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Glycogen Phosphorylase in the Aorta and the Iliac Artery in Rabbits

(glycogen phosphorylase/aorta/iliac artery)

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We estimated glycogen phosphorylase activities in the aorta and iliac artery in rabbits and the following results were obtained : 1) In parallel with the amounts of protein and glycogen, the levels of total glycogen phosphorylase and glycogen phosphorylase a decreased from the arch of aorta to the iliac artery. 2) The level of glycogen phosphorylase b was higher in the arch of aorta than in the thoracic and abdominal aortae and the iliac artery.

Kirk (1) estimated the glycogen phosphorylase activities in various types of human vascular tissues and found higher activities of this enzyme in the pulmonary artery than in the thoracic aorta and that higher levels were also seen in the brachial artery and inferior vena cava. Judging from these results, it would appear that the activity of this enzyme varies in different blood vessels. However, change in the activity of glycogen phosphorylase from the arch of aorta to the peripheral blood vessels in mammals has apparently not been documented. Therefore, we checked the enzymatic levels of the aorta and iliac artery and estimated the activity of this enzyme of the heart for a comparison.

MATERIALS AND METHODS

Normal, adult rabbits of both sexes were allowed food *ad libitum* before the experiments and were sacrificed by an intravenous administration of pentobarbital sodium (5 mg/kg). 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 these animals. All of these materials were washed with a chilled solution of physiological saline (0—4°C, pH 7.0—7.4) to remove all blood. For the assay of glycogen phosphorylase, segments of each material were homogenized in a cold 10 mM Tris malate-buffered solution (pH 6.1) in a Polytron homogenizer (10 mg protein/ml). For the analysis of glycogen, KOH (30% : final concentration) was added to these segments. Using the tissue preparations, the glycogen phosphorylase activity was determined by the method of Shimazu and Amakawa (2), the glycogen content was estimated according to the method of Fong *et al.*(3), and the amount of protein was

measured by the method of Lowry *et al.*(4).

RESULTS

Protein in the tissue preparations was determined to calculate glycogen phosphorylase activities (units/mg protein). One unit of the enzyme was defined as that amount which caused the liberation of 1 μmole Pi in 1 min. As shown in Fig. 1 (a : closed circles), higher amounts were found in the heart than in the aorta and iliac artery. In addition, the average values exhibited from the arch of aorta to the iliac artery showed a downward curve. Since the arterial wall has three layers (intima, media and adventitia), the protein components of the aorta and iliac artery are distributed among these layers and are associated with the extra and intra-cellular structures. Therefore, it is probable that such changes of the amount of this substance relate with the architecture of their arteries.

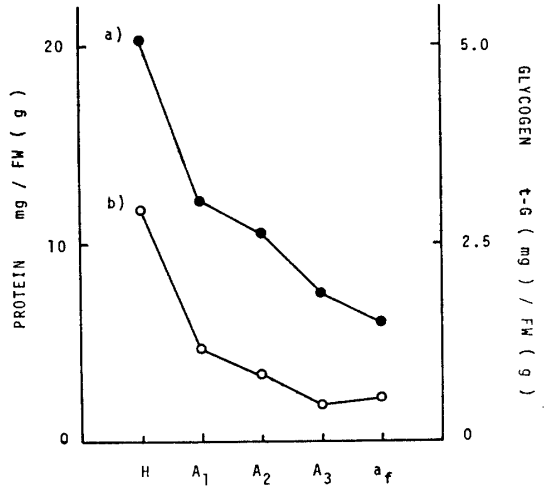

Fig. 1. Amounts of protein and glycogen in arterial tissues. t-G : total glycogen, FW : fresh weight, H : the heart, A_1 : the arch of aorta, A_2 : the thoracic aorta, A_3 : the abdominal aorta, a_f : the iliac artery, a) Protein ●——● average of 30 experiments, b) Glycogen ○——○ average of 6 experiments

The glycogen contents of the tissue preparations were estimated from the relationships between the levels of glycogen phosphorylase and its substrate (glycogen). The results are shown in Fig. 1 (b : open circles). When expressed in terms of mg of glycogen of the wet tissue, higher values of glycogen were also found in the heart than in the aorta and iliac artery. Concerning vascular tissues of rabbits, the level of glycogen progressively declined along the aortic pathway (the arch of aorta ⟶ the thoracic aorta ⟶ the abdominal aorta). However, the level in the iliac artery increased by about 20% in comparison with that of the abdominal aorta.

In relation to vascular tissue in cattles, two types of glycogen phosphorylase were demonstrated by Mohme-Lundholm (5). These enzymes are glycogen phosphorylase a and b. As the enzyme a is active, whereas the enzyme b is enzymatically inactive, the activities of type a in the tissue preparations were estimated. The results are given in Fig. 2 (a : open circles). When each

average obtained was plotted, a slope of the enzymatic level was observed from the heart to the iliac artery.

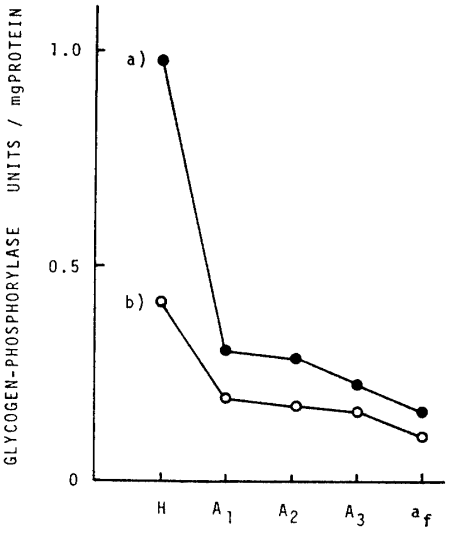

Fig. 2. Activities of glycogen phosphorylase in arterial tissues. H : the heart, A_1 : the arch of aorta, A_2 : the thoracic aorta, A_3 : the abdominal aorta, a_f : the iliac artery, a) total-glycogen phosphorylase ●——● average of 5 experiments b) glycogen phosphorylase a ○——○ average of 5 experiments

In the presence of 1 mM adenosine 5′-monophosphate (5′-AMP), glycogen phosphorylase b is active and can be determined. Accordingly, by the addition of 5′-AMP to the incubation medium, it is possible to determine the total glycogen phosphorylase activity including both active (the enzyme a) and inactive (the enzyme b) form. With this procedure, the total activities of the tissue preparations were then measured. As shown in Fig. 2 (b : closed circles), higher activities of total glycogen phosphorylase were found for the heart than the aorta and iliac artery.

In each cardiovascular tissue, the level of enzyme b was determined from the difference between the activities of the total glycogen phosphorylase and the enzyme a. As is evident from Fig. 3, the heart was rich but the aorta and iliac artery were poor in the enzyme b. Since the enzyme b is converted into the enzyme a, it would be expected that the level of the enzyme a in a constantly working muscle is able to increase remarkably with stimulation of cardiac glycolysis.

DISCUSSION

Phosphorylase a participates in the first step of glycogenolysis. In this part of the reaction, the endogenous carbohydrate (glycogen) is broken down to glucose. Concerning vascular tissues of mammals, most intracellular glucose is metabolized to pyruvate or lactate. Consequently, the main product is lactate. However, to some extent, lactate is oxidized to CO_2 and H_2O via the Krebs cycle.

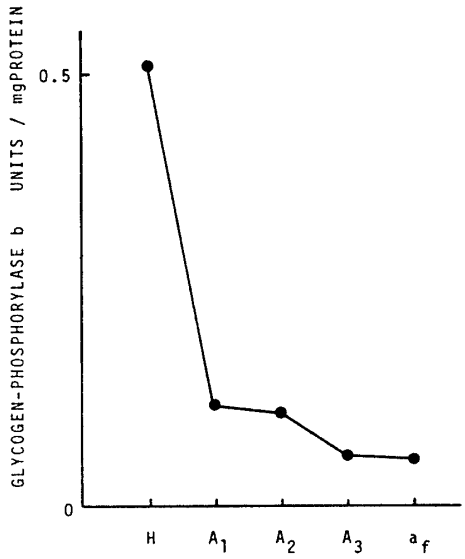

Fig. 3. Levels of phosphorylase b in arterial tissues. H : the heart, A_1 : the arch of aorta, A_2 : the thoracic aorta, A_3 : the abdominal aorta, a_f^* : the iliac artery, Average of 5 experiments

In our studies, both levels of glycogen phosphorylase a and glycogen were considerably higher in the heart than in the aorta and iliac artery. Of the arterial tissues, these levels were higher in the arch of aorta than in the other arteries (the thoracic aorta, the abdominal aorta and the iliac artery). Moreover, among the arterial preparations, the arch of aorta was rich in phosphorylase b. Therefore, when glycogen phosphorylase of the aorta and iliac artery is activated, the conversion of phosphorylase b into the active form (phosphorylase a) probably is more evident in the arch of the aorta.

Glycogenolytic enzyme activity in smooth muscle has also been estimated by many investigators (6—9). In rabbit aorta, phosphorylase a activity increases under anaerobic conditions (6) and is stimulated by phenylephrine, angiotensin, histamine or K^+ (7). On the other hand, in rabbit colon, the glycogenolytic activity increases when incubated in a glucose-free buffer (8—9). Therefore, under such various conditions, we have to further check on the enzymatic activity from the arch of aorta to the iliac artery.

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