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Glycolytic Enzymes in Rabbit Aorta and Iliac Artery

(glycolytic enzymes/aorta/iliac artery)

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We estimated activities of glycolytic enzymes in rabbit aorta and iliac artery and found higher activities of these enzymes in the heart itself than in the aorta and iliac artery. In the aorta, the activities declined from the arch of aorta to the abdominal aorta, while in the iliac artery, the levels of hexokinase, phosphoglucoisomerase and pyruvate kinase increased considerably and those of other glycolytic enzymes decreased in comparison with the activities of the abdominal aorta. The lactate level also decreased along the aortic pathway, but levels showed a fair increase in the iliac artery, as compared with those of the abdominal aorta.

Many different enzymes participate in the metabolism of carbohydrates, fats and proteins in arteries (1). It is the carbohydrates which are mainly utilized for the energy production. In this process, the major metabolic pathway is glycolysis, and not the Krebs cycle, as the rate of oxygen consumption in arterial tissues is very small (2).

In relation to glycolysis, changes in the activities of glycolytic enzymes from the arch of aorta to the peripheral blood vessels in mammals are poorly understood. We determined the levels of these glycolytic enzymes in the aortic and iliac pathway and for a comparison, we estimated the enzymatic activities of the heart.

MATERIALS AND METHODS

Healthy, adult rabbits of both sexes were fed laboratory chow ad libitum before the experiments. Pentobarbital sodium (30 mg/kg) was given i. p. and subsequently, a solution of physiological saline (at 15—20°C) was perfused via a marginal vein in the ear, to remove all the blood from a carotid artery. When all blood had been replaced with the perfusate, the cardiac and arterial tissues (the heart, the arch of aorta, the thoracic aorta, the abdominal aorta and the iliac artery) were immediately excised from the animals to assay the activities of the following glycolytic enzymes.

The activity of hexokinase was determined by the method of Joshi and Jagannathan (3) and that of phosphoglucoisomerase was estimated according to the method of Bodansky (4). Phosphofructokinase was solubilized and activated

by the method of Mansour *et al.* (5) who pointed out that this enzyme in the sheep heart was present in an inactive, insoluble form of the homogenates. After these procedures, the activity was measured by the method of Ling *et al.* (6). Activity of pyruvate kinase was assayed according to the method of Kimberg and Yielding (7) and that of lactate dehydrogenase was determined by the method of Bergmeyer and Bernt (8). Throughout the studies, one unit of each enzyme was defined as the amount of enzyme that catalyzes the formation of 1μ mole of product per min.

In addition, the lactate level was checked by the method of Gutmann and Wahlefeld (9), while the protein content was determined by the method of Lowry *et al.* (10).

RESULTS AND DISCUSSION

Hexokinase and Phosphoglucoisomerase

In the initial step of glucose degradation, intracellular glucose is converted into glucose 6-phosphate by hexokinase. When we estimated the enzymatic activities in various materials (Fig. 1), higher values were found in the heart

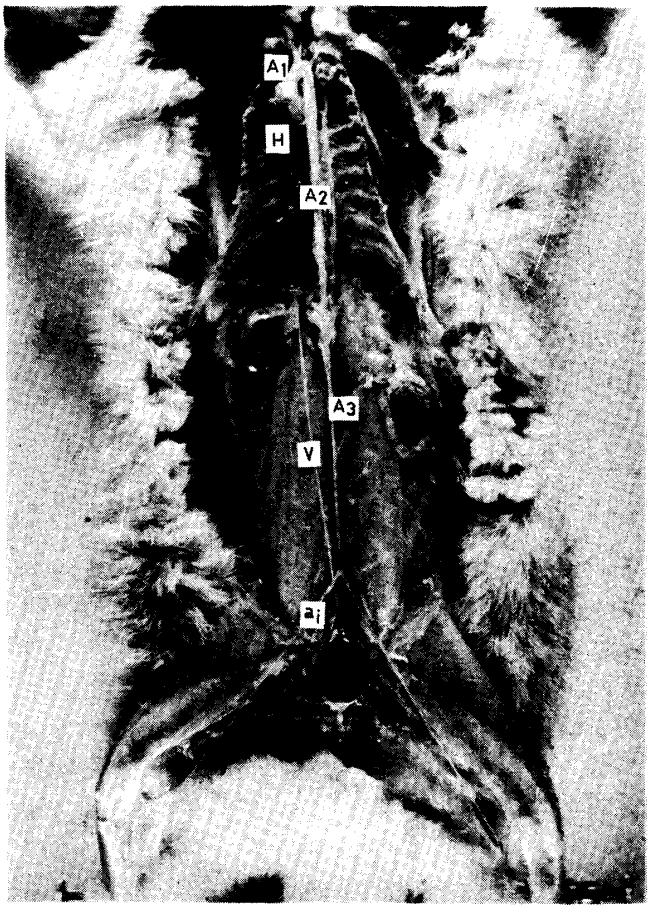

Fig. 1. The cardiovascular system in the rabbit perfused with a solution of physiological saline at 15–20°C. A_1 : Arcus aortae (the arch of aorta), A_2 : Aorta thoracica (the thoracic aorta), A_3 : Aorta abdominalis (the abdominal aorta), a_i : Arteria iliaca externa (the iliac artery), V : Vena cava caudalis, H : the heart.

than in the aorta and iliac artery. Concerning the arterial tissues, the level of hexokinase progressively declined along the aortic pathway in the order of (the arch of aorta → the thoracic aorta → the abdominal aorta). However, the level in the iliac artery increased by about 17 % in comparison with that of the abdominal aorta, (Fig. 2.a). The activities of hexokinase were

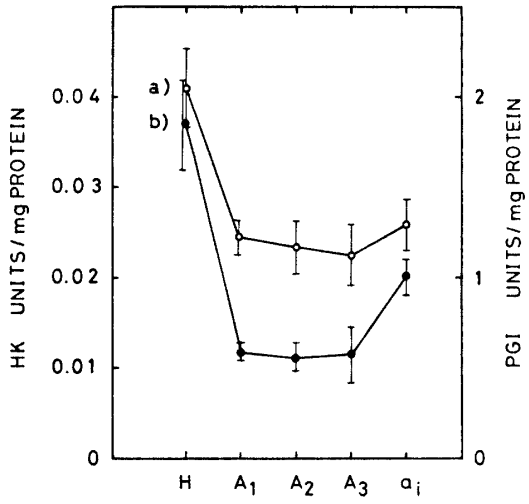

Fig. 2. The activities of hexokinase (left) and phosphoglucoisomerase (right). H : the heart A_1 : the arch of aorta A_2 : the thoracic aorta A_3 : the abdominal aorta a_i : the iliac artery a) Hexokinase (HK) ○——○ average of 6 experiments b) Phosphoglucoisomerase (PGI) •——• average of 6 experiments. Vertical bars : ±standard deviation.

lower than those of the other glycolytic enzymes (phosphoglucoisomerase, phosphofructokinase, pyruvate kinase and lactate dehydrogenase).

On the other hand, in bovine mesenteric artery, Lundholm *et al.* found that when oxygen tension was low, glucose transport from the incubation medium to the arterial tissues was considerably increased (11). Therefore, under such anaerobic conditions, it would be expected that hexokinase becomes a rate determining enzyme, if the rate of the sugar transport is higher than that of the phosphorylation.

Phosphoglucoisomerase is the catalytic enzyme in the interconversion of glucose 6-phosphate to fructose 6-phosphate. As shown in Fig. 2(b), the heart was rich in enzymatic activity, while levels in the aorta and iliac artery were low. However, values in the iliac artery increased by about 90 %, as compared with values in the abdominal aorta.

Phosphofructokinase

Phosphofructokinase is the enzyme responsible for the conversion of fructose 6-phosphate to fructose 1,6-diphosphate and plays a leading role as the regulator of glycolysis. Regarding animal phosphofructokinase (12—13), the activity is affected by various factors (pH, ATP, fructose 6-phosphate, fructose 1,6-diphosphate and other metabolites). Acidification facilitates the dissociation

of this enzyme to a dimeric state (an inactive form : two protomers), whereas alkalinization to pH 8 stimulates the association of the dimers, which promotes the increase in the tetrameric and polymeric states (active forms of phosphofructokinase). Similarly, fructose 6-phosphate (a substrate) or fructose 1,6-diphosphate (a product) induces the association process. Consequently, under such conditions, the molecular weight of phosphofructokinase increases in the order, the dimers < the tetramers < the polymers.

ATP is one of the substrates of phosphofructokinase. According to the review reported by Hofmann (12), the action of this substrate is complicated. That is, the enzymatic activity increases when ATP concentration is low, it decreases when the concentration is high. Here, the inhibitory action of ATP is suppressed by 5'-AMP and 3', 5'-cyclic AMP and others (alkalinization to pH 8, fructose 6-phosphate, fructose 1,6-diphosphate, etc.).

Accordingly, in our studies, a relatively large amount of fructose 6-phosphate (2 mM) was added to the assay mixtures (at pH 8), in order to determine the full activity of phosphofructokinase. Using this procedure, a slope of the enzymatic level was obtained from the heart to the iliac artery, when each average measured was plotted. The result is shown in Fig. 3.

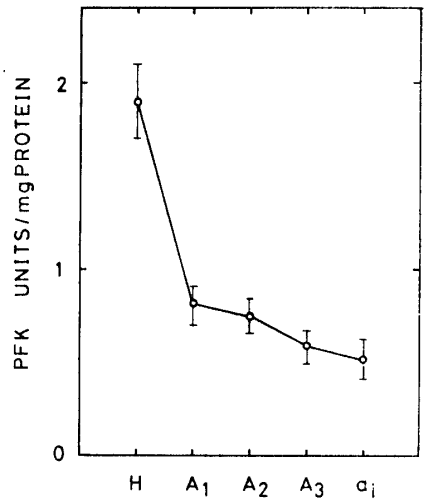

Fig. 3. The activities of phosphofructokinase (PFK). H : the heart A_1 : the arch of aorta A_2 : the thoracic aorta A_3 : the abdominal aorta a_i : the iliac artery. Average of 5 experiments. Vertical bars : ±standard deviation.

Pyruvate Kinase and Lactate Dehydrogenase

These enzymes participate in the production of pyruvate and lactate. Since arterial tissues utilize considerable amounts of glucose and produce large amounts of lactate (14), we studied the activities of pyruvate kinase and lactate dehydrogenase. As is evident from Fig. 4, higher activities of these enzymes were observed in the heart. In the aorta, both the activities continuously decreased along the aortic pathway. On the other hand, in the iliac artery, the activity of pyruvate kinase increased by about 30 %, whereas that of lactate dehydrogenase further decreased by about 10 %, when compared with

these activities in the abdominal aorta.

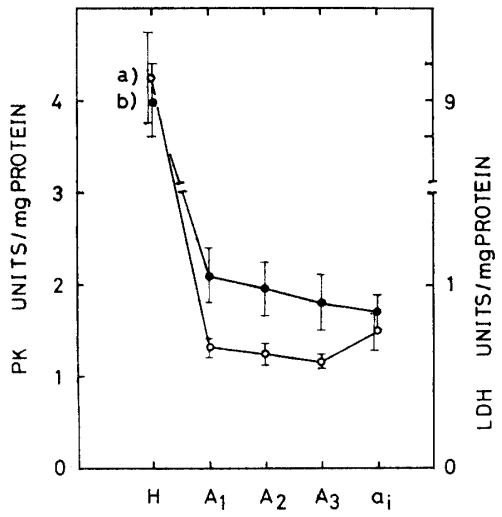

Fig. 4. Activities of pyruvate kinase (left) and lactate dehydrogenase (right). H : the heart A_1 : the arch of aorta A_2 : the thoracic aorta A_3 : the abdominal aorta a_i : the iliac artery a) Pyruvate kinase (PK) ○——○ average of 8 experiments b) Lactate dehydrogenase (LDH) •——• average of 8 experiments. Vertical bars : ± standard deviation.

Lactate

The lactate level also decreased along the aortic pathway, but increased in the iliac artery, when compared with levels in the abdominal aorta, (Fig. 5). In this figure, the solid line (a) shows the lactate contents of the present

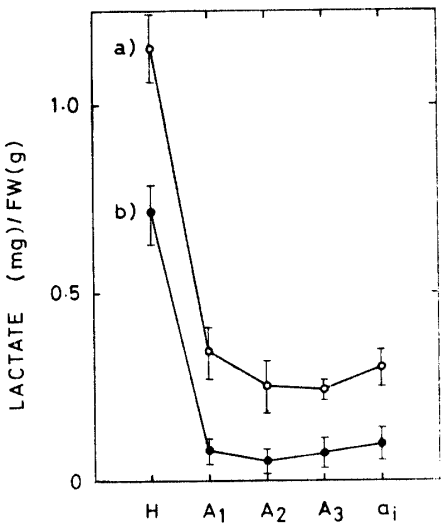

Fig. 5. Contents of lactate. H : the heart A_1 : the arch of aorta A_2 : the thoracic aorta A_3 : the abdominal aorta a_i : the iliac artery a) the estimating values were obtained immediately after administration of pentobarbital sodium (5 mg/kg i. v.) ○——○ average of 6 experiments b) lactate levels were measured at the end of perfusion with physiological saline •——• Average of 7 experiments. Vertical bars : ±standard deviation.

materials obtained immediately after the animals were sacrificed by giving pentobarbital sodium (5 mg/kg i. v.), while the solid line (b) represents the lactate level estimated at the end of the perfusion with physiological saline solution. When the solid line (a) is compared with (b), we may conclude that lactate in the cardiovascular tissues is leaky under such a perfusion.

Other Remarks

In all cases, higher values of these glycolytic enzymes were found in the heart, indicating that glycolysis in the constantly working heart is strikingly active. Since all of the activities declined along the aortic pathway, it appears that a slope of the enzymatic level exists from the arch of aorta to the abdominal aorta. Of the iliac artery, however, the levels of hexokinase, phosphoglucoisomerase and pyruvate kinase increased considerably in comparison with those of the abdominal aorta.

It has been reported that the intima and the inner one third of the media are lacking in vasa vasorum (15). Accordingly, the oxygen supply in this part of arterial wall may be poor. Particularly, under anaerobic conditions, the lactate appears to accumulate in the inner layer and leak into the circulating blood. This is supported by the present results which showed a decrease in the lactate level in cardiovascular tissues perfused with a solution of physiological saline.

Contraction of vascular smooth muscle is accompanied with lactate production (16). Taking all these data together, it can be presumed that the glycolytic enzymes are stimulated by contractions of the arterial muscle.

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