, has a reduced ability to oxidize lactate when returned to oxidative metabolism.

The content of the tricarboxylic acid cycle intermediates in ischemic and reperfused hearts

During ischemia the succinate content in the heart increased by 6.67 μmol/g dry wt. (Table III). Under oxygen deprivation the span from citrate to α-ketoglutarate in the tricarboxylic acid cycle appears to be inoperative, and α-ketoglutarate formed in the alanine aminotransferase reaction is the primary succinate source [6,24] (Fig. 3). Thus, the net α-ketoglutarate production is obviously equal to the net alanine one, and in our case was 8.63 μmol/g dry wt. during 30 min ischemia (Table II). Taking into account the decrease in myocardial aspartate during the ischemic period (Table II), it appears that 1.19 μmol/g dry wt. of α-ketoglutarate was consumed in the coupled aspartate aminotransferase reaction. Aspartate utilization in the purine nucleotide cycle seems unlikely because the adenylosuccinate synthase reaction should be inhibited by energy deficiency in ischemia [25]. Only a minute amount of α-ketoglutarate could be used for glutamate formation in

TABLE III

EFFECTS OF TOTAL ISCHEMIA AND REPERFUSION ON TISSUE CONTENTS OF SOME TRICARBOXYLIC ACID CYCLE INTERMEDIATES IN ISOLATED GUINEA-PIG HEARTS

For the experimental protocol see Materials and Methods. Values are the mean ± S.E. (in μmol/g dry wt.) from 6 experiments.

	Succinate	Malate	Citrate	Fumarate	α-Ketoglutarate
Steady state	1.32 ± 0.22	0.57 ± 0.07	0.59 ± 0.08	0.08 ± 0.01	0.36 ± 0.04
Ischemia	7.99 ± 0.90 [a]	2.01 ± 0.38 [a]	0.62 ± 0.14	0.11 ± 0.02	0.07 ± 0.02 [b]
Reperfusion	1.29 ± 0.28 [c]	0.64 ± 0.09 [c]	0.49 ± 0.06	0.07 ± 0.01	0.26 ± 0.08 [c]

[a] $P < 0.05$, from the steady state.
[b] $P < 0.02$, from the steady state.
[c] $P < 0.05$, from ischemia.

the glutamate dehydrogenase reaction, since a pronounced glutamate fall was observed in ischemic myocardium (Table II). This means that about 7.44 μmol/g dry wt. of α-ketoglutarate should be spent on the replenishment of the tricarboxylic acid cycle intermediates, which is in good accord with the revealed succinate synthesis. Despite the anaplerosis coupled with the alanine formation, the myocardial α-ketoglutarate content decreased in ischemia (Table III). This implies rapid conversion of α-ketoglutarate to succinate. The decrease in succinate tissue content at the beginning of the reperfusion was caused mainly by its leakage into the perfusate (about 5.60 μmol g dry wt., Fig. 2b) and to a lesser degree by oxidation in the tricarboxylic acid cycle.

The citrate and fumarate concentrations did not change significantly during the total ischemia (Table III), but malate increased rapidly, reaching a value which was 4-fold higher than the initial one. The malate increment (approxim. 1.40 μmol/g dry wt. per experimental period of ischemia, Table III) was near the calculated formation of oxaloacetate in the aspartate aminotransferase reaction. This is accounted for the fact that in ischemia practically all oxaloacetate is converted to malate by virtue of increased $NADH/NAD^+$ ratio, and the oxaloacetate tissue content remained low in spite of its continuous replenishment by the coupled transaminations [6]. We did not determine the oxaloacetate concentration in the present study, since its pool is only a minor part of that of malate [26]. In contrast to succinate, the malate release into the myocardial effluent was observed neither by means of NMR nor enzymatically. By the end of the reperfusion the malate content in the cardiac muscle reduced to the preischemic value, testifying to its oxidation and probably simultaneous disposal catalyzed by the $NADP^+$-dependent malic enzyme [6,27].

Discussion

The data obtained prove that disturbances of energy metabolism in total myocardial ischemia are associated with the rise in the tissue levels of alanine, malate, and succinate. The formation of these metabolites is caused by almost stoichiometric consumption of glutamate and aspartate in the alanine and aspartate aminotransferase reactions which present an anaplerotic mechanism. Indeed, the net amount of alanine formed during ischemia really approximated the net glutamate loss (Table II). This fact is consistent with the results of the previous animal studies [15,28,29] and agrees with the positive correlation between myocardial

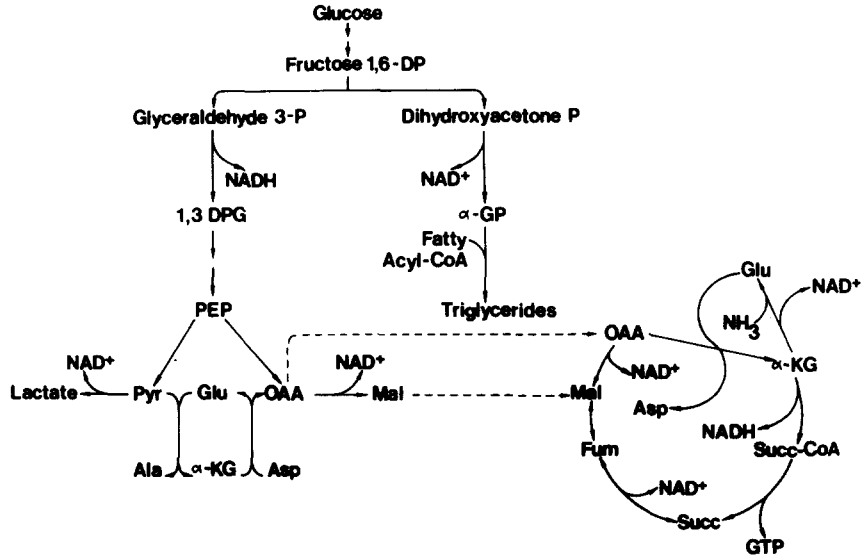

Fig. 3. Metabolic pathways illustrating coupling of alanine and succinate formation with glycolysis and the reactions of the tricarboxylic acid cycle in ischemic myocardium.

glutamate uptake and alanine release in patients with chronic ischemic heart disease [12]. An increase in malate formation observed during the ischemic period in this study corresponded to the anaplerotic flux aspartate → oxaloacetate → malate, and the succinate production was comparable to the α-ketoglutarate yield in glutamate transamination (Tables II and III).

An analysis of the intermediary metabolism in the anoxic and ischemic myocardium suggested that succinate derived from glutamate is an end product of the tricarboxylic acid cycle (Fig. 3) [23,27–29,30]. Elimination of succinate and alanine synthesis during anoxia by aminooxyacetate, an inhibitor of alanine and aspartate aminotransferase, provides further evidence for amino acid catabolism in response to oxygen deprivation [15,29]. Augmented succinate formation also indicates anaerobic mitochondrial energy production through substrate level phosphorylation occurring at the convertion of succinyl CoA to succinate [31] and oxidation of NADH by fumarate [32]. These nonglycolytic sources of energy probably play a compensatory role in the adaptation of diving animals [33] and man [34] to hypoxia. The physiological significance of these pathways has been confirmed by the ability of exogenous glutamate, aspartate and α-ketoglutarate to improve the performance of anoxic or ischemic myocardium via stimulation of mitochondrial synthesis of ATP and GTP coupled to succinate and alanine formation [15,35,36].

It should be noted that alanine and succinate accumulation in energy-depleted myocardium (Tables I–III) was accompanied by the release of these metabolites, as lactate, from post-ischemic hearts at the early phase of the reperfusion (Figs. 1 and 2). The return to oxidative metabolism and restoration of ATP and phosphocreatine levels during further reperfusion led to a decrease in the myocardial contents of alanine and succinate to the preischemic values and a cessation of their leakage into the perfusate (Figs. 1 and 2, Tables I–III). Lactate and alanine rapidly pass through myocardial cell membranes, while membrane kinetics of succinate is not well known. This dicarboxylic acid is normally confined to mitochondrial, and its efflux in the extramitochondrial space occurs by exchange with malate or inorganic phosphate [37]. A significant elevation of succinate in plasma was documented during acute hypoxia in rats [38] and in man either after acute hypoxia [39] or after an exhausting exercise [34]. The diffusion of succinate simultaneously with lactate and alanine from hypoxic rabbit papillary muscles into incubation media was reported in Ref. 29. These findings may be attributed to an effect of hypoxia on succinate transport across cellular membranes. However, an augmentation of succinate release was demonstrated during oxygenative incubation of papillary muscles with the added succinate precursors, aspartate, malate and α-ketoglutarate [29]. Therefore, the appearance of succinate in the extracellular space may reflect not only a membrane damage, but also increased rates of its synthesis. The ability of succinate and alanine, in contrast to that of malate, to diffuse into myocardial effluent indicates the valuability of these compounds as markers of lesions of cardiac oxidative metabolism.

The data of the present report show that succinate and alanine are accumulated and released from ischemic myocardium in by far less amounts than lactate. Their formation and release may probably be more pronounced in myocardial ischemic injury in vivo or in heart perfusion with fatty acids or ketone bodies [40]. A formation of alanine in transamination of glutamate with pyruvate requires a system to maintain the oxidation-reduction balance of the glycolytic pathway. A reoxidation of NADH may principally occur in two cytosolic reactions catalyzed by the glycerolphosphate dehydrogenase and the malate dehydrogenase (Fig. 3). Increased synthesis of triglycerides in ischemia accompanied by α-glycerolphosphate expenditure promotes the formation of the latter from dihydroxyacetone phosphate and, thus, reoxidation of NADH [41]. The production of oxaloacetate from aspartate is obviously insufficient to generate the required amount of NAD^+ in the malate dehydrogenase reaction (1.44 μmol of malate vs. 8.63 μmol of alanine, Tables II and III). The phospho*enol*pyruvate carboxykinase reaction may serve as an additional source of oxaloacetate. The existence of this anaplerotic route has been reported for different muscle types including myocardium [42]. Tissue

acidosis as well as an accumulation of alanine and succinate promotes the activation of this enzyme [44]. The reduction of oxaloacetate, derived from phospho*enol*pyruvate, to malate may also diminish the deficiency of NAD^+. On the other hand, the oxidation of α-ketoglutarate, formed in the alanine aminotransferase reaction, into succinate in mitochondria requires NAD^+ for the α-ketoglutarate dehydrogenase. In ischemic myocardium at least three mitochondrial reactions may lead to generation of NAD^+: (1) the conversion of oxaloacetate to malate caused by increased $NADH/NAD^+$ ratio; (2) the oxidation of NADH by fumarate reductase system producing ATP; (3) the reductive amination of α-ketoglutarate by NAD-dependent glutamate dehydrogenase (Fig. 3). It is difficult to evaluate the contribution of these potential mechanisms from the obtained data. However, the previous study on succinate synthesis in oxygen-deprived rat heart mitochondria suggested that two molecules of NAD^+ formed by the reduction of oxaloacetate and fumarate are utilized in the α-ketoglutarate dehydrogenase reaction [43]. In addition, malic enzymes can take part in the regulation of NADH and NADPH-linked metabolic pathways in cardiac muscle [6,26,27,40], but its significance in ischemia remains unclear.

The data of the present study substantiate an accumulation of creatine in the heart during the total ischemia occurring in phosphocreatine depletion and its subsequent release into the perfusate upon the early phase of the reperfusion (Table I, Figs. 1 and 2). Irreversible loss of creatine due to its release into the cardiac effluent may be a likely explanation of a decreased capacity to regenerate phosphocreatine and consequently of insufficient recovery of the cardiac function particularly when the ATP content is significantly reduced [4,7,45]. Unlike alanine and succinate, diffusing across the cell membranes [11,12,15,23,28], the creatine efflux occurs most likely due to a sarcolemmal disruption [10]. Thus, creatine detection in the coronary perfusates may also indicate the increased membrane permeability and the loss of cell volume regulation.

We believe that an establishment of a more close relationship between energy state of the myocardium and the formation of the studied compounds will be perspective in further studies of metabolic and functional disturbances in ischemia. The results of this and the previous works [46,47] demonstrate that ^{1}H-NMR can be a promising tool in monitoring of myocardial metabolism complementary to ^{31}P-NMR measurements.

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References

1 Danforth, W.H., Naegle, S. and Bing, R.J. (1960) Circ. Res. 8, 965–971.
2 Liedtke, A.J. (1981) Prog. Cardiovasc. Dis. 23, 321–336.
3 Rovetto, M.J., Whitmer, J.T. and Neely, J.R. (1973) Circ. Res. 32, 699–711.
4 Schaper, J., Mulch, J., Winkler, B. and Schaper, W. (1979) J. Mol. Cell. Cardiol. 11, 521–541.
5 Swain, J.L., Sarbina, R.L., McHale, P.A., Greenfield, J.C. and Holmes, E.W. (1983) Am. J. Physiol. 242, H818–H826.
6 Peuhkurinen, K.J., Takala, T.S.E., Nuutinen, E.M. and Hassinen, I.E. (1983) Am. J. Physiol. 244, H281–H288.
7 Taegtmeyer, H., Roberts, A.F.C. and Raine, A.E.G. (1985) J. Am. Coll. Cardiol. 6, 864–870.
8 Steenbergen, C., DeLeeuw, G. and Williamson, J.R. (1978) J. Mol. Cell. Cardiol. 10, 56–67.
9 Williamson, J.R., Ford, C., Illingworth, J. and Safer, B. (1976) Circ. Res. 38, 139–151.
10 Reimer, K.A., Jennings, R.B. and Hill, M.L. (1981) Circ. Res. 49, 901–911.
11 Mudge, G.H., Mills, R.M., Taegtmeyer, H., Gourlin, R. and Lesch, M. (1976) J. Clin. Invest. 58, 1185–1192.
12 Thomassen, A.R., Neilsen, T.T., Bagger, J.P. and Henningsen, P. (1983) Clin. Sci. 64, 33–40.
13 Langendorff, O. (1895) Pflügers Arch. Gesamte Physiol. 61, 291–332.
14 Neely, J.R., Rovetto, M.J., Whitmer, J.T. and Morgan, H.E. (1973) Am. J. Physiol. 225 (3), 651–658.
15 Pisarenko, O.I., Solomatina, E.S., Ivanov, V.E., Studneva, I.M., Kapelko, V.I. and Smirnov, V.N. (1985) Basic Res. Cardiol. 80, 126–134.
16 Lamprecht, W. and Trautschold, I. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), Vol. 4, pp. 2101–210, Academic Press, New York.
17 Bernt, E., Bergmeyer, H.U. and Mollering, H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), Vol. 4, pp. 1772–1776, Academic Press, New York.
18 Bernt, E. and Bergmeyer, H.U. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), Vol. 4, pp. 1704–1708, Academic Press, New York.
19 Grassl, M. (1974) in Methods of Enzymatic Analysis

(Bergmeyer, H.U., ed.), Vol. 4, pp. 1682–1685, Academic Press, New York.
20 Bergmeyer, H.U., Bernt, E., Mollering, H. and Pfleiderer, G. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) Vol. 4, pp. 1696–1700, Academic Press, New York.
21 Hohorst, H.J. (1963) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), pp. 266–270, Academic Press, New York.
22 Williamson, J.R. and Corkey, B. (1969) Methods Enzymol. 13, 439–513.
23 Taegtmeyer, H., Peterson, M.B., Ragavan, V.V., Ferguson, A.G. and Lesch, M. (1977) J. Biol. Chem. 252, 5010–5018.
24 Pisarenko, O.I., Solomatina, E.S. and Studneva, I.M. (1986) Biochim. Biophys. Acta 885, 154–161.
25 Tornheim, K. and Lowenstein, J.M. (1972) J. Biol. Chem. 247, 162–169.
26 Hiltunen, J.K. and Hassinen, I.E. (1977) Int. J. Biochem. 8, 505–509.
27 Peuhkurinen, K.J., Nuutinen, E.M., Pietilainen, E.P., Hiltunen, J.K. and Hassinen, I.E. (1983) Biochem. J. 208, 577–581.
28 Sanborn, T., Gavin, W., Berkowitz, S., Perille, T. and Lesch, M. (1979) Am. J. Physiol. 237, 535–541
29 Taegtmeyer, H. (1978) Circ. Res. 43, 808–815.
30 Fréminet, A., Leclerc, L., Poyart, C., Huel, C. and Gentil, M. (1980) J. Physiol. (Paris) 76, 113–117.
31 Hunter, F.E. (1949) J. Biol. Chem. 177, 361–372.
32 Sanadi, D.R. and Fluharty, A.L. (1963) Biochemistry 2, 523–528.
33 Hochachka, P.W. and Storey, K.B. (1975) Science 187, 613–621.
34 Hochachka, P.W. and Dressendorfer, R.H. (1976) Eur. J. Appl. Physiol. 35, 235–242.
35 Bittl, J.A. and Shine, K.I. (1983) Am. J. Physiol. 245, H406–H419.
36 Matsuoka, S., Jarmakani, J.M., Young, H.H., Uemura, S. and Nakanishi, T. (1986) J. Mol. Cell Cardiol. 18, 897–906.
37 LaNoue, K., Nicklas, W.J. and Williamson, J.R. (1970) J. Biol. Chem. 245, 102–111.
38 Fréminet, A. and Leclerc, L. (1980) J. Physiol. (Paris) 76, 677–691.
39 Iles, R.A., Barnett, D., Strunin, L., Strunin, J.M., Simpson, B.R. and Cohen, R.D. (1972) Clin. Sci. 42, 35–45.
40 Peuhkurinen, K.J. (1982) Biochim. Biophys. Acta 721, 124–134.
41 Neely, J.R. and Morgan, H.E. (1974) Annu. Rev. Physiol. 36, 413–421.
42 Nolte, J., Brdiczka, D. and Pette, D. (1972) Biochim. Biophys. Acta 284, 497–507.
43 Pisarenko, O.I., Khlopkov, V.N. and Ruuge, E.K. (1986) Biochem. Internat. 12, 145–154.
44 Hochachka, P.W. and Mustafa, T. (1972) Science 176, 1056–1066.
45 Bailey, I.A., Seymour, A.L. and Radda, G.K. (1981) Biochim. Biophys. Acta 637, 1–7.
46 Brown, T.R., Kincaid, B.M. and Ugurbil, K. (1982) Proc. Natl. Acad. Sci. USA 79, 3523–3526.
47 Ugurbil, K., Petein, M., Maidan, R., Michurski, S., Cohn, J.N. and From, A.H. (1984) FEBS Lett. 167, 73–77.