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Short Communication

Levels of Glycogen Phosphorylase in Rabbit Aorta during a Prolonged Incubation

(total glycogen phosphorylase/glycogen phosphorylase a/glycogen phosphorylase b/rabbit aorta)

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During a prolonged incubation (for 3 hr at 37°C under aerobic conditions), the level of total glycogen phosphorylase and that of glycogen phosphorylase were significantly reduced. The estimated values of total glycogen phosphorylase were 70–60 %, while those of glycogen phosphorylase were 40–25% of the originals determined immediately after sacrifice of the animals. In the case of the experiments, however, the level of glycogen phosphorylase b was unchanged. Therefore, it may be concluded that the reduction of total gycogen phosphorylase activity during the prolonged incubation was caused by decrease in the activity of enzyme a.

To study arterial metabolism *in vitro*, most investigators have mounted isolated strips in a bath medium at 30–40°C under aerobic or anaerobic conditions (1–5). However, under such conditions, we found that the levels of glycolytic enzymes in the freshly prepared strips of rabbit aorta were reduced with time (6). From these findings, it appears that the incubation procedure may influence the metabolic rate of carbohydrates. Therefore, we must take precautions against drawing conclusions on the *in vivo* carbohydrate metabolism from the results of *in vitro* experiments. In this paper, we considered a related problem concerning the reduction of glycogenolytic enzyme in rabbit aorta during a prolonged incubation in a bath medium.

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. The aortic arch (A_1), the proximal (A_{2p}) and distal (A_{2d})parts of the thoracic aorta and those (A_{3p}, A_{3d}) of the abdominal aorta were immediately excised. Subsequently, the aortic preparations were cut into spiral shaped strips. Using the method of Karaki and Urakawa (7), the adventitia of all the materials was removed from the intima-media layers. The aortic strips were mounted in 20 ml of a bath medium aerated with 95 % O_2 and 5 % CO_2 at 37°C. In this case, resting tension of 1 gm was applied to each strip in the bath

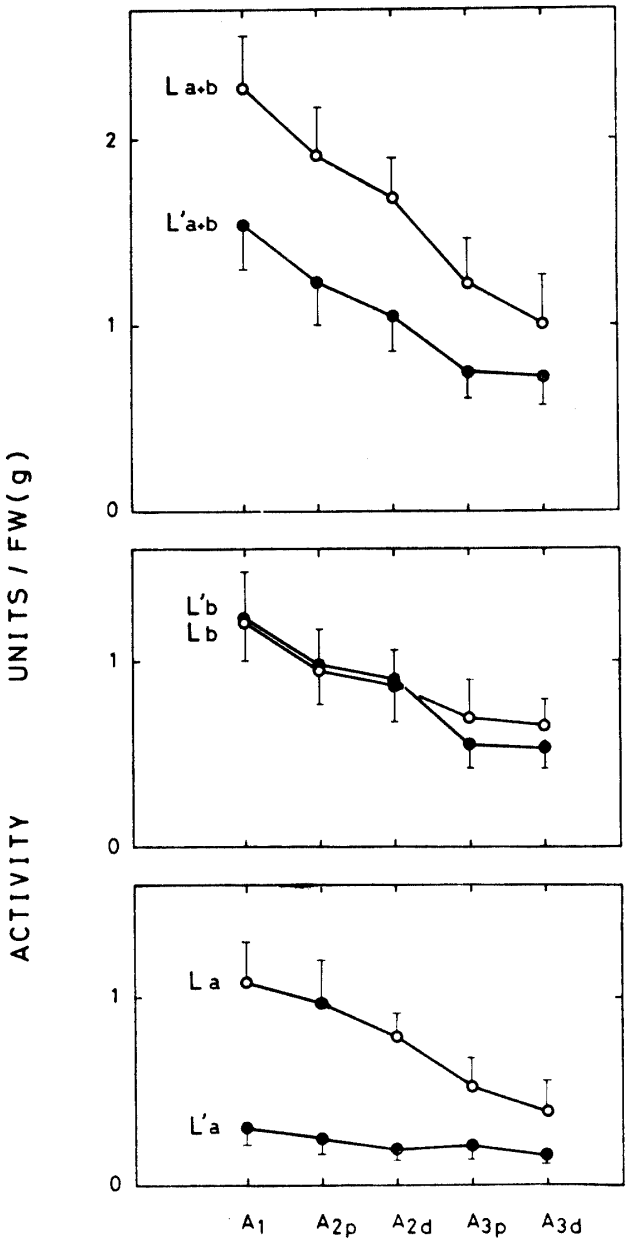

Fig. 1. The levels of glycogen phosphorylase in different areas of rabbit aorta. On the aortic strips prepared immediately after the sacrifice of the animals, La+b is the level of total glycogen phosphorylase and La, Lb are those of glycogen phosphorylase a and b. On the strips mounted in the bath medium, L'a+b is the level of total glycogen phosphorylase and L'a, L'b are those of enzyme a and b.
A_1 : the aortic arch, A_{2p} : the proximal area of thoracic aorta, A_{2d} : the distal area of thoracic aorta, A_{3p} : the proximal area of abdominal aorta, A_{3d} : the distal area of abdominal aorta. Ordinates : glycogen phosphorylase activity (units/FW(g)). FW : fresh weight frozen by dry ice-acetone. Average of 9–22 tests. Vertical bars : ±standard deviation.
The statistical analyses on the difference estimated between two levels : La+b, L'a+b ($p < 0.005$), Lb, L'b ($p > 0.05$), La, L'a ($p < 0.005$).

medium prepared by the method of Namm (1). Under such conditions, all strips were incubated for 3 hr. 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). Using the homogenized preparations, the activity of glycogen phosphorylase a was determined by the method of Bergmeyer *et al.* (8). The total activity (a+b) of glycogen phosphorylase was simultaneously estimated by addition of 1 mM adenosine 5′-monophosphate to the assay mixture and was represented by the activity of glycogen phosphorylase a. The level of glycogen phosphorylase b was also calculated by the difference between the activities of the total glycogen phosphorylase and glycogen phosphorylase a. Throughout the experiments, one unit of glycogen phosphorylase a was defined as that amount which catalyzed the formation of 1 μmole of glucose-1-phosphate per min.

When the aortic strips were incubated for 3 hr, the total level (L′a+b) decreased by 30—40 % in comparison with the level of the starting materials ($p < 0.05$, Fig. 1 (La+b, L′a+b)). On the other hand, in this procedure, the activity of glycogen phosphorylase a in each of the different areas (L′ a) decreased by 60—75 % of the original ($p < 0.05$, Fig. 1 (La, L′a)). However, the level of enzyme b was not significantly changed during the incubation for hours in the bath medium (Fig. 1 (Lb, L′b)). It is then deduced from the present study that such a reduction of the total activity observed during the prolonged incubation (3 hr) was caused by decrease in the activity of enzyme a. Therefore, the incubation may affect the magnitude of the subsequently investigated rate of carbohydrate metabolism.

In the strips mounted in the bath medium, O_2 and glucose diffuse through the extracellular space to the cell membrane and penetrate into the intracellular space. Thus, in order to attain the equilibrium of these transports, the aortic tissue should be incubated for 3 hours. Indeed, according to Namm (1), rabbit aortic strips were incubated for at least 3 hr before starting studies on the activation of glycogen phosphorylase. However, since the present results indicate that the activities of the total glycogen phosphorylase and glycogen phosphorylase a were significantly reduced under such experimental conditions, the enzymatic level determined in the experiments of Namm would be attenuated in comparison with the actual level *in vivo*.

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