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Activity of Glycogen Phosphorylase in Rabbit Aorta during Contraction

(glycogen phosphorylase/aorta/adrenaline)

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Rabbit aortic strips were subjected to a load of 1 gm of tension and mounted in a bath medium at 37°C, aerated with 5 % CO_2 and 95 % O_2. Adrenaline (10^{-4} M) was added to the bath medium to contract these strips. In the aortic strips (the aortic arch, and the thoracic aorta), activation of glycogen phosphorylase was observed during contraction. The activity of glycogen phosphorylase a in the contracting aortic arch was increased by 31 % of the control activity, while the increase in the enzymatic activity in the contracting thoracic aorta was 28 %. However, the activity of the abdominal aorta was unchanged during contraction. From these findings, it appears that glycogenolysis increases in the contracting aortic arch and thoracic aorta.

In relation to vascular tissues, Mohme-Lundholm demonstrated two types of glycogen phosphorylase (1). These enzymes are glycogen phosphorylase a and b. The former is active and participates in the first step of glycogenolysis, whereas the latter is inactive. Accordingly, in mammalian arteries, the level of enzyme a has been studied under different conditions. Concerning rabbit aorta, the glycogenolytic enzyme was activated under anaerobic conditions and was also stimulated by contracting agents (angiotensin, histamine, isoproterenol, phenylephrine and KCl) (2). In addition, glycogenolysis in rabbit thoracic aorta was augmented by a contracting agents (adrenaline) (3).

We estimated basal levels of enzyme a and b in rabbit aorta and obtained the following results. The activities of the total glycogen phosphorylase (a + b) and enzyme a declined along the aortic pathway(4). This finding suggested that in different parts of the aorta, the enzymatic activity probably varies during contraction. Thus, we attempted to determine the activity of glycogen phosphorylase a in the contracting aortic arch, thoracic aorta and abdominal 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. Then, aortic

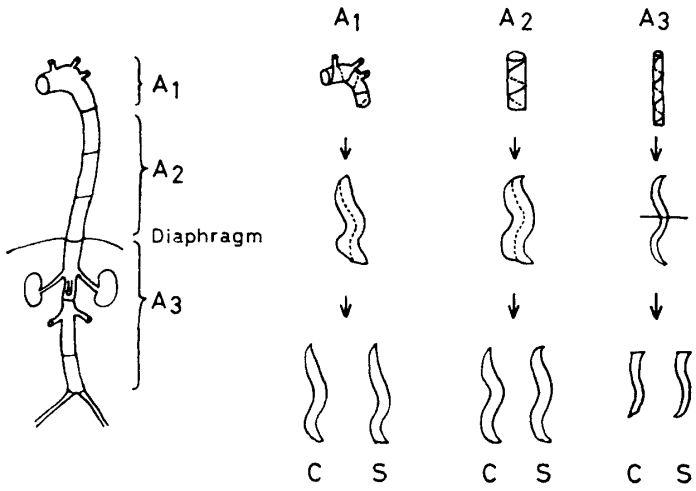

Fig. 1. Preparation of the aortic strips. A_1 : the aortic arch, A_2 : the thoracic aorta, A_3 : the abdominal aorta. Each spiral strip was bisected longitudinally (dotted lines in A_1 and A_2) or transversely (a solid line in A_3). One of the paired strips (S) was contracted by adrenaline, while the other (C) served as the control.

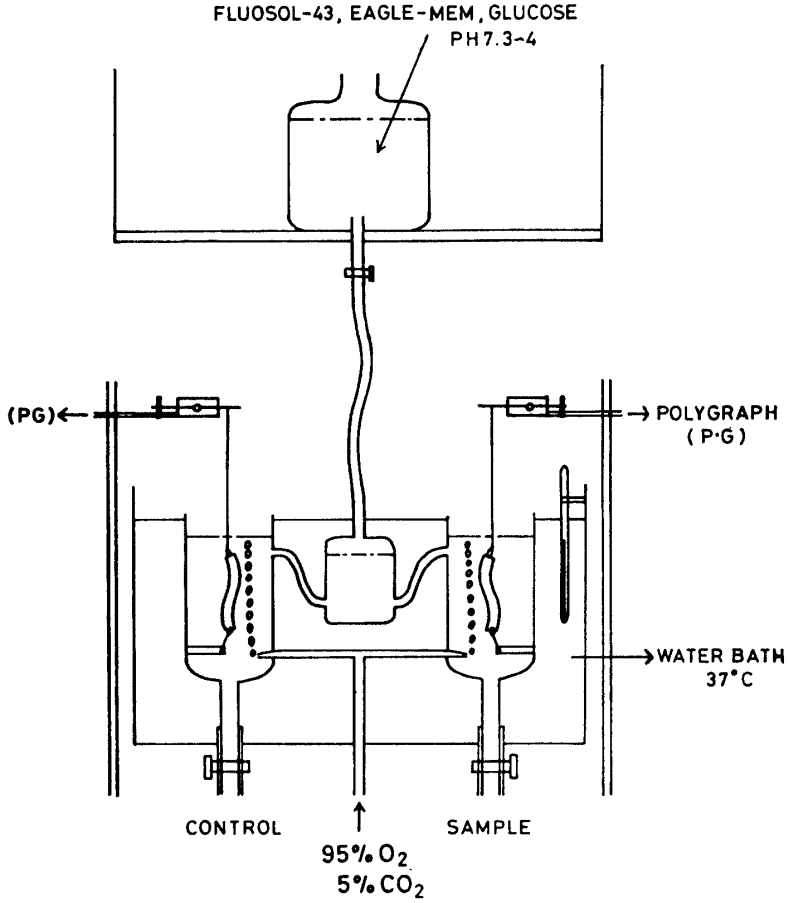

Fig. 2. Scheme of the incubation baths used for mounting the aortic strips. Control : a C- strip in one side. Sample : a S-strip in the other side.

arch, thoracic aorta and abdominal aorta were immediately excised. All the materials were washed with a solution of physiological saline (15°—20°C). As shown in Figs. 1 and 2, the aortic segments were cut into spiral shaped strips and mounted in 18 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 incubated in the bath medium for 30 min before adrenaline (10^{-4} M) was added.

The bath medium (Fluosol-43^R) had the following composition (w/v %); Perfluorotributylamine, 28; polyoxypropylene-polyoxyethylene copolymer, 3.2; NaCl, 0.60; KCl, 0.034; $CaCl_2$, 0.028; $MgCl_2$, 0.02; $NaHCO_3$, 0.21; glucose, 0.18 ; hydroxyethyl starch, 2.70. Since Clark pointed out the high oxygen solubility in perfluorochemicals(5), Fluosol-43^R serves adequately as artificial blood for experimental studies of metabolism.

When adrenaline was added to the bath, 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 had attained the maximum, the strips were frozen using dry ice-acetone. Subsequently, the activity of glycogen phosphorylase a was estimated by the method of Shimazu and Amakawa(6) and the amount of protein was determined according to the method of Lowry *et al.*(7).

Fluosol-43^R was obtained from The Green Cross Corporation, Osaka, Japan.

RESULTS AND DISCUSSION

1. Contracting Action of Adrenaline on the Aortic Strips

When a small amount of adrenaline (within 10^{-6}M) was added to the bath medium, there was a positive linear relationship between the aortic contractility and the dose level. On the other hand, in the supramaximal dose (10^{-4} M), the maximum contraction could be obtained. As shown in Fig. 3 (A_1,A_2 and A_3), each contraction was in a steep ascent within 2 min and slowly developed between 2 and 10 min. After that, all strips exhibited tonic contraction.

According to the report of Namm(2), the activity of glycogen phosphorylase a in rabbit aorta was increased in proportion to the tension development. Namely, when the tension was the maximum, the enzymatic activation was the highest. Therefore, in the present study, the aortic strips were contracted by supramaximal stimulation to estimate the highest enzymatic activity.

2. The Total Activity of Glycogen Phosphorylase

The total activity (a + b) of glycogen phosphorylase in the aortic strips was determined in the presence of adenosine 5′-monophosphate (5′-AMP) and was represented by the activity of glycogen phosphorylase a. Prior to the estimation of the total activity, the activity of glycogen phosphorylase a was estimated and plotted against the concentration of 5′-AMP. Fig. 4 shows a

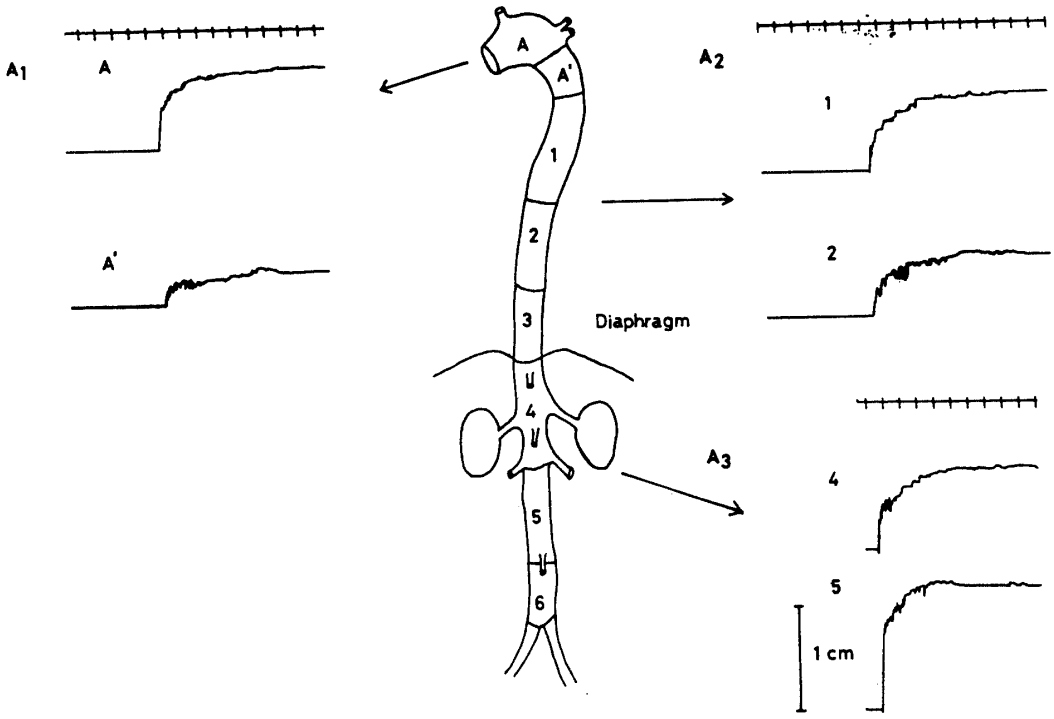

Fig. 3. Contraction of the aortic strips stimulated with supramaximal dose of adrenaline (10^{-4} M). Times were recorded at intervals of one min. A_1 : the aortic arch, A_2 : the thoracic aorta, A_3 : the abdominal aorta.

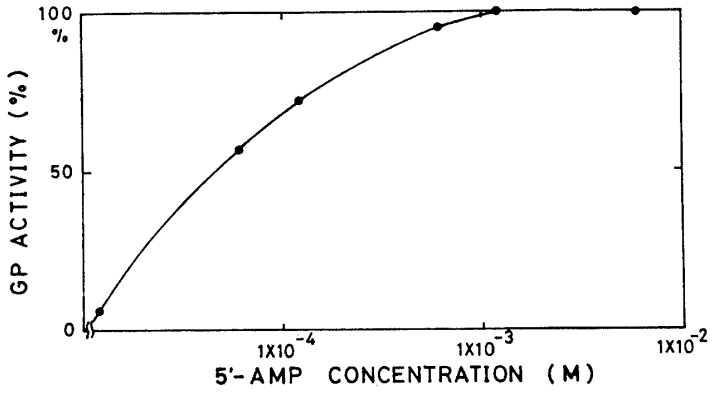

Fig. 4. A dependence of glycogen phosphorylase a on the concentration of 5′-AMP. Ordinates : the maximum activity was expressed in terms of 100%. Abscissae : the concentration of 5′-AMP. GP : total glycogen phosphorylase.

typical example of the aortic arch. The activity ascended in a convex curve in proportion to the increase in 5′-AMP. This dependence of the enzymatic activity on 5′-AMP was estimated in the thoracic and abdominal aorta. Since the highest levels of this enzyme in the aortic strips were observed with over 1 mM 5′-AMP, the total activity was determined at the excess level of this nucleotide.

Within the limits of the experiments, the total activity was not significantly changed before or after mounting the strips in the bath medium. As shown in Table I, higher values were found in the aortic arch than in the

TABLE I. *Total Activities of Glycogen Phosphorylase in the Aortic Strips*

	U/mg Protein	U/mg Protein
A_1	0.306(±0.018)	0.308(±0.006)
A_2	0.289(±0.023)	0.290(±0.022)
A_3	0.223(±0.017)	0.222(±0.022)

Left column : the activities were estimated 30 min after mounting the strips in the bath medium. Right column : the activities determined immediately after sacrifice. A_1 : the aortic arch, A_2 : the thoracic aorta, A_3 : the abdominal aorta.

thoracic and abdominal aorta, indicating that the total level of this enzyme varied at different parts of the aorta.

3. The Activity of Glycogen Phosphorylase a

When the aortic strips were mounted in the bath medium for 30 min, the activity of glycogen phosphorylase a in the aortic arch was decreased by about 30 % of the original, as determined immediately after sacrificing the animals, whereas the activity in the thoracic and abdominal aorta was not significantly changed (Fig. 5). This finding suggests that glycogen phosphorylase a in the aortic arch is unstable as compared with that in the thoracic and abdominal areas.

The level of glycogen phosphorylase b in the aortic strips was also determined from the difference between the activities of the total glycogen phosphorylase (Table I) and the glycogen phosphorylase a (Fig. 5 (b)). As is evident

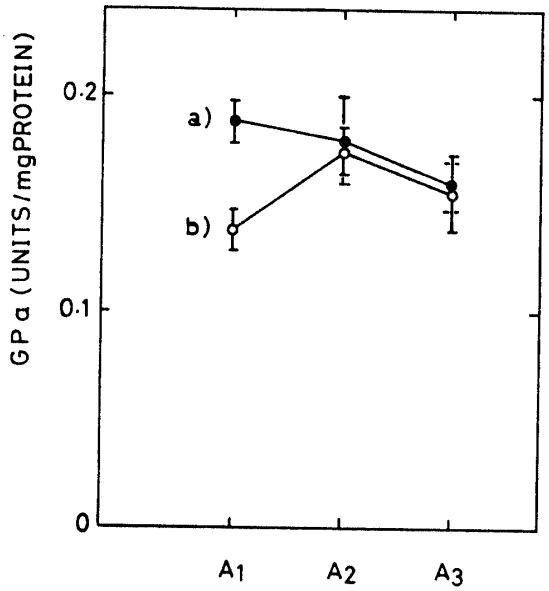

Fig. 5. Activities of glycogen phosphorylase a in the aortic strips. a : the activities were determined immediately after sacrifice. b : the activities estimated 30 min after mounting the strips in the bath medium (the control activities).
Ordinates : activity of glycogen phosphorylase (GPa). A_1 : the aortic arch, A_2 : the thoracic aorta, A_3 : the abdominal aorta.

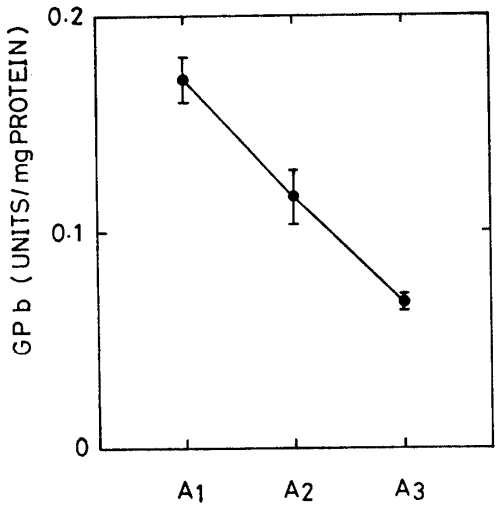

Fig. 6. Glycogen phosphorylase b (GPb) level (30 min after mounting the strips in the bath medium).
Ordinates : glycogen phosphorylase b level. A_1 : the aortic arch, A_2 : the thoracic aorta, A_3 : the abdominal aorta.

from Fig. 6, the glycogen phosphorylase b was dominant in order of the aortic arch, the thoracic aorta and the abdominal aorta. Since glycogen phosphorylase b is converted into enzyme a, the level of glycogen phosphorylase a in the aortic strips would be increased if glycogenolysis is indeed stimulated during the aortic contraction.

4. Activation of Glycogen Phosphorylase in the Aortic Strips during Contraction

Activation of glycogen phosphorylase in the aortic strips was observed during contraction. The activity of glycogen phosphorylase a in the contracting aortic arch was increased by 31 % of the control activity, while the increase in the enzymatic activity of the contracting thoracic aorta was 28 % (Fig. 7). However, the activity in the abdominal aorta was unchanged during contraction. These findings indicate that in the aortic arch and thoracic aorta, conversion of glycogen phosphorylase b into the enzyme a was stimulated during contraction. Accordingly, in this process, the glycogenolytic activity would be accelerated in the aortic arch and thoracic aorta, but not in the abdominal aorta.

In contracting smooth muscle, several investigators have observed activation of glycogen phosphorylase(2,8-10). As the enzymatic activation would be affected by different factors (isotonic or isometric contraction, a contracting agent and developing or sustaining tension), studies on these factors are required to compare our results with the data of other investigators.

In our experiments, it was unexpected that contraction of the abdominal aorta was not associated with the activation of glycogen phosphorylase, because considerable amounts of carbohydrates in arterial tissues are utilized by glucose uptake and/or by glycogenolysis. Therefore, under such an isotonic contraction

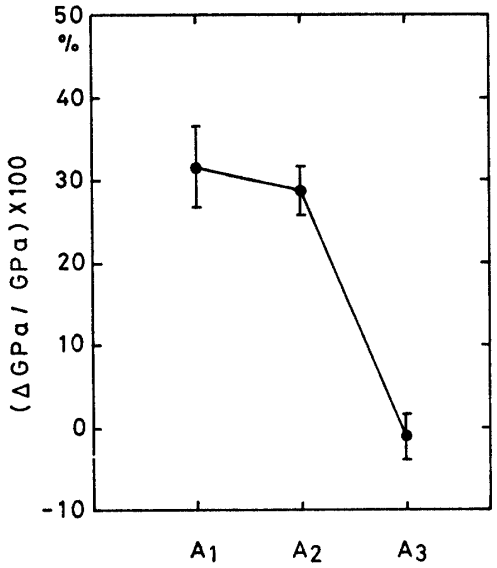

Fig. 7. Net increase in the activity of glycogen phosphorylase a (△ GPa) expressed in terms of the percentage of its control value (GPa). Ordinates: the ratio expressed in terms of the percentage. A_1 : the aortic arch, A_2 : the thoracic aorta, A_3 : the abdominal aorta.

of the aorta, the carbohydrate metabolism of the abdominal aorta appears to be different from that of the aortic arch and thoracic aorta.

According to the report of Andersson(10), bovine mesenteric arteries contracted isotonically by high K^+ (126.7 mM) did not show any stimulation of the glycogen phosphorylase. This evidence indicates that contraction of arterial smooth muscle is not necessarily associated with stimulation of this enzyme. The relation between the contraction and the enzymatic activity remains obscure.

When smooth muscle is contracted, ATP and creatine phosphate are decomposed. Accordingly, such energy rich compounds must be supplied as a consequence of the contraction. Glycogen would thus be utilized in the aortic arch and thoracic aorta while glucose uptake would be augmented in the abdominal aorta in order to replenish the fuel.

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