CHANGES IN ACTIVITY AND ISOENZYME COMPOSITION OF LACTATE DEHYDROGENASE DURING A SINGLE CARDIAC CONTRACTION

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The total activity and isoenzyme composition of frog myocardial lactate dehydrogenase vary during a single cardiac cycle. The total lactate dehydrogenase activity is high at the end of systole and the end of diastole, and low at the beginning of systole and at the beginning of diastole. Changes in the relative proportions of different molecular forms of lactate dehydrogenase observed at various stages of the single cardiac cycle are mainly on account of isoenzymes of muscle type and are exhibited as changes in the size and intensity of staining of the bands on electrophoresis. Particularly sharp changes in the assortment of molecular forms of lactate dehydrogenase are observed at the beginning of systole, when the bands of the hybrid enzymes separate into clearly distinguishable narrower bands. It is assumed that migration of ions on excitation is one possible cause of the changes in activity and properties of the enzyme observed during the cycle.

Comparison of changes in metabolism in the myocardium with the phases of electrical and mechanical activity of the heart is an important step toward the elucidation of the biochemical processes lying at the basis of the contractile activity of heart muscle. In this connection the writers have previously studied the dynamics of changes in the concentration of certain substrates of carbohydrate metabolism during a single cardiac contraction. The results showed that the concentration of glycogen and of pyruvic and lactic acids varies considerably at different phases of contraction of the heart muscle [1]. To investigate the mechanism of these changes, in the present study the activity and isoenzyme composition of lactate dehydrogenase (LDH) in the frog myocardium was investigated. This enzyme is responsible for the final reaction of glycolysis, catalyzing the conversion of pyruvic into lactic acid. Although the principal pathway of pyruvate conversion in the heart is oxidation in the Krebs' cycle, the lactate-dehydrogenase reaction largely determines the subsequent fate of the pyruvate formed by glycolysis. The role of this reaction increases in certain physiological states associated with hypoxia.

Each organ and tissue has its own characteristic assortment of LDH isoenzymes, in connection with differences in the functional role of the H- and M-subunits [7], which differ in their enzymic properties [5, 8].

Isoenzymes of muscle type can function in the presence of high concentrations of pyruvic and lactic acids, as are usually found in hypoxia.

The total activity and isoenzyme composition of LDH was determined in order to explain the changes observed in the concentrations of pyruvic and lactic acids during a single cardiac contraction and to obtain some idea whether aerobic or anaerobic metabolism is predominant in the myocardium at particular phases of its contraction.

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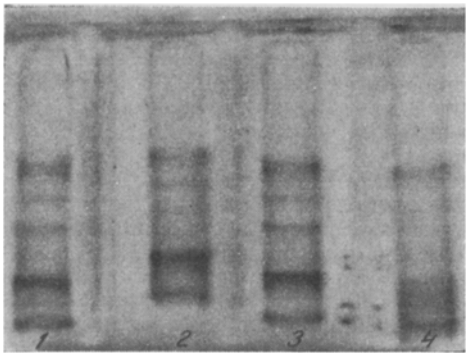

Fig. 1. LDH isoenzymes at various stages of cardiac contraction (electrophoresis of extracts of frogs' hearts in polyacrylamide gel): 1) beginning of diastole; 2) end of diastole; 3) end of systole; 4) beginning of systole.

EXPERIMENTAL METHOD

The heart was fixed in a certain phase of the contraction cycle by means of a special device controlled by the R wave of the ECG and enabling the heart to be instantaneously frozen at any specified moment of the cycle [2]. The frozen tissue was ground into a powder, and extracted with 4 volumes of 0.15 M phosphate buffer, pH 7.4. LDH activity was determined in the extracts spectrophotometrically, using pyruvic acid as the substrate. To determine the isoenzyme spectrum of LDH, the method of electrophoresis in polyacrylamide gel as described by Davis [6] was used in the modification of Safonov and Safonova [3], with subsequent specific staining by Brody's method [4].

EXPERIMENTAL RESULTS AND DISCUSSION

The results show that LDH activity varies during the cardiac cycle, which in the frog lasts for 1600-2000 msec. The maximum LDH activity (340.45±16.1 units/min/mg tissue) was observed at the end of systole. Toward the beginning of diastole, the activity fell (265.38±13 units/min/mg), and then rose again to 304.56±11.8 units/min/mg toward the end of diastole. At the beginning of systole, the activity of the enzyme was lower (265.55±9.8 units/min/mg) than at the end of diastole.

Determination of the relative percentages of subunits of the muscular and cardiac type in heart muscle extract, and fractionation of the extract by electrophoresis in polyacrylamide gel showed that during the cycle changes take place in the ratio between the various molecular forms of LDH. LDH in the frog's heart is represented by 5 isoenzymes (Fig. 1). In all 4 phases of the cardiac cycle, isoenzymes of cardiac type predominate, notably the isoenzyme H_3M. The change in the assortment of LDH isoenzymes during the cycle is mainly due to isoenzymes of muscular type: activity of the isoenzyme H_4 is the same at all phases of the cycle, while activity of the isoenzyme H_3M changes only very slightly. Particularly sharp changes in the assortment of molecular forms of LDH occur at the beginning of systole. In this period the content of isoenzymes of muscular type was very low, especially in the case of hybrid forms H_2M_2 and HM_3. A characteristic feature of this phase of the cardiac cycle is splitting of the bands of the hybrid isoenzymes on electrophoresis. The isoenzyme H_3M is shown as several bands lying close together, while the other hybrid forms each appear as two bands, lying close together. Because of the low activity of these isoenzymes, it is difficult to distinguish these subsidiary bands on the photograph, but in fresh specimens they are clearly visible.

The causes of the quantitative changes in the assortment of LDH isoenzymes during the cardiac cycle, as well as the causes of splitting of the bands of the hybrid forms of LDH during electrophoresis of the cardiac extract at the beginning of systole must next be considered. Splitting of the bands of hybrid isoenzymes observed at the beginning of systole cannot be regarded as a technical error because the quantity of protein applied to the gel was the same in the case of all extracts obtained from the hearts fixed at different moments of the cardiac cycle. Electrophoresis of these extracts was always carried out simultaneously, in the same apparatus, and at the same ionic strength and pH. Splitting of the bands at the beginning of systole was observed in all experiments. In all probability, splitting of the bands was observed because of the existence of several conformational states of hybrid forms of LDH in this phase of the cycle. Equilibrium is usually established on the side of the thermodynamically most stable conformation of the enzyme, so that it is difficult to detect other conformational states of the enzyme experimentally under ordinary conditions. However, by changing the salt composition of the medium, different conformational states of hybrid forms of LDH can be obtained [9]. Very probably at the beginning of systole, during depolarization of the membrane and a change in the concentration of K^+ and Na^+ ions in the cell, a situation arises which favors the onset and persistence (for several tens of milliseconds) of different conformations of hybrid LDH isoenzymes.

So far as changes in the quantitative ratio between LDH isoenzymes during the cardiac cycle are concerned, it is hardly conceivable that the rate of synthesis of the individual subunits, which are polypeptides

of high molecular weight, could change significantly in the course of a few tenths of a second. More probably at individual moments of the cycle, because of changes in the intracellular medium, the conditions for the last stage of formation of the enzyme, namely the assembling of the enzymically active tetramer from the subunits, are changed.

The experiments thus showed that during small time intervals, measured in tenths of a second, changes in the total activity and assortment of LDH isoenzymes take place. Because of differences in the enzymic properties of isoenzymes of muscular and cardiac type [10], a change in the relative percentages of the molecular forms of LDH, reflecting significant changes in metabolism in the course of the cycle, affects the specific pattern of conversion of the end products of glycolysis, namely pyruvic and lactic acids. The decrease in LDH activity and the beginning of systole, on account of isoenzymes of muscular type, may be the main cause of the increase in pyruvic acid concentration discovered previously, accompanied by a simultaneous decrease in the concentration of glycogen, but by no change in the lactic acid concentration. In the remaining phases of the cardiac cycle, LDH in all probability does not play a decisive role in the conversions of pyruvic acid.

It can accordingly be postulated that excitation, with the associated migration of K^+ and Na^+ ions, gives rise to substantial changes even in those enzymic processes which are not directly connected with contraction of the myofibrils.

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