PHOSPHORYLATION OF FRUCTOSE IN RAT SKELETAL MUSCLE AND LIVER IN HYPOXIA

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In an earlier investigation [12] it was shown that oxygen hunger led to reduced glucokinase activity in skeletal and cardiac muscle. As, therefore, cells are less able to utilize glucose for energy metabolism in hypoxia, it was thought important to examine the metabolism of fructose under the same conditions. The first "key" in the metabolism of fructose is of course catalyzed by the enzyme fructokinase:* D-Fructose + ATP → D-fructose-1-phosphate + ADP.

The effect of hypoxia resulting from low barometric pressure on fructokinase activity was now examined in skeletal muscle and liver—tissues in which there is both glucokinase and fructokinase activity [6, 8].

METHOD

Male albino rats, weighing between 190 and 260 g, were used in the investigation.

The animals were examined in two groups, a control group of 16 animals and an experimental group of 17 animals which were exposed to conditions producing oxygen hunger.

Hypoxia was produced by keeping the animals for 90 min in a chamber in which the atmospheric pressure was reduced to 190 mm Hg, a pressure equivalent to that experienced at a height of 10,000 m above sea level.

When the rats were removed from the chamber they were at once anesthetized with ether and killed by decapitation. The tissues to be examined were removed rapidly but carefully, cleared of blood, fixed in liquid oxygen and ground in a mortar. The suspension was extracted for 1 h in the cold with 0.15 M KCl solution in proportions of 1 : 2 for skeletal muscle and 1 : 4 for liver. The mixtures were then centrifuged for 5 min at 3000 rpm.

Fructokinase activity was determined from the quantity of fructose destroyed after incubation of the extracts for 20 min at 37°C in a medium containing K-phosphate buffer pH 7.4 ($5 \cdot 10^{-2}$ M), fructose ($2.2 \cdot 10^{-3}$ M), $MgCl_2$ ($5 \cdot 10^{-3}$ M), KCl ($1.5 \cdot 10^{-2}$ M), NaF ($5 \cdot 10^{-2}$ M), ATP ($5 \cdot 10^{-3}$ M) and extract with a protein content of 2.13-4.65 mg per test. Proteins were precipitated and hexosephosphoric esters removed with $CdSO_4$—NaOH. Fructose was estimated by the Selivanoff reaction as modified by Roe [7] and protein, by the biuret method [4]. Enzyme activity was expressed in milliunits, i.e. millimicromoles of fructose destroyed per milligram test protein per minute of incubation.

RESULTS

The examinations made on normal (control) rats showed that fructokinase activity in liver (11.64 ± 0.98 mU) was more than twice that of skeletal muscle extract (4.80 ± 0.54 mU). As other authors [1, 3] have found, individual values for fructokinase activity varied considerably.

In the hypoxic rats fructose activity was 11.03 ± 0.88 mU in the liver and 4.50 ± 0.47 mU in skeletal muscle.

Fructokinase activities in skeletal muscle and liver were thus very similar in the two groups, normal and hypoxic ($P > 0.6$).

*ATP: D-fructose-1-phosphotransferase (2.7.1.3) according to "Classification and Nomenclature of Enzymes (1962).

Unlike glucokinase activity [2], therefore, fructokinase activity is not reduced in oxygen hunger. This difference in the behavior of glucokinase and fructokinase in acute hypoxia, which is known to be accompanied by increased production of adrenocortical hormones [5, 9, 10], is apparently to be explained by differences in the hormonal control of the two enzymes and is completely in line with observations that fructokinase activity is not controlled by glucocorticosteroids or insulin [1, 3

This evidence of the maintenance of fructokinase activity in hypoxia definitely indicates the need for further investigation on the effect the administration of fructose, which is readily utilizable by the tissues, has on animal tolerance of hypoxia.

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