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Activity of Phosphofructokinase during Lactate Accumulation in Rabbit Aorta

(phosphofructokinase/lactate/rabbit aorta)

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When rabbit aortic strips were incubated under anaerobic conditions (at 37°C for 3 hours), the lactate level increased remarkably to about 10 times the level observed under aerobic conditions. In the process of lactate accumulation, the activity of phosphofructokinase increased by 5 –30 % of the enzymatic level observed under aerobic conditions. When adrenaline was added to the bath medium, the aortic muscle contraction was somewhat weak and the activation of phosphofructokinase was less remarkable in comparison with observations under aerobic conditions. This finding suggests that lactate accumulation in rabbit aorta suppresses the activation of phosphofructokinase in the contracting materials.

In the arterial wall, lactate accumulation is dominant under anaerobic conditions. This finding is known as the Pasteur effect. Since one of glycolytic enzymes, phosphofructokinase is stimulated under anaerobic conditions (1), it would appear that this enzyme plays a leading role in the regulation of glycolysis and the site of operation of the Pasteur effect. However, according to the review of Hofmann (2), increase in H^+ inactivates phosphofructokinase in animal tissues. Accordingly, it is suggested that a remarkable accumulation of lactate in the arterial wall would influence the Pasteur effect, because of a dislocation of the pH optimum of phosphofructokinase. To assess changes in the enzymatic level during lactate accumulation, we studied the activity of phosphofructokinase in resting or contracting rabbit aorta after incubating the materials in a hypoxic medium. Our findings were compared with those obtained under aerobic conditions.

MATERIALS AND METHODS

Healthy, adult rabbits of both sexes fed a laboratory chow diet *ad libitum* before the experiments were given pentobarbital sodium (30 mg/kg i. p.) and then exsanguinated from a carotid artery. The aortic arch (A_1), the proximal (A_{2p}) and distal (A_{2}) areas of the thoracic aorta and those (A_{3}, A_{3d}) of the abdominal aorta were immediately excised. Subsequently, the aortic materials were cut into spiral shaped strips which were then bisected longitudinally. Using the method of Karaki and Urakawa (3), the adventitia of all the materials was removed from the intima-media layers. One of each pair of

the strips served as the control and the other was brought into contact with adrenaline.

The aortic strips were mounted in 20 ml of a bath medium either bubbled with 5 % CO_2 and 95 % N_2 or aerated with 5 % CO_2 and 95 % O_2 at 37°C. In this case, a resting tension of 1 gm was applied to each strip. Under such conditions, all strips were equilibrated in the bath medium of Krebs-Henseleit solution (pH 7.3—7.4) for 3 hours before the addition of adrenaline.

When adrenaline was added to the bath, the tension produced was recorded by means of a force-displacement transducer (TD-111 S, JD-111 S, Nihon Koden Kogyo Co., Tokyo) connected to the RM-251 multipurpose polygraph. Aortic muscle was contracted for 10 minutes by a dose of 5×10^{-6} M of this catecholamine. Within this time, the tension development attained the maximum. These strips were then frozen using dry ice-acetone.

The lactate production (μmole/g) was calculated from the increase in lactate content of a frozen strip and of the bath medium. To measure the lactate level (4), the bath medium was previously concentrated by a method of freeze drying.

To estimate the activity of phosphofructokinase, the frozen strips,measuring on the average 30 mg., were suspended in 20 volumes of 1—4°C solution containing a final concentration of 30 mM potassium phosphate buffer, 1 mM EDTA and 30 mM KF at pH 6.8. The suspensions were homogenized in a Polytron homogenizer and then centrifuged for 40 min at 10,000×g at 2°C. Using the supernatants thus obtained, the activity of phosphofructokinase was assayed by the method of Ling *et al.* (5). Throughout the studies, one unit of each enzyme was defined as the amount of enzyme that catalyzes the formation of 1 μmole of product per minute.

RESULTS AND DISCUSSION

Aortic Contractility

When adrenaline was added to the bath medium, contractility of the aortic muscle was somewhat poor, under anaerobic conditions.

Fig. 1 shows examples of the aortic strips. When the gas mixture was switched from N_2—CO_2 (95—5 %) to O_2—CO_2 (20.9—0.03 %), the tension development was restored to the same level as observed under aerobic conditions.

Lactate

In the resting aorta, lactate produced during a prolonged incubation (3 hours) remarkably increased under anaerobic conditions. As is evident from Fig. 2 (c_1, c_2), the levels of this metabolite in the different areas were about 10 times those observed under aerobic conditions (Pasteur effect).

In the contracting aorta, the lactate levels increased by only 1—3 % under anaerobic conditions (Fig. 2 (c_2, s_2)) but were augmented by 30—50 % in the medium aerated with oxygen (Fig. 2 (c_1, s_1)). In the hypoxic medium, the

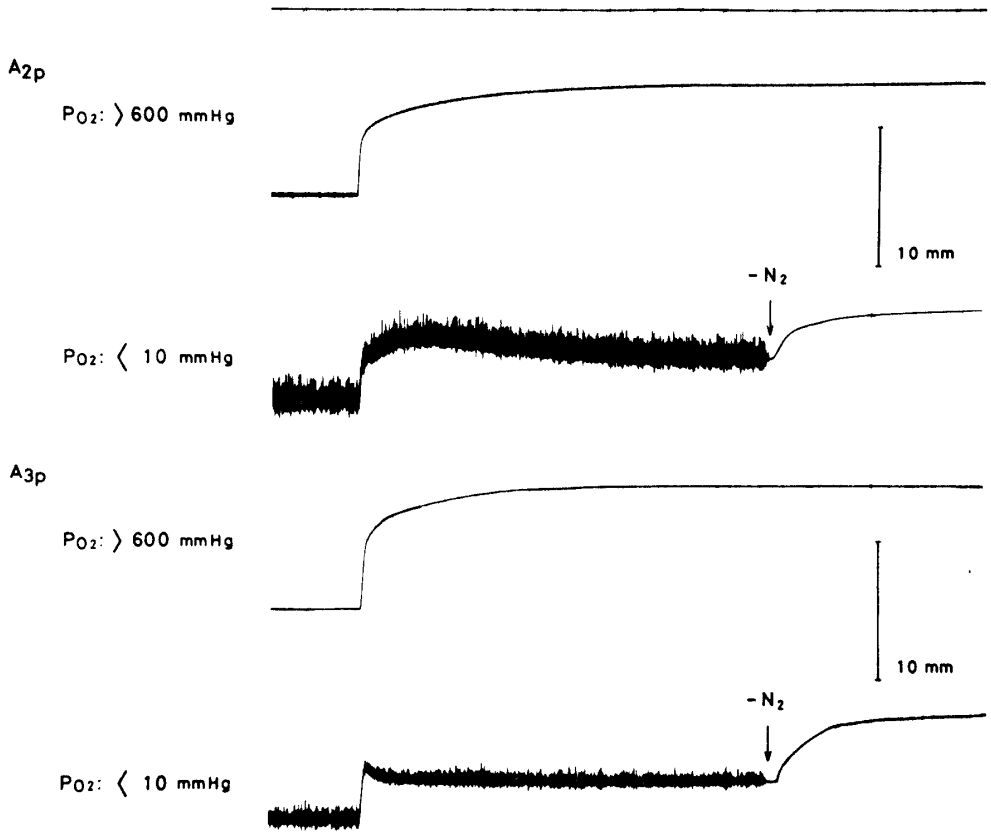

Fig. 1. Aortic contractility. Top : proximal area of the thoracic aorta. Bottom : proximal area of the abdominal aorta. Po_2 : partial pressure of oxygen estimated by a gas analyzer (Meter ABL II, Copenhagen). $Po_2 > 600$ mm Hg under aerobic conditions. $Po_2 < 10$ mm Hg under anaerobic conditions. $-N_2$: the gas mixture was switched from N_2-CO_2 (95−5 %) to O_2-CO_2 (20.9−0.03 %).

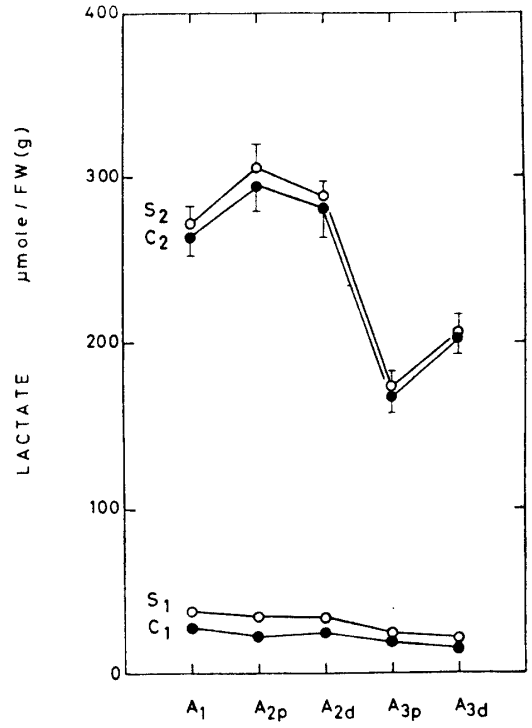

Fig. 2. Levels of lactate produced for 3 hours and 10 minutes. C_1, S_1 : aerobic conditions. C_2, S_2 : anaerobic conditions. C_1, C_2 : the levels in the resting aorta. S_1, S_2 : the levels in the contracting aorta. FW : fresh weight in dry ice-acetone. The values of C_1 or S_1 : average of 26−30 tests. The values of C_2 or S_2 : average of 10−14 tests. Vertical bars : ± standard error.

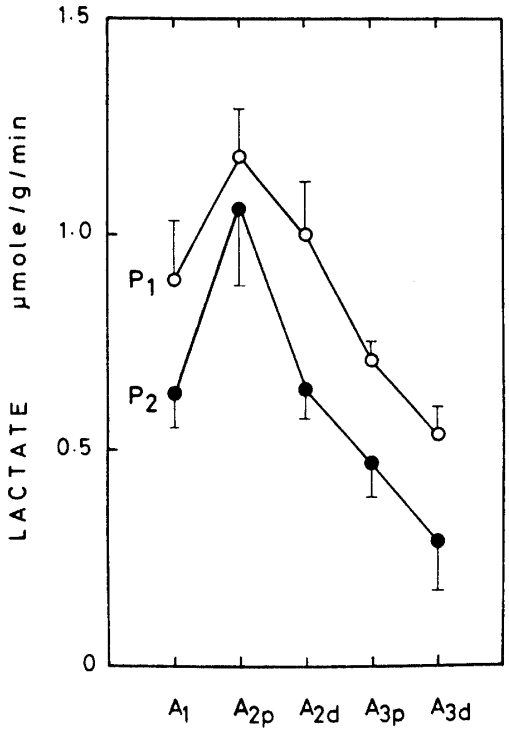

Fig. 3. The net increase in lactate production. Open circles (p_1) : aerobic conditions. Closed circles (p_2) : anaerobic conditions. Vertical bars : ±standard error. Referring to Fig. 2, all the values were calculated from the difference between two levels of lactate in the resting and in the contracting aorta.

control levels (Fig. 2 (c_2)) were high to the extent that the increasing percentages of lactate decreased.

In each experiment, net increase in lactate production during shortening was calculated from the difference between two levels of lactate in the resting and in the contracting aorta. As shown in Fig. 3, higher values were found in the proximal area of thoracic aorta (A_{2p}) than in other areas (A_1, A_{2d}, A_{3p}, A_{3d}). However, the lactate production in these areas observed under anaerobic conditions (Fig. 3 (p_2)) was lower than that obtained under aerobic conditions (Fig. 3 (p_1)).

Phosphofructokinase

In the resting aorta, the levels of phosphofructokinase increased under anaerobic conditions, as compared with findings in the case of aerobic medium. Fig. 4 (c_1, c_2) shows these results. The increasing percentages in these instances were 20—30 % in the aortic arch, 10—20 % in the thoracic aorta (A_{2p}, A_{2d}) and 5—10 % in the abdominal aorta (A_{3p}, A_{3d}). This finding shows that the enzymatic activation varied in the different areas. Since glycogenolytic activity (6) and glucose uptake (7) in mammalian arteries are stimulated under anaerobic conditions, we cannot rule out the contribution of glycogenolytic enzyme and membrane transport of sugar to the accumulation of lactate.

In the contracting aorta, the activation of phosphofructokinase under anaerobic conditions (Fig. 4 (p_2)) was less than that observed under aerobic conditions (Fig. 4 (p_1)). Therefore, it seems most likely that such a reduction in the enzymatic activation follows a decrease in the pH of these materials, in parallel with the lactate accumulation.

Little is known of the mechanism controlling the Pasteur effect. The

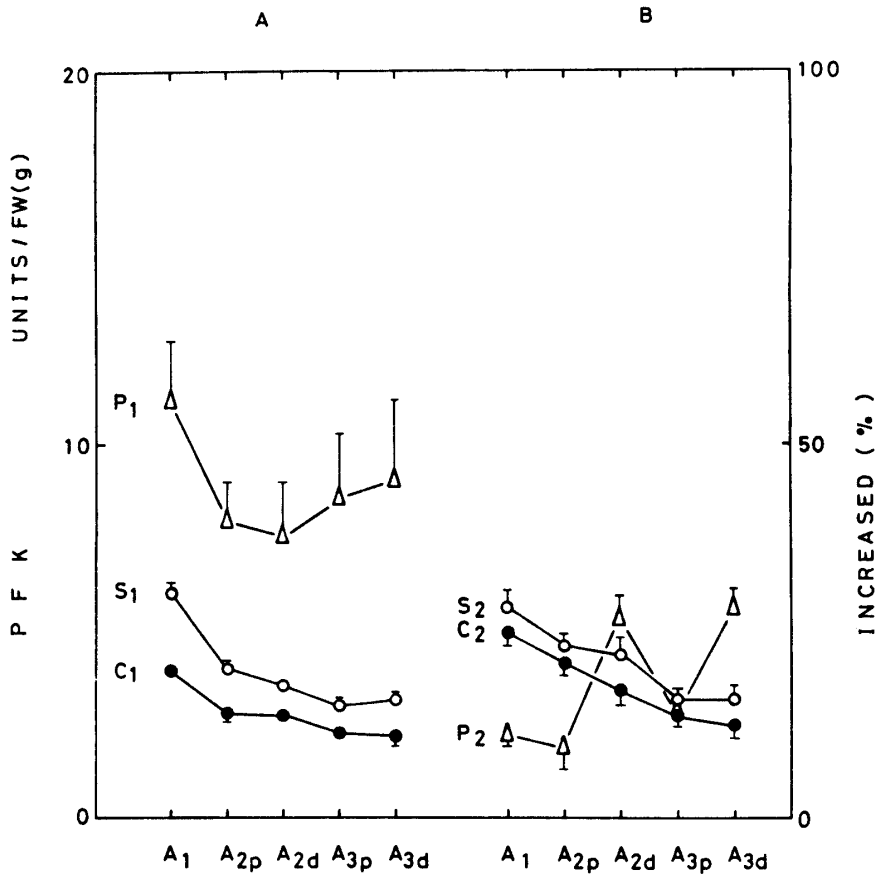

Fig. 4. The levels of phosphofructokinase in different areas of rabbit aorta. A : aerobic conditions. B : anaerobic conditions. C_1, C_2 : the levels in the resting aorta. S_1, S_2 : the levels in the contracting aorta. P_1 : the increasing percentages (under aerobic conditions). P_2 : the increasing percentages (under anaerobic conditions). FW : fresh weight frozen in dry ice-acetone. The values of C_1 or S_1 : average of 24–28 tests. The values of C_2 or S_2 : average of 8–9 tests. Vertical bars : ± standard error.

activity of phosphofructokinase in biological systems is suppressed by H^+ or by metabolic products such as citrate and fatty acid. Accordingly, this observation, together with our findings that the enzymatic activation in the contracting rabbit aorta (Fig 4 (p_1 ,p_2)) was less remarkable under anaerobic conditions raises the question of whether or not the rate of glycolysis in mammalian arteries may be restrained by these metabolites that acidify the materials.

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