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Activities of Glycolytic Enzymes in Rabbit Aorta during Contraction

(glycolytic enzymes/aorta/adrenaline)

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After removal of the adventitia from the rabbit aorta, the preparations were cut into spiral strips and mounted in a bath medium at 37°C, aerated with 5 % CO_2 and 95 % O_2 under a load of 1 gm of tension. When adrenaline was added to the bath medium to contract these strips, the effect of this drug on glycolytic enzymes in the materials was studied. In the experiments, most of the enzymatic activities were augmented in comparison with those of the controls. However, in the statistical analyses, hexokinase and phosphofructokinase in the aortic arch (A_1) and in the distal part of abdominal aorta (A_{3d}) were significantly activated. Therefore, it appears that during the aortic contraction, glycolysis in these areas (A_1, A_{3d}) would be accelerated by such enzymatic activations.

According to Needleman and Blehm (1), adrenaline stimulates lactate production and reduces ATP content in rabbit contracting thoracic aorta. This finding suggests that the glycolytic process would be enhanced in requirement of calorie output of the contracting smooth muscle. Since the rate of glycolysis in arterial tissue is accelerated by glycolytic enzymes, such an increase in lactate levels is probably dependent on the activation of these enzymes.

To evaluate activities of glycolytic enzymes in contracting aorta, we previously determined the levels in resting rabbit aorta and concluded that the activities declined from the aortic arch to the abdominal aorta (2). These results supported those of Morrison *et al.* (3), who pointed out that higher lactate levels in swine aorta were found in the aortic arch rather than in the thoracic aorta. In consideration of the basic enzymatic activities, we estimated these levels in different parts of the contracting aorta.

MATERIALS AND METHODS

Normal, adult rabbits of both sexes were fed laboratory chow *ad libitum* before the experiments. Pentobarbital sodium (30 mg/kg) was given i. p. and the animals were sacrificed by bleeding from a carotid artery. As shown in Fig. 1, the aortic arch (A_1), the proximal (A_{2p}) and distal (A_{2d}) parts of the

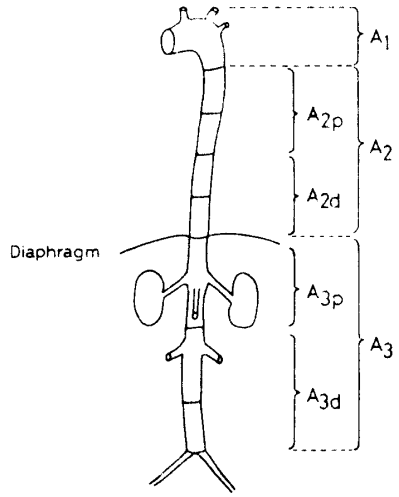

Fig. 1. Sections of rabbit aorta. A_1 : the aortic arch, A_2 : the thoracic aorta (A_{2p} : the proximal part, A_{2d} : the distal part), A_3 : the abdominal aorta (A_{3p} : the proximal part, A_{3d} : the distal part).

thoracic aorta and those (A_{3p}, A_{3d}) of the abdominal aorta were immediately excised. Using the method of Karaki and Urakawa (4), the adventitia of all the materials was removed from the intima-media layers. Subsequently, the aortic preparations were cut into spiral shaped strips. These strips were bisected longitudinally in the aortic arch (A_1) and thoracic aorta (A_{2p}, A_{2d}), or transversely in the abdominal aorta (A_{3p}, A_{3d}). 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 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 with the bath medium for at least 3 hrs before adrenaline was added.

The bath medium prepared by the method of Namm (5) had the following composition (millimolar concentration) : NaCl, 119 ; KCl, 4.7 ; KH_2PO_4, 1.18 ; $MgSO_4$, 1.7 ; $NaHCO_3$, 14.9 ; $CaCl_2$, 2.0 ; dextrose, 5.5 ; and sucrose, 50. When adrenaline (10^{-6}—10^{-9}M) was added to the bath medium, the tension produced was recorded by an isotonic transducer (TD-111 S, JD-111 S) connected to a 2 channel polygraph (RM-251). After aortic muscle contraction by this drug (5×10^{-6}M) had attained the maximum, the strips were frozen, using dry ice-acetone and the following glycolytic enzymes were then assayed.

The activity of hexokinase was determined by the method of Joshi and Jagannathan (6) and that of phosphoglucose isomerase was estimated according to the method of Bodansky (7). The activity of phosphofructokinase was measured by the method of Ling *et al.* (8). The level of pyruvate kinase was assayed according to the method of Gutman and Bernt (9) and that of lactate dehydrogenase was determined by the method of Bergmeyer and Bernt (10). 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 min.

RESULTS AND DISCUSSION

Contracting Action of Adrenaline in the Aortic Strips

When the aortic strips were stimulated by adrenaline ($10^{-6}-10^{-9}$M), the contractile response progressively developed within a few minutes in proportion to the amounts of this drug and attained to the maximum 5 min after the stimulation. Fig. 2 shows an example of the aortic arch. The maximum

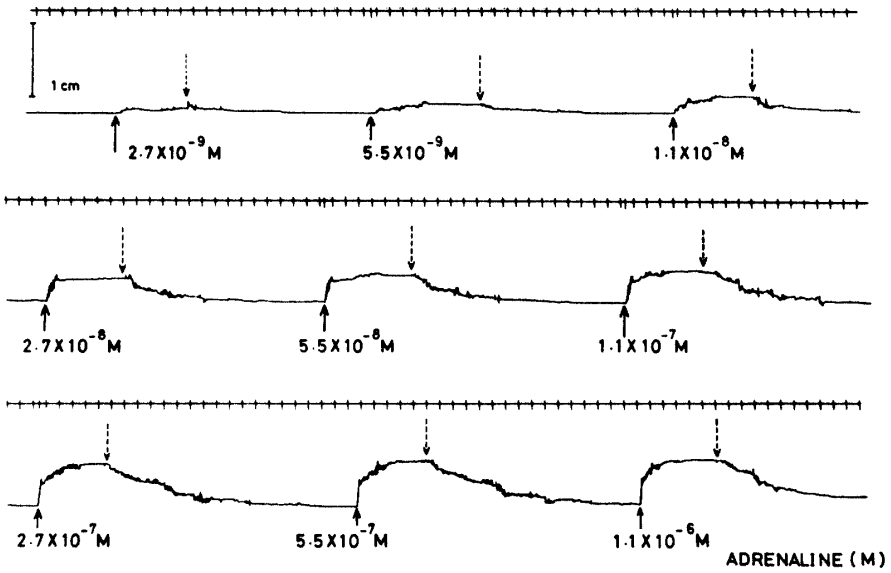

Fig. 2. Contractlile responses of a spiral strip of rabbit aorta in various concentrations of adrenaline. Arrows (solid line) : addition of adrenaline to the strip mounted in the bath medium. Arrows (dotted line) : adrenaline was removed.

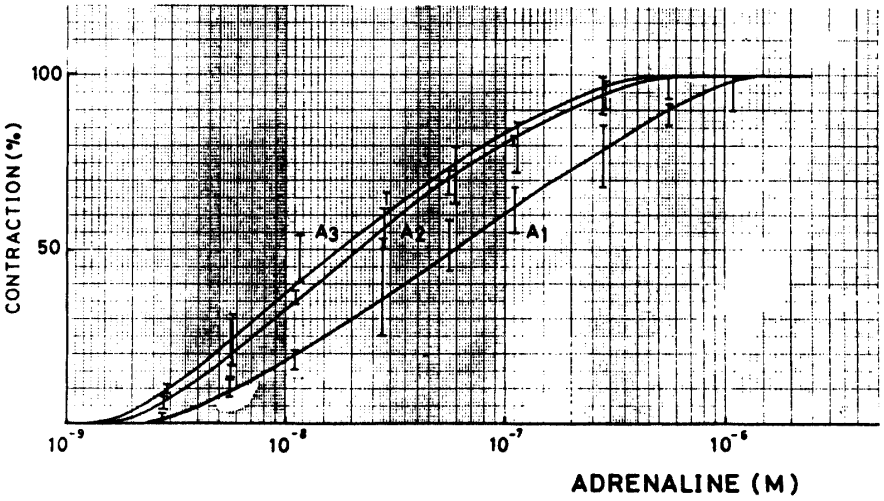

Fig. 3. Dose-response curves of the aortic strips. A_1 : the aortic arch, A_2 : the thoracic aorta, A_3 : the abdominal aorta. Vertical bars : the upper and lower limits.
Each point shows the average of 3 experiments on the A_1 and that of 4 experiments on the A_2 and A_3.

contractions of the strips produced over 10^{-6} M of this catecholamine were expressed in terms of 100 % to indicate varying degrees of smooth muscle contractility with each different molarity of this drug. Fig. 3 shows these dose response curves. The threshold for this response was higher in the aortic arch than in the thoracic and abdominal aorta, while in the range of $10^{-6}-10^{-9}$ M of adrenaline, higher contraction percentages were found in the thoracic and abdominal aorta than in the aortic arch. The differences of the dose-response curves among the aortic strips may be due to the different affinities for this catecholamine.

Activities of Glycolytic Enzymes in the Aortic Strips without Contraction

In the control experiments, each activity of the glycolytic enzymes declined from the aortic arch (A_1) to the distal part of thoracic aorta (A_{2d}), but with the exception of phosphoglucose isomerase in the distal part of abdominal aorta (A_{3d}), the other enzymatic levels in this part (A_{3d}) increased by 20—80 % in comparison with those in the proximal part (A_{3p}). Fig. 4 (a) shows these results.

Averages of the enzymatic activities between the proximal and distal parts of thoracic or abdominal aorta are shown in Table I (Exp. I). On the phosphoglucose isomerase, pyruvate kinase and lactate dehydrogenase, the average levels

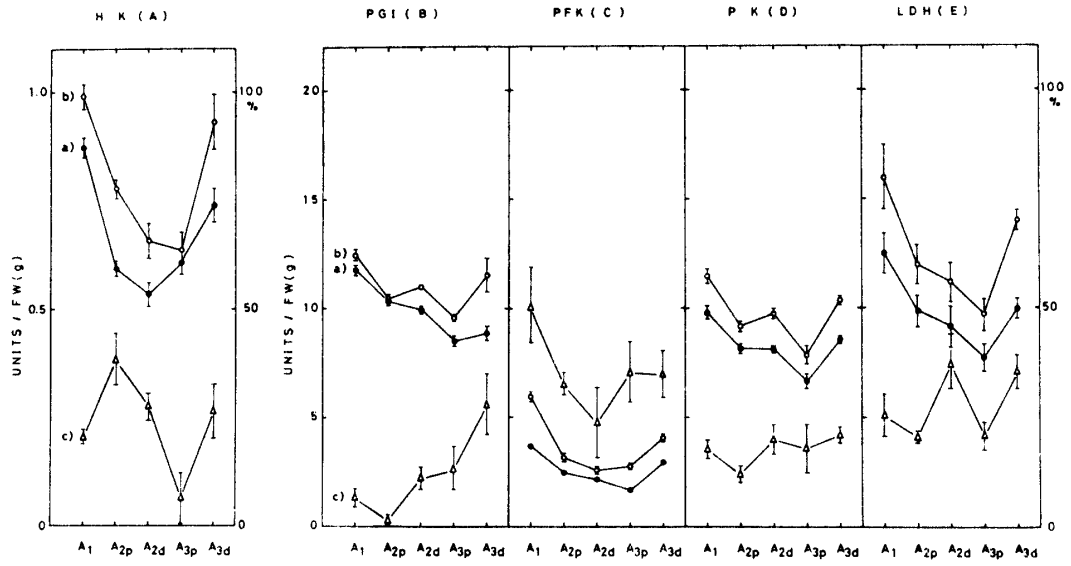

Fig. 4. The activities of the glycolytic enzymes. HK : hexokinase, PGI : phosphoglucose isomerase, PFK : phosphofructokinase, PK : pyruvate kinase, LDH : lactate dehydrogenase.
A_1 : the aortic arch, A_{2p} : the proximal part of thoracic aorta, A_{2d} : the distal part of thoracic aorta, A_{3p} : the proximal part of abdominal aorta, A_{3d} : the distal part of abdominal aorta.
a : the control activities, b : the activities in the contracting strips, c : the increasing percentages of the enzymatic levels in the contracting strips.
Ordinates : left- the enzymatic activities (units/FW(g)), right-the increasing percentages of the enzymatic levels (%). FW : fresh weight frozen by dry ice-acetone.
Average of 8—12 tests. Vertical bars : ± standard error.

TABLE I. *Comparison between the Activity of Hexokinase and Those of the Other Glycolytic Enzymes*

	A_1	A_{2p}	A_{2d}	$\frac{A_{2p}+A_{2d}}{2}$	A_{3p}	A_{3d}	$\frac{A_{3p}+A_{3d}}{2}$
Exp. I.							
HK	0.8750±0.025 (1)	0.5944±0.025 (1)	0.5317±0.037 (1)	0.563±0.031 (1)	0.6083±0.037 (1)	0.7409±0.045 (1)	0.675±0.041 (1)
PGI	11.724±0.347 (13.4)	10.309±0.238 (17.3)	9.9643±0.300 (18.7)	10.137±0.269 (18.0)	8.4978±0.278 (14.0)	8.8899±0.333 (12.0)	8.694±0.306 (13.0)
PFK	3.6520±0.145 (4.17)	2.4100±0.214 (4.05)	2.1220±0.153 (3.99)	2.266±0.184 (4.02)	1.6460±0.127 (2.71)	2.9380±0.130 (3.97)	2.292±0.129 (3.34)
PK	9.7460±0.366 (11.1)	8.1800±0.211 (13.8)	8.1000±0.242 (15.2)	8.140±0.226 (14.5)	6.6820±0.330 (11.0)	8.5400±0.184 (11.5)	7.611±0.257 (11.3)
LDH	12.522±0.939 (14.3)	9.8740±0.724 (16.6)	9.1400±1.002 (17.2)	9.507±0.863 (16.9)	7.7280±0.672 (12.7)	9.9240±0.544 (13.4)	8.826±0.608 (13.1)
Exp. II.							
HK	1.0107±0.072 (1)	0.9270±0.021 (1)	0.8417±0.021 (1)	0.8840±0.015 (1)	0.6980±0.073 (1)	0.7689±0.042 (1)	0.7330±0.035 (1)
PGI	16.972±1.202 (16.8)	14.433±0.905 (15.6)	13.438±0.391 (16.0)	13.936±0.444 (15.8)	12.162±0.730 (17.4)	13.583±0.546 (17.7)	12.872±0.441 (17.6)
PFK	12.987±0.642 (12.8)	9.5770±0.522 (10.3)	7.3070±0.614 (8.68)	8.442±0.568 (9.49)	5.8000±0.754 (8.31)	6.4300±0.935 (8.36)	6.115±0.844 (8.34)
PK	16.263±0.733 (16.1)	13.160±0.545 (14.2)	11.223±0.101 (13.3)	12.192±0.323 (13.8)	10.230±0.377 (14.7)	11.547±0.320 (15.0)	10.889±0.349 (14.9)
LDH	19.007±1.311 (18.8)	16.063±1.516 (17.3)	14.257±0.394 (16.9)	15.160±0.958 (17.1)	12.453±0.445 (17.8)	13.587±0.516 (17.7)	13.020±0.481 (17.8)

Exp. I : the enzymatic activities in the aortic strips incubated in the bath medium at 37°C for 3 hrs (the controls).
Exp. II : the enzymatic activities in the aortic strips determined immediately after sacrificing the animals.
The values in the parentheses : the ratio of the enzymatic levels to respective activity of hexokinase.
HK : hexokinase, PGI : phosphoglucose isomerase, PFK : phosphofructokinase, PK : pyruvate kinase, LDH : lactate dehydrogenase.
A_1 : the aortic arch, A_{2p} : the proximal part of thoracic aorta, A_{2d} : the distal part of thoracic aorta, A_{3p} : the proximal part of abdominal aorta, A_{3d} : the distal part of abdominal aorta.
$A_{2p}+A_{2d}/2$: the average values between the proximal and distal parts of thoracic aorta.
$A_{3p}+A_{3d}/2$: the average values between the proximal and distal parts of abdominal aorta.

declined from the thoracic aorta to the abdominal aorta. On the other hand, these averages also determined immediately after sacrificing the animals are shown in Table I (Exp. II). All of the levels descended to the abdominal aorta. Since the values indicated in Table I (Exp. II) were higher than those represented in Table I (Exp .I), it appears that the enzymatic activities in the different parts faded out during the prolonged incubation in the bath medium at 37°C under a 1 gm load of tension.

Activities of Glycolytic Enzymes in the Aortic Strips during Contraction

As one of properties of vascular muscle contraction, electromechanical or pharmaco-mechanical coupling is a main event and finally leads to the muscle tension development. Throughout either coupling, it would be expected that glycolytic activity in the muscle is augmented, because glycolysis is the major metabolic and energy producing pathway.

As shown in Fig. 4 (b), most of the enzymatic levels increased during contraction. When such a rise in each activity was expressed in terms of the percentage of its control, the level of phosphoglucose isomerase in the proximal part of thoracic aorta (A_{2p}) was unchanged, whereas the other activities in the different parts increased by 10—40%.

As is evident from Table I, the activities of hexokinase and phosphofructokinase were relatively lower than those of the other glycolytic enzymes. Accordingly, it is likely that these two enzymes mainly regulate the glycolytic rate of the contracting aorta. In the statistical analyses, both of these enzymes in the aortic arch (A_1) and in the distal part of abdominal aorta (A_{3d}) were significantly activated during contraction.

In a previous study, the activation of glycogen phosphorylase in the contracting strips was observed in the aortic arch and thoracic aorta, but not in the abdominal aorta (11). Together with this finding, it may be concluded that glycogenolysis as well as glycolysis in the aortic arch were stimulated, while glycogenolysis in the thoracic aorta and glycolysis in the distal part of abdominal aorta were augmented during contraction. Here, referring to Fig. 4 (b), we found that the distal part of abdominal aorta (A_{3d}) exceeded the thoracic aorta (A_{2p}, A_{2d}) in the activity of hexokinase. This result favors the interpretation that as a consequence of the aortic contraction, the part of A_{3d} would utilize glucose through the carbohydrate uptake rather than glycogenolysis in order to replenish the fuel.

Other Remarks

As described above, Needleman and Blehm (1) observed an increase in lactate content, when rabbit thoracic aorta was contracted by adrenaline. Apart from their study, we determined the activities of glycolytic enzymes in different parts of the contracting aorta and found the increasing levels of most glycolytic enzymes. However, as shown in Table II, some of these augmentations were statistically insignificant. Therefore, it is difficult to say that such a rise in the lactate content is related to all enzymatic activations. In our studies, the increase in phosphofructokinase activity in the contracting thoracic aorta was 20—40 %. Since this percentage was insignificant, the lactate deposit in the contracting aorta may depend on the stimulation of glycogenolysis but not that of glycolysis.

On the other hand, however, these same authors (1) noted that the lactate content in the strips of thoracic aorta slightly decreased, when contracted by KCl. Therefore, it appears that aortic contraction is not necessarily associated

TABLE II. *Statistical Analyses on the Increasing Percentages of the Enzymatic Levels in the Contracting Strips*

	A_1	A_{2p}	A_{2d}	A_{3p}	A_{3d}
	%	%	%	%	%
HK	*20.1±2.8	*38.8±5.9	27.1±3.8	6.7±5.8	*26.4±6.5
PGI	6.8±2.0	1.4±1.3	*11.1±2.7	*13.4±5.1	*28.9±6.2
PFK	*50.2±8.4	32.3±3.1	23.8±8.6	*35.9±7.4	*35.3±5.4
PK	*18.1±1.9	*12.5±2.0	*20.5±3.0	18.3±5.2	*21.6±1.7
LDH	25.9±4.8	20.6±1.5	37.6±6.8	21.0±3.0	*35.7±3.7

* : statistical significance ($p<0.05$)
HK : hexokinase, PGI : phosphoglucose isomerase, PFK : phosphofructokinase, PK : pyruvate kinase, LDH : lactate dehydrogenase.
A_1 : the aortic arch, A_{2p} : the proximal part of thoracic aorta, A_{2d} : the distal part of thoracic aorta, A_{3p} : the proximal part of abdominal aorta, A_d : the distal part of abdominal aorta.

with the stimulation of glycolytic enzymes. Under these conditions, the reserved ATP would be preferentially utilized to contract the muscle.

ATP content in the strips of thoracic aorta was reduced by contracting agents such as adrenaline and KCl (1). thus, the hydrolysis of ATP in the aortic contraction is more predominant than the synthesis of this nucleotide. According to Scott *et al.* (12), about half of the total ATP synthesis in swine arterial tissue is probably derived from aerobic glycolysis. If the nucleotide in rabbit aorta is similarly synthesized during lactate production, it is possible that the ATP production is enhanced, when the tissue is contracted by adrenaline.

In addition, we can not rule out the possible effects of calcium ion on the glycogenolytic and glycolytic enzymes, because the divalent cation plays an important role on smooth muscle contraction. According to the review of Lundholm *et al.* (13), calcium ion in some mammalian smooth muscles was a requisite for an increase in the levels of cyclic AMP which in turn activates glycogen phosphorylase. The movements of calcium ion in different areas of the contracting aorta are now being investigated.

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