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Short Communication

Levels of ATP in Different Areas of Contracting Rabbit Aorta under Aerobic Conditions

(ATP/contracting rabbit aorta/aerobic conditions)

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Using pharmacological approaches, the levels of ATP in different areas of contracting rabbit aorta were found to be increased over the findings in the controls. The percentages were 170 % in the aortic arch, 230–360 % in the thoracic aorta and 30–80 % in the abdominal aorta. This evidence suggests that the levels of ATP in the contracting aorta are probably not reduced under aerobic conditions.

The levels of ATP and CrP in contracting bovine mesenteric artery were found to be reduced under aerobic or anaerobic conditions (1–3). However, according to a report of Needleman and Blehm (4), the concentration of ATP in contracting rabbit thoracic aorta was enhanced with addition of oxygen. These findings suggest that the content of this nucleotide during vascular contraction is not necessarily reduced in the presence of oxygen. For confirmation, we assessed the levels of ATP in different areas of contracting rabbit aorta, under aerobic conditions.

Healthy, adult rabbits of both sexes fed a laboratory chow diet *at 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_{2d}) areas of the thoracic aorta and those (A_{3p}, A_{3d}) of the abdominal aorta were immediately excised. Subsequently, the aortic materials were cut into spiral strips and bisected longitudinally. Using the method of Karaki and Urakawa (5), 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 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 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, JD-111 S, Nihon Koden Kogyo Co., Tokyo) connected to the RM-251 multipurpose polygraph. The aortic muscle contracted for 10 minutes by

a dose of 5×10^{-6} M of this catecholamine. With time, the tension development attained the maximum. These strips were then frozen using dry ice-acetone.

To estimate the amounts of ATP (6), the frozen strips, measuring on the average 30 mg., were suspended in 20 volumes of 2°C solution of 1.0 N perchloric acid. These suspensions were homogenized in a Polytron homogenizer and then centrifuged for 10 minutes at 2°C and 3000×g. Using a third of the volume of perchloric acid used for the first extraction, the sediments were washed with 1.0 N perchloric acid three times. Subsequently, the supernatants thus obtained were combined and adjusted to pH 6.0—6.5 with 2.0 M KOH. After this procedure, these solutions were allowed to stand overnight in an ice bath and the clear supernatants were used for the assay of this nucleotide.

In the first series of experiments, the original levels of ATP in different areas of rabbit aorta were determined immediately after exsanguination. As indicated in Fig. 1 (a), these levels of this nucleotide were higher in the

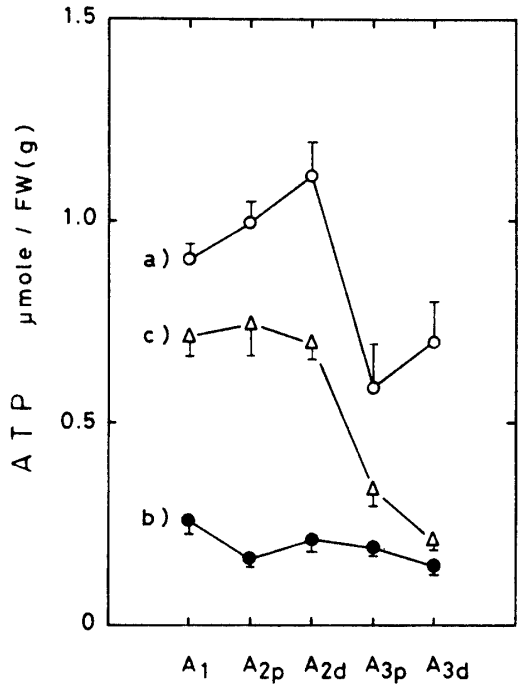

Fig. 1. The levels of ATP in different areas of rabbit aorta. Ordinates : ATP (μmole/FW (g)). FW : fresh weight frozen by dry ice-acetone. a : the levels of ATP in the original materials. b : the levels of ATP estimated after the prolonged incubation (3 hours and 10 minutes). c : the levels of ATP in the contracting materials. Average of 14—16 tests. Vertical bars : ± standard error.

distal area of thoracic aorta than in the other areas. Since ATP is synthesized *via* Embden-Meyerhof pathway and *via* Krebs cycle, it should be emphasized that the estimated values reflect the activities of these metabolic pathways in the original materials.

When the aortic strips were incubated in the bath medium for 3 hours and 10 minutes at 37°C under a 1 gm load of tension, the levels of ATP in different areas of the resting aorta (the controls) decreased by 70—80 % of the original (Fig. 1 (a)), thereby indicating that such a prolonged incubation caused a decomposition of this nucleotide.

During elevation of tone, the levels of ATP rose over the controls. As shown in Fig. 1 (c), a marked increase in the nucleotide level was observed in the aortic arch and thoracic aorta. In each of the materials, the increasing percentages amounted to 170 % in the aortic arch, 230—360 % in the thoracic aorta (A_{2p}, A_{2d}) and 30—80 % in the abdominal aorta (A_{3p}, A_{3d}).

In a contracting vascular muscle, ATP and CrP are decomposed to liberate free energy. Accordingly, as a consequence of the muscle contraction, such energy-rich compounds are supplied in the metabolic process of carbohydrates. We found that the ATP content in the aortic strips was augmented during contraction, therefore, the synthesis of ATP in different areas of the contracting aorta is probably more predominant than the hydrolysis of this nucleotide.

According to Scott *et al.* (7). 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, it is possible that the ATP production is enhanced when aerobic glycolysis is stimulated during contraction. Indeed, in a previous study, we observed that phosphofructokinase (a possible rate limiting enzyme of glycolysis) was significantly facilitated in all areas of the contracting aorta, under aerobic conditions (8). This finding favours the interpretation of an augmented ATP production. However, since oxygen uptake in a mammalian smooth muscle is accelerated during tension development (9), it can be expected that the calorigenic level of contracting rabbit aorta is also augmented and the oxidative phosphorylation enhanced under aerobic conditions.

We conclude that levels of ATP in contracting mammalian arteries are not necessarily reduced under aerobic conditions. Further studies are underway to evaluate whether ATP levels in contracting arteries are dependent on species and the anatomic sites.

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