EFFECT OF CHOLESTEROL ON ACTIVITY OF SOME MITOCHONDRIAL ENZYMES OF THE LIVER IN VITRO

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Incubation of intact liver mitochondria and of mitochondria fragmented by ultrasound for 30 min at 37°C in isolation medium with cholesterol in a concentration of $2 \times 10^{-3}$ M leads to the activation of hexokinase, malate dehydrogenase, and glutamate dehydrogenase and inhibits the activity of $\beta$-hydroxybutyrate, glycerophosphate, and lactate dehydrogenases. The effect is greater on enzymes of the intact mitochondria.

KEY WORDS: liver mitochondria; action of ultrasound; cholesterol; mitochondrial enzymes.

The study of the action of cholesterol, a substance playing a key role in the genesis of atherosclerosis, on the activity of the enzymes responsible for the metabolic disturbances in atherosclerosis is of great interest [1-5, 8].

This paper gives the results of an investigation of the action of cholesterol in vitro on the activity of the enzymes hexokinase (HK) and lactate (LD), glycerophosphate (GPD), malate (MD), $\beta$-hydroxybutyrate ($\beta$-HD), and glutamate dehydrogenases (GD).

EXPERIMENTAL METHOD

Experiments were carried out on the liver of intact rabbits. The animal was decapitated and the mitochondria separated from the liver tissue by fractional centrifugation in 0.25 M sucrose in Tris-versene buffer, pH 7.4. The suspension of washed mitochondria was divided into two parts. One part was immediately fragmented with ultrasound (0-2°C, 5 min, 20 kHz, 1.25 A, 500 W, GUZ-1.5 generator).

Finely dispersed, thrice recrystallized cholesterol was added to equal volumes of the sonicated and unsonicated suspensions of mitochondria in the isolation medium in a final concentration of $2 \times 10^{-3}$ M. The weight of the cholesterol sample was calculated from its concentration in the liver of rabbits with experimental atherosclerosis. Parallel control tests were carried out with sonicated and unsonicated mitochondria. All four samples were incubated in Ringer-phosphate buffer in a Warburg apparatus at 37°C for 30 min, after which the tubes were placed in ice. Unsonicated mitochondria also were fragmented with ultrasound.

The activity of HK [6], LD [11], GPD [9], MD [14], $\beta$-HD [13], and GD [10] was determined spectrophotometrically.

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental results are given in Table 1. Under the experimental conditions described the HK activity of the mitochondrial fraction increased after incubation with cholesterol by 63% in the case of sonicated and 65% for unsonicated mitochondria.
Incubation at 37°C for 30 min led to a decrease in the GPD activity in all samples. The addition of cholesterol inhibited the enzyme activity of the sonicated mitochondria by 37.1%.

The LD activity of the fragmented mitochondria was unchanged during incubation with cholesterol, but that of the intact mitochondria fell by 21.7% during incubation.

The conditions of incubation had an activating effect on MD. This may be attributable to the better solubilization of the enzyme and to the effects of the temperature factor. Addition of cholesterol increased the MD activity of the sonicated and intact mitochondria.

CD activity rose sharply in the presence of cholesterol. The degree of activation was higher in the fragmented mitochondria. Treatment with ultrasound evidently facilitates the access of cholesterol to the enzyme located in the mitochondrial matrix.

An increase in β-HD activity was observed as a result of incubation. The enzyme is "mounted" in the mitochondrial cristae and is insoluble; to unmask its activity, besides treatment with ultrasound [13], various activators are used [12]. The activation of β-HD by 763.6% compared with its initial activity must be borne in mind and used as a method of determining enzyme activity. Contact with cholesterol reduced the β-HD activity of the fragmented mitochondria by 37.1% and that of the intact mitochondria by 70%.

Cholesterol is evidently an "aggressive" agent affecting the activity of the enzymes studied. The normal cholesterol content in the mitochondria varies from 1.2 to 13%[7]. An excess of exogenous cholesterol in the medium may perhaps lead to chemical reaction between it and the lipoprotein membrane of the mitochondria, as the stronger response reaction of HK, MD, GD, β-HD, and LD of the intact mitochondria indicates.

**LITERATURE CITED**