ENZYMIC HETEROGENEITY OF THE RAT MYOCARDIUM

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UDC 612.172.015.1

An uneven distribution of activity of oxidoreductases in the rat ventricular myocardium was demonstrated by a histoenzymic method. The irregular distribution of activity of lactate, malate, and α-glycerophosphate dehydrogenases was most marked close to the apex of the heart, where it was expressed as "spotty" areas of increased activity against a generally uniform background of formazan deposits. Activity of mitochondrial α-glycerophosphate dehydrogenase in all parts of the ventricles was characterized by an irregular distribution in the sarcoplasm, with an increase in the direction from intercalated disk to nucleus. Irregularity of distribution of β-hydroxybutyrate dehydrogenase activity was discovered in some animals and was sharply defined in all parts of the myocardium. The intensity of the reaction for detection of succinate dehydrogenase and of NAD- and NADP-diaphorases varied only slightly.

KEY WORDS: oxidoreductases; rat myocardium.

In previous papers [2, 3] the writers drew attention to the distinctive character of distribution of activity of certain enzymes in the rat heart. The object of the present investigation was to study the histoenzymic pattern of activity of some oxidoreductases in different parts of the ventricular myocardium.

EXPERIMENTAL METHOD

The ventricular myocardium of noninbred male albino rats of three age groups was used as the test object: 1) young rats aged 3 months and weighing 120 g (6 animals), 2) sexually mature rats aged 1 year and weighing 300-330 g (12 animals), and 3) old rats aged 2 years and weighing 500-540 g (8 animals). The rats were decapitated, the heart was quickly extracted and kept in melting snow, cut into two halves in the frontal or horizontal plane, and frozen in liquid nitrogen; serial sections were cut in a cryostat. Sections 10 μ in thickness were glued to slides and tests carried out to detect the activity of NAD-dependent dehydrogenases: lactate (LD), malate (MD), β-hydroxybutyrate (β-HBD), and α-glycerophosphate (cytoplasmic α-GPD) [9]. For detecting activity of NAD- and NADP-diaphorases, reduced forms of pyridine nucleotides were used. Activity of succinate dehydrogenase (SD) and of NAD-independent α-GPD (mitochondrial α-GPD) was determined by the method of Nachlas et al. [10]. Nitro-BT was used as electron acceptor. Some sections were incubated in a floating state. The level of enzyme activity was determined from the intensity of formazan deposition and was assessed visually by a plus system, from ± (traces) to 4+ (very high).

EXPERIMENTAL RESULTS

The experimental results showed that in the muscle cells of the ventricles as a whole activity of SD, LD, and NAD- and NADP-diaphorases was high (3+, 4+), activity of β-HBD and mitochondrial α-GPD was very irregular (from ± to 3+ or 4+), MD activity was average (2+), and activity of cytoplasmic α-GPD was weak (±, 1+).

Laboratory of Age Pathology, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 4, pp. 487-489, April, 1976. Original article submitted June 11, 1975.

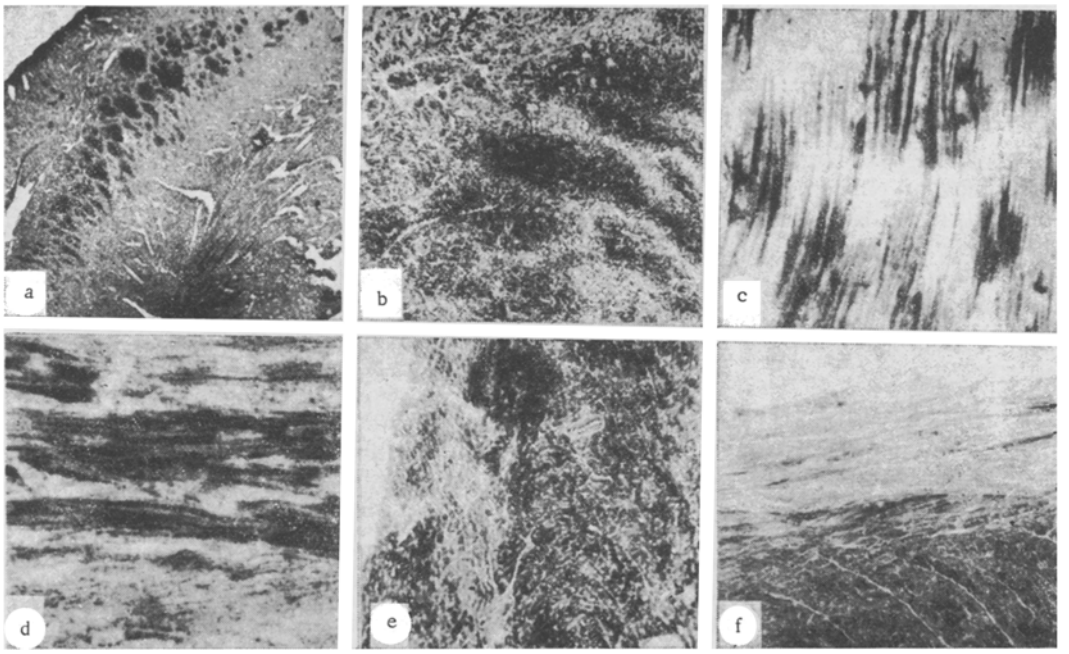

Fig. 1. Activity of oxidoreductases in myocardium of rat ventricles: a) reaction for MD: spots of increased enzyme activity in thickness of myocardium of left ventricle (magnifying glass); b) reaction for LD: spots of increased enzyme activity in thickness of left ventricular myocardium (70 ×); c) reaction for mitochondrial α-GPD: increased enzyme activity in direction from intercalated disk to nucleus (500 ×); d) reaction for β-HBD: unequal enzyme activity in adjacent muscle cells of left ventricle (400 ×); e) reaction for β-HBD: unequal distribution of enzyme activity in left ventricular myocardium (50 ×); f) reaction for β-HBD: low enzyme activity in subendocardial layer of right ventricle (50 ×).

Meanwhile, some irregularity of distribution of enzymic activity among the different regions or layers of the ventricles, and even in the muscle cells themselves, was found to be a characteristic feature of the mycocardium of rats of all age groups studied. The heterogeneity thus observed had a number of special features with respect to individual enzymes.

In the reaction for LD, MD, and cytoplasmic α-GPD, against a generally uniform background of formazan deposits, "spots" of increased activity were found in the thickness of the myocardium of the left and right ventricles, mainly in the lower third of the heart, and they were particularly clearly visible in sections cut in the frontal plane (Fig. 1a, b). These areas contained different numbers of cells and fibers, arranged in bundles, and they were topographically similar as regards the reactions for all three enzymes mentioned above. Incubation of the floating sections yielded similar results.

Mitochondrial α-GPD activity was distinguished by a special and constant pattern: It varied almost from traces in the intercalated disks to relatively high (2+, 3+) in the center of the cells, around the nuclei (Fig. 1c). The activity of this enzyme varied appreciably from cell to cell, and also often between adjacent groups of muscle fibers.

The intensity of the reaction for SD and the two diaphorases varied chiefly only as regards the content of finely granular formazan in the cells, which was more or less distinguishable against the background of the darkly stained "linear" formazan deposits [3].

In the reaction for β-HBD the character of distribution of enzyme activity frequently was different in sections of the hearts of different rats of the same age group placed side by side on the same slide and incubated together. In some animals (3 young, 4 sexually mature, 3 old) activity of the enzyme in the myocytes was relatively uniformly high (2+, 3+) throughout the plane of the section; in the rest it varied sharply from traces to high over the whole myocardium in bundles arranged side by side, and sometimes even in two neighboring cells (Fig. 1d, e). In the presence of such heterogeneity in the subendocardial layers of both ventricles, weak β-HBD activity as a rule was discovered (Fig. 1f).

Heterogeneity of distribution of enzyme activity evidently points to variation in the utilization of substrates in different bundles of muscle fibers and in different parts of the myocardium at any given moment of time. This conclusion agrees with the fact that the distribution of glycogen in the myocardium [1], as the substrate of energy metabolism, is irregular. Considering the direct dependence of the contractile function of the myocardium on its metabolic activity [8], the possibility cannot be ruled out that the enzymic heterogeneity is connected with functional heterogeneity of the contracting myocardium [4]. In view of the important role of nonesterified fatty acids [11] in energy metabolism and of β-HBD in the lipid metabolism of heart muscle [6], it can be assumed that it is β-HBD activity that reflects the state of myocardial function to a certain degree and that the heterogeneity of its distribution characterizes the reserves of adaptability of the myocardial muscle fibers to the changing mechanical demands of the heart. The irregular distribution of β-HBD activity in the myocardium of some animals and the absence of this enzyme in others do not confirm the view that the heart muscle cells can be divided, on the basis of their metabolic properties, into histogenetically fixed types, like skeletal muscle fibers [7], and they require further study with an analysis of the functional state of the myocardium before the beginning of the investigation.

The constantly high activity of SD, the key enzyme of the Krebs' cycle, suggests that the citric acid cycle, where utilization of different substrates used mainly for energy purposes converges, guarantees the production of high-energy compounds required for activity of the heart in all of its cells.

These results require discussion in connection with the specificity of detection of so-called soluble dehydrogenases, dependent upon pyridine nucleotides. Diffusion of the enzyme from the section into the incubation solution with reduction of the exogenous coenzyme present in it, followed by the development of a diaphorase reaction masking the activity of the specific dehydrogenase studied [5], has been shown to be possible.

The results of the present investigation point to the impossibility of such an error in this case. In fact, irregular β-HBD activity even in adjacent muscle cells, in the presence of high and uniform NAD-diaphorase activity in the same areas of the myocardium does not confirm the possibility of development of a diaphorase reaction and, consequently, any marked diffusion of the enzyme located in the mitochondria. Further evidence against the absence of a diaphorase reaction is given by the relatively weak, but irregularly localized activity of MD and cytoplasmic α-GPD. In this case, reduction of NAD probably does not take place because of the weak activity of the diffusing enzyme.

The data on enzymic heterogeneity of the myocardium must be remembered during any biochemical, and of necessity total, investigation of oxidative enzymes and during the quantitative analysis of the results of histoenzymic reactions by cytospectrophotometric methods.

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