Biochemical Studies on Energy Metabolism in the Conduction System of Bovine Heart

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SUMMARY

The conduction system of bovine heart was studied to clarify the characteristics of its energy metabolism. Results showed that its mitochondrial oxygen uptake was much lower than that of ordinary heart muscle with succinate as substrate but similar to that of ordinary heart muscle with glutamate+malate as substrate. The activity of succinate dehydrogenase was lower than that in ordinary heart muscle. The total activity of isozymes of lactate dehydrogenase was lower in the specialized heart muscle than in ordinary heart muscle. The main isozyme of lactate dehydrogenase in the specialized heart muscle was LDH 1 (H_4). The cytoplasmic $NAD^+/NADH$ ratio was much higher in the specialized heart muscle than in ordinary heart muscle. These results suggest that in the specialized heart muscle energy is mainly derived from aerobic metabolism.

Additional Indexing Words:

Mitochondrial oxygen uptake Succinate dehydrogenase Lactate
dehydrogenase Isozyme of lactate dehydrogenase $NAD^+/NADH$
ratio Ordinary heart muscle Specialized heart muscle

THE cardiac conduction system has a central function in propagation of impulses. This tissue is known to differ from ordinary heart muscle both morphologically and physiologically, and thus it is thought that it probably also differs from the latter metabolically. In fact, certain metabolic differences have been found. Histochemical studies have shown that its glycogen content is higher,^{1),2)} and its succinate dehydrogenase activity is much lower than those in ordinary heart muscle.^{3),4)} Moreover, oxygen uptake of slices of the specialized heart muscle is lower than that of slices of ordinary heart muscle.^{5),6)} Furthermore, the specialized heart muscle has

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a greater capacity to survive anoxia.⁷⁾ These findings suggested that in the specialized heart muscle energy might be mainly supplied by anaerobic metabolism.

In this study we compared the characteristics of energy metabolism in the cardiac conduction system and in ordinary heart muscle. For this purpose, we examined energy generation in these tissues by measuring mitochondrial respiration, the activity of succinate dehydrogenase, the activity and isozyme pattern of lactate dehydrogenase, and the cytoplasmic NAD^+/NADH ratio.

MATERIALS AND METHODS

In ungulates, the cardiac conduction system can easily be separated from the surrounding myocardium,⁸⁾ and in this study we used bovine heart for experiments, because its conduction system was large and discrete. The heart was rapidly removed at the slaughterhouse and brought to the laboratory on ice, and the conduction system was dissected out as soon as possible in a cold room. Fig. 1 is a photograph of the conduction system of a bovine heart; it is composed from an atrioventricular node, a bundle of His, and right and left bundle branches. Ordinary heart muscle was obtained from the left ventricular wall.

Mitochondria were prepared as described by Chance and Hagihara.⁹⁾ Specialized or ordinary heart muscle was finely minced in cold isolation medium (0.21 M mannitol-0.07 M sucrose, 0.2 mM EDTA, adjusted to pH 7.4 with Tris base at 4°C), and incubated with Nagarse (crystalline bacterial alkaline proteinase) for 20 min at 4°C. The resulting suspension was diluted 2-fold and homogenized

Fig. 1. Specialized heart muscle from bovine heart. The specialized heart muscle is composed of an atrioventricular node (AVN), bundle of His (His), and right and left bundle branches (RBB and LBB).

in a glass teflon homogenizer. The homogenate was centrifuged for 5 min at 500 g and the supernatant obtained was centrifuged for 20 min at 12,000 g in a refrigerated centrifuge. The precipitate was suspended in the isolation medium and promptly used for analysis of mitochondrial respiration. Mitochondrial respiration was measured polarographically in reaction medium containing 10 mM phosphate buffer (pH 7.4), 0.3 M mannitol, 0.01 M KCl, and 0.25 mM EDTA. Succinate or glutamate+malate was used as substrate. The following indicators of mitochondrial respiration were determined by the procedure of Chance and Williams¹⁰⁾: mitochondrial oxygen uptake (natoms oxygen uptake/mg protein/min) and the P/O ratio (n mole ATP produced/n atom oxygen consumed).

Succinate dehydrogenase (SDH) activity was measured with a 2 wavelength spectrophotometer (Hitachi 356) at 550–600 nm in the following assay medium¹¹⁾: 60 mM phosphate buffer (pH 7.6), 1 mM KCN, 72 μ M 2, 6-dichlorindophenol (DCIP), 0.1 mM phenazine methosulphate (PMS), 0.75 mM CaCl_2 , 10 μ M phospholipase C, and 20 mM succinate. Enzyme activity was expressed as nmoles DCIP reduced/mg protein/min.

Lactate dehydrogenase (LDH) activity was measured spectrophotometrically at 340 nm in 0.1 M phosphate buffer (pH 7.4), 0.1 mM NADH and 1 mM Na pyruvate.¹²⁾ Enzyme activity was expressed as moles NADH oxidized/mg protein/min. Disc gel electrophoresis was performed to resolve the isozymes of LDH. After electrophoresis, LDH activity on the gel was located by incubating the gel in 20 ml of 0.1 M phosphate buffer (pH 7.4) containing 2.3 Gm of NAD and 2 mg of PMS. The densitogram was recorded with a dual-wavelength TLS scanner (Schimazu CS-900).

The redox state of the cytoplasm was calculated from the following equation: $\text{NAD}^+/\text{NADH} = \text{pyruvate}/\text{lactate} \times 1/K$ ($K = 1.11 \times 10^{-4}$).¹³⁾ Lactate and pyruvate contents were measured at 460 nm with a fluorescence spectrophotometer (Hitachi MPF-2)¹⁴⁾ using the following assay media: 60 mM phosphate buffer (pH 7.6), 0.1 mM NADH, 0.5 μ g LDH for lactate assay.¹⁵⁾

The protein concentrations of mitochondrial and supernatant fractions were measured by the method of Lowry et al¹⁶⁾ with crystalline bovine serum albumin as a standard.

Results are expressed as mean $\pm$ S.D. of the number of experiments indicated.

RESULTS

Table I shows the values for various parameters of respiration of mitochondria isolated from specialized and ordinary heart muscles. The P/O ratios of both types of muscle were nearly the theoretical values of 2.0 for succinate and 3.0 for glutamate+malate. These results also show that the preparations of mitochondria of both types of muscle were as active for oxidative phosphorylation. The oxygen uptakes (natoms oxygen uptake/mg protein/min) of mitochondria of specialized and ordinary heart muscles were 126.5 and 220.2, respectively, with succinate as substrate and 138.5 and 148.6, respectively, with glutamate+malate as substrate. Oxygen uptake of mitochondria of the specialized heart muscle was significantly lower than

Table I. Parameters of Respiration of Mitochondria of Heart Muscles

Heart Muscle	Substrate	Q_{O_2}	P/O
Specialized (n=9)	Succinate	126.5 ± 30.4	1.8 ± 0.2
Ordinary (n=20)	„	220.2 ± 32.8	2.2 ± 0.2
		$p < 0.001$	$p < 0.001$
Specialized (n=14)	Glutamate+Malate	138.9 ± 21.8	2.8 ± 0.2
Ordinary (n=23)	„	148.6 ± 20.2	2.9 ± 0.2
		N.S.	N.S.

Values are means $\pm$ SD for the number of experiments shown in parentheses. P values indicate significance of differences between values for the 2 heart muscles. N.S.=not significant; Q_{O_2} =nanomoles oxygen uptake/mg protein/min.

Fig. 2.

Fig. 3.

Fig. 2. (left). Succinate dehydrogenase activities of bovine heart. □, ordinary heart muscle; ▨, specialized heart muscle. The P value shows the significance of the difference between the 2 activities.

Fig. 3 (right). Lactate dehydrogenase activities of bovine heart. □, ordinary heart muscle; ▨, specialized heart muscle. The P value shows the significance of the difference between the 2 activities.

that of mitochondria of ordinary heart muscle with succinate as substrate, but not significantly different from the latter with glutamate+malate as substrate. Oxygen uptake by mitochondria of the specialized heart muscle was similar with succinate and glutamate+malate as substrates.

Since oxygen uptake by mitochondria of the specialized heart muscle was very low with succinate as substrate, we measured their succinate dehydrogenase activity. Fig. 2 shows that the SDH activity (nmoles DCIP reduced/mg protein/min) of the specialized heart muscle (29.4) was significantly lower than that of ordinary heart muscle (81.5).

LDH activity is an index of anaerobic metabolism. Fig. 3 shows that the LDH activity (moles NADH oxidized/mg protein/min) in specialized heart muscle (0.37) was significantly lower than that in ordinary heart muscle (2.55). Fig. 4 shows densitograms of the isozymes of LDH separated by electrophoresis. Specialized heart muscle gave 1 main band, ordinary heart muscle gave 2, and a mixture of the 2 heart muscles gave 2 major bands in the positions of those of the ordinary heart muscle. These results show that the main component in specialized heart muscle is LDH 1 (H_4), whereas the main components in ordinary heart muscle are LDH 1 (H_4) and LDH 2 (H_3M_1).

As shown in Table II, the $NAD^+/NADH$ ratio in the cytoplasm was 1251 in specialized heart muscle and 77 in ordinary heart muscle. The

Fig. 4. Densitometric trace of LDH isozymes of bovine heart. (a), (b), and (c) are tracings of isozymes in specialized heart muscle, ordinary heart muscle, and a mixture of the 2, respectively.

Table II. Cytoplasmic $NAD^+/NADH$ Ratios of Heart Muscles

Heart Muscle	$NAD^+/NADH$
Specialized (n=15)	1251
Ordinary (n=15)	77

higher NAD^+/NADH ratio in the specialized heart muscle provides support for the idea that this muscle is an aerobic tissue.

DISCUSSION

Mitochondria have a central role in generating ATP and are also important in energy metabolism. The mitochondria of specialized heart muscle have not previously been isolated. Therefore, we isolated them and investigated their respiration. Fig. 5 is a schematic representation of the electron transfer systems of specialized heart muscle and ordinary heart muscle. Since succinate dehydrogenase activity is low in specialized heart muscle, electron transfer from succinate to the respiratory chain is slower in specialized heart muscle than in ordinary heart muscle. On the other hand, since mitochondrial oxygen uptake is similar in the 2 types of heart muscle with glutamate+malate as substrate, the rates of electron transfer from NADH-dependent substrates to the respiratory chain seem to be similar in these muscles. Furthermore, the rates of electron transfer from succinate and from NADH-dependent substrates seem to be similar in specialized heart muscle.

Since the LDH reaction strongly favors lactate formation from pyruvate, the activity of LDH can be thought to express the width of the pathway of lactate formation. Histochemical studies have shown that the LDH activity of specialized heart muscle is low or the same as that of ordinary heart muscle.^{1),3)} In isolated tissues LDH must be measured rapidly, because it is unstable. Our spectrophotometric measurements of LDH activity in freshly isolated preparations showed that formation of lactate was less in specialized

Fig. 5. Schematic representation of the electron transfer systems of mitochondria of specialized and ordinary heart muscles. The rate of electron transfer from NADH-dependent substrates to the respiratory chain was similar in specialized heart muscle and in ordinary heart muscle, but the rate of electron transfer from succinate to the respiratory chain was slower in specialized heart muscle than in ordinary heart muscle.

heart muscle than in ordinary heart muscle. There are 5 isozymes of LDH, each composed of 4 subunits of 2 parent molecules, the H-type and M-type; the H-type is found in aerobic tissues, such as cardiac muscle, and M-type is found in the more anaerobic tissues, such as voluntary skeletal muscle. The cellular proportion of these subunits are changed by various factors: i.e., differentiation, hormonal responses, loss of the nerve supply, various diseases and change in oxygen tension.^{17),18)} A low oxygen tension favors synthesis of the H-type.^{19),20)} Thus the isozyme pattern of LDH reflects the metabolism of a given tissue. Our findings on the isozyme patterns of LDH indicate that the specialized heart muscle is an aerobic tissue.

The factor controlling the direction of the LDH reaction is the NAD^+/NADH ratio. We found that the cytoplasmic NAD^+/NADH ratio was high in specialized heart muscle. In other words, the redox state of the cytoplasm of the specialized heart muscle may be higher than that of ordinary heart muscle. Therefore, the LDH reaction should favor pyruvate formation from lactate more in specialized heart muscle than in ordinary heart muscle.

It has been considered that in specialized heart muscle energy is supplied mainly by anaerobic metabolism from the following reasons: (i) Oxygen uptake by slices of the conduction tissue is low.^{5),6)} (ii) Succinate dehydrogenase activity is low in specialized heart muscle.^{3),4)} (iii) The glycogen content of the specialized heart muscle is high.^{1),2)} (iv) Specialized heart muscle can survive anoxia.⁷⁾ However, we found that oxygen uptake by mitochondria of the specialized heart muscle was almost the same to that of mitochondria of ordinary heart muscle with NADH -dependent substrates. Moreover, although the succinate dehydrogenase activity of mitochondria from specialized heart muscle was low, their oxygen uptakes with succinate and with glutamate+malate were almost the same. These findings eliminate reasons (i) and (ii) for concluding that specialized heart muscle depends mainly on anaerobic metabolism. It is well known that increase in glycolysis is an important compensatory mechanism in myocardial ischemia.²¹⁾ Myocardial glycogen can be broken down during enhanced glycolysis and anaerobic ATP generation to meet the needs of the tissue in anoxia.²²⁾ The high glycogen content of specialized heart muscle may similarly make specialized heart muscle more resistant to anoxia. Therefore, reasons (iii) and (iv) do not prove that the specialized heart muscle gets its energy mainly from anaerobic metabolism in aerobic state. On the contrary we conclude from our results on mitochondrial respiration, LDH activity, the LDH isozyme pattern and the cytoplasmic NAD^+/NADH ratio that the specialized heart muscle is mainly supplied with energy from aerobic metabolism rather than anaerobic metabolism.

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