Acute or Chronic Exercise Alters Angiotensin II-Induced Contraction of Rat Aorta

Tetsuya IZAWA, Masako MORIKAWA*, Michiko INOUE*,
Takudou MIZUTA, Hitoshi YAMASHITA[†], Hideki OHNO[†],
and Takao KOMABAYASHI*

*Department of Health and Sports Sciences, The University of
Electro-Communications, Chofu, Tokyo, 182 Japan*

**Department of Pharmacology, Tokyo University of Pharmacy
and Life Sciences, Hachioji, Tokyo, 192-03 Japan*

*[†]Department of Hygiene, National Defense Medical College,
Tokorozawa, 359 Japan*

Summary Chronic exercise substantially shifted the dose-response curve for angiotensin II (ANG II)-induced contractions to the right with increases in the EC₅₀ value in both endothelium-intact and endothelium-denuded aortae. An acute endurance exercise also caused a 3-fold increase in the EC₅₀ value in both endothelium-intact and endothelium-denuded aortae from sedentary control rats. Whereas, no substantial increase in the EC₅₀ value was observed in trained rats after an acute endurance exercise. Thus, chronic exercise attenuates ANG II receptor-mediated contraction of rat aortae. An acute exercise also caused the reduced responses to ANG II in sedentary control rats but not in chronically exercise-trained rats.

Key words: aorta, angiotensin II, exercise.

Physical exercise training has been shown to alter the vascular responsiveness of coronary arteries to vasoactive substances. Oltman *et al.* [1] suggested that exercise training alters adenosine and α -adrenergic receptor-signal transduction events in coronary vascular smooth muscle. Moreover, Delp *et al.* [2] observed that exercise training-induced alterations in responses of rat abdominal aorta to norepinephrine were due to an endothelium-dependent mechanism involving α_2 -adrenergic receptors. On the other hand, arterioles also respond to angiotensin

Received on July 17, 1995; Accepted on October 16, 1995

Correspondence should be addressed to: Tetsuya Izawa, Department of Health and Sports Sciences, The University of Electro-Communications, 1-5-1, Chofugaoka, Chofu, Tokyo, 182 Japan.
Fax: +81-424-98-1194, E-mail: izawa@prime.e-one.uec.ac.jp

II (ANG II), a potent vasoconstrictor which circulates in the blood and becomes elevated during exercise [3–5], and this increase in ANG II has been suggested to contribute to vascular responses to exercise. Very recent study shows that ANG II contributes to the increase in mean arterial blood pressure and redistribution of cardiac output during dynamic exercise [5]. However, it is unclear whether an acute or chronic endurance exercise may cause alterations in vascular contractile responses to ANG II, and no direct evidence has so far been obtained. Therefore, we investigate the effects of acute and chronic dynamic exercises on contractile responses to ANG II in rat aortae.

Methods

Animal care and exercise training program. Male Wistar rats (Japan SLC) with an initial body weight of 140–150 g were used for the experiments. The animals were randomly divided into four groups: control (6 rats); control-exercising (6 rats); trained (6 rats); trained-exercising (6 rats). All groups of rats were housed three to a cage in a room with the temperature controlled at 24°C and a 12:12-h light-dark cycle. They were given free access to food and water. All animals were cared for in accordance with the guiding principles in the care and use of animals based upon the Declaration of Helsinki. Animals in control were not subjected to treadmill running. Control-exercising rats also remained untrained until the week before the experiment, and then they were given four preliminary exercise sessions, lasting <20 min, to acquaint them with running on the treadmill at different day. Thereafter, to evaluate the effect of an acute endurance exercise, they ran on a rodent treadmill set an angle of 5° for 90 min at 30 m/min, and were sacrificed by decapitation immediately after completion of this exercise session. Trained rats ran on the treadmill 5 d/week for 9 weeks, as described previously [6]. Briefly, the running time and speed were increased progressively until, after 6–7 weeks, the rats ran continuously for 90 min/d at 30 m/min. Trained rats were sacrificed at least 48 h after their last exercise session (90 min at 30 m/min at an angle of 5°). Trained-exercising rats were also subjected to exercise training. However, to evaluate the effect of an acute endurance exercise, they were sacrificed immediately after completion of their last exercise session (90 min at 30 m/min at an angle of 5°).

Isometric tension recordings. Isometric tension recording was performed according to the procedure described in our previous study [7]. Following removal of thoracic aortae, all adipose and connective tissues were removed from adventitia in buffer A of the following composition (in mM): 120.3 NaCl, 4.8 KCl, 1.8 CaCl₂, 1.3 MgSO₄, 5.6 glucose, 10 N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid, pH 7.4. The solution was gassed with 100% O₂. Then a segment 2 mm in width and 10 mm in length was exactly isolated. Two segments were prepared from one animal; one was endothelium-intact and the other endothelium-denuded. During all procedures, special care was taken not to damage the endothelium. Endothelial cells were removed by gentle rubbing of the aortae. To test whether endothelial

cells were removed, we confirmed that acetylcholine did not elicit relaxation in a rubbed aorta. One end of the segment was ligated to the transducer and the other to the stainless holder. The segment was suspended in an organ bath with 5 ml of buffer A at 37°C. All muscle segments were submitted to a resting tension of 1 g and allowed to equilibrate for 1 h before the beginning of the experiment. The contractile responses were recorded isometrically with a force displacement transducer (Orientech T-7-8-240) connected to a polygraph (Nihon Kohden). Dose-response relationships were determined by cumulative addition of ANG II or KCl. Recovery periods of 15 min were allowed between dose-response tests so that resting tension returned to the baseline before administration of the next agent. Bathing medium was replaced every 5 min during recovery periods.

Expression of data and statistical analysis. Concentration-response curves were evaluated using three-way analysis of variance. Differences observed were further assessed by Duncan's multiple comparison. The concentration of ANG II required to produce a half-maximal response (EC_{50}) was derived from a four-parameter logistic equation. Maximal tension was presented as absolute value in mg per mm² of segments. The significance of differences between mean values of citrate synthase activity, EC_{50} , and maximal tension was assessed by unpaired *t*-tests. Analysis of variance was used to determine whether significant differences existed among EC_{50} values for endothelium-intact and endothelium-denuded rings from groups. All data are expressed as means $\pm$ SE. Results were considered statistically significant at $p < 0.05$.

Results and Discussion

Effects of chronic endurance exercise. The final mean body weight (g) of the trained (220 ± 3) or trained-exercising (216 ± 5) was significantly less than that of control (260 ± 4) or control-exercising (257 ± 5). The activity (U/mg protein) of citrate synthase in skeletal muscle was significantly higher in the trained (0.194 ± 0.029) and trained-exercising (0.241 ± 0.004) than in control (0.141 ± 0.011) and control-exercising (0.165 ± 0.006). This indicates an increase in skeletal muscle oxidative capacity and characteristic of effective exercise training.

Figure 1 shows the dose-dependent curves of endothelium-intact and endothelium-denuded aortae in response to ANG II in control and trained rats. The maximal tension and EC_{50} values after chronic endurance exercise and an acute endurance exercise (described in the next session) are summarized in Table 1. Chronic exercise substantially shifted the dose-response curve for ANG II-induced contractions to the right, with a 2.7-fold increase in the EC_{50} value in endothelium-intact aortae. Similarly, in endothelium-denuded aortae, chronic exercise shifted the curve to the right, with a 3.3-fold increase in the EC_{50} values. Thus, chronic exercise attenuated the sensitivity of rat aortae to ANG II. It is well known that exposure of specific receptors to high concentrations of agonist results in desensitization related to a down-regulation in receptor number and/or receptor uncoupling. Therefore, it seems likely that the attenuated sensitivity to ANG II after chronic

Fig. 1. Dose-response curves for ANG II in endothelium-intact or endothelium-denuded aortae isolated from control (C) and trained (TR) rats. Significant differences ($p < 0.05$): * C vs. TR.

Table 1. Maximum contraction and the concentration required to produce a half-maximum response (EC_{50}) for ANG II in endothelium-intact and endothelium-denuded aortae isolated after an acute or chronic endurance exercise.

	Maximum tension (mg/mm ²)		EC_{50} (nM)	
	Endothelium-intact	Endothelium-denuded	Endothelium-intact	Endothelium-denuded
Control (6)	5.1 ± 1.1	4.1 ± 0.3	2.1 ± 0.2	2.0 ± 0.2
Control-exercising (6)	5.2 ± 0.7	$8.1 \pm 0.4^{\dagger, \ddagger}$	$6.2 \pm 0.7^{\dagger}$	$6.2 \pm 0.9^{\dagger}$
Trained (6)	4.6 ± 0.6	4.4 ± 0.8	$5.6 \pm 0.5^*$	$6.6 \pm 0.7^*$
Trained-exercising (6)	4.4 ± 0.8	$9.9 \pm 0.9^{\dagger, \ddagger}$	5.2 ± 0.6	5.1 ± 0.3

Values are mean $\pm$ SE. Control group remained sedentary, control-exercising group remained untrained but ran on a treadmill set an angle of 5° for 90 min at 30 m/min just before they were killed. Animals of trained group ran on the treadmill 5 d/week for 9 weeks, and were sacrificed at least 48 h after their last running; 90 min at 30 m/min at an angle of 5° . Trained-exercising rats were sacrificed immediately after completion of their last running; 90 min at 30 m/min at an angle of 5° . Symbols indicate significant t difference ($p < 0.05$): *Control vs. trained; † control vs. control-exercising or trained vs. trained-exercising; ‡ endothelium-intact vs. endothelium-denuded. Numbers in parentheses indicate numbers of rats tested.

endurance exercise is due to an alteration in ANG II receptor-mediated activation, e.g. receptor uncoupling and/or down-regulation resulting from repeated exposure to ANG II during chronic exercise. Because there were no significant differences in

60 mM KCl-induced contractile responses between control and trained rats (data not shown) and maximal tension was unaltered (Table 1), chronic exercise may cause ANG II receptor uncoupling rather than receptor down-regulation.

Delp *et al.* [2] suggested that chronic exercise training-induced alterations in responses of rat abdominal aortae to norepinephrine were due to an endothelium-dependent mechanism involving α_2 -adrenergic receptors. However, this was not the case in this study: after chronic endurance exercise, maximal tension and reduced sensitivity of aortae in response to ANG II in the absence of endothelium were similar to those in the presence of endothelium (Table 1). Thus, chronic endurance exercise-induced alterations in contractile responses to ANG II would not be associated with endothelium-dependent vasodilation. Although the exact reason for the discrepancy between the results of Delp *et al.* [2] and the present findings is unclear, it is possible that this discrepancy is associated with the differences in aortae employed to measure vascular responses, e.g. thoracic (present study) versus abdominal aortae (the study of Delp *et al.*). Indeed, it has been shown that in rat thoracic aorta, exercise training causes no significant changes in noradrenaline-induced vasomotor responses [8] but that in rat abdominal aorta, a significant increase in EC_{50} to noradrenaline is observed after exercise training [2]. Moreover, coronary arteries from exercise-trained pigs have been shown to develop 50–60% less maximal tension in response to noradrenaline than do controls [1], but no change in maximal tension of rat abdominal aorta in response to noradrenaline has been observed after exercise training [2].

Effect of an acute endurance exercise. As shown in Fig. 2 and Table 1, an acute endurance exercise shifted the dose-response curve for ANG II-induced contractions to the right, with a 3-fold increase in the EC_{50} value in endothelium-intact and endothelium-denuded aortae from control-exercising rats. Thus, an acute endurance exercise also caused the reduced sensitivity of aortae to ANG II in control-exercising rats. This suggests that an acute endurance exercise caused a functional desensitization in response to ANG II in sedentary untrained rats. Indeed, the high circulating concentrations of hormones or catecholamines during dynamic exercise have been shown to induce functional desensitization [9, 10], and receptor uncoupling and down-regulation have been observed after an acute exercise [11, 12]. However, in trained-exercising rats, no significant difference in the dose-response curve for ANG II-induced contractions was observed after an acute endurance exercise (Fig. 2 and Table 1). This phenomenon may be due to the result that a functional desensitization of aortae had already happened in trained-exercising rats as suggested in previous section.

In contrast, maximal tension in endothelium-denuded aortae significantly increased after an acute endurance exercise in both control-exercising and trained-exercising rats (Table 1). This result can not be explained by the desensitization of receptors and conflicts with the data by Howard *et al.* [10] who showed the reduced maximal tension of rabbit aortae in response to α_1 -adrenergic agonist after a single bout of exercise. The reason for this discrepancy and the precise mechanism

Fig. 2. Dose-response curves for ANG II in endothelium-intact or -denuded aortae isolated after acute endurance exercise in control (C) and trained (TR) rats. The data for resting rats were obtained from Fig. 1. Significant differences ($p < 0.05$): ^aResting vs. exercising; ^bC vs. TR.

underlying the enhancement of ANG II-induced maximal tension after an acute endurance exercise are unclear. However, the enhancement of ANG II-induced maximal tension after an acute endurance exercise was not due to, at least, a general enhancement of the contractile mechanism, because no significant difference in 60 mM KCl-induced tension after an acute endurance exercise between control-exercising and trained-exercising rats was observed irrespective of the presence or absence of endothelium (data not shown).

On the other hand, the fact that maximal tension after an acute endurance exercise was less in endothelium-intact aorta than in endothelium-denuded aorta presents another interesting possibility that an acute exercise may increase the levels of endothelium-dependent relaxation factors, i.e. nitric oxide and prostaglandins. Indeed, possible increases in blood flow, cyclic wall stretch, and catecholamines in response to dynamic exercise would stimulate the endothelium and facilitate the

spontaneous release of nitric oxide from the endothelium [13, 14]. Moreover, it is suggested that endothelium-dependent relaxation factor(s) released spontaneously from the endothelium is responsible for endothelium-dependent inhibition of the contractions induced by ANG II [15].

Conclusion

Chronic endurance exercise caused the attenuation of the vascular sensitivity to ANG II receptor-mediated activation. This attenuation would not result from endothelium-dependent inhibition of contraction induced by ANG II. An acute endurance exercise also caused the reduced sensitivity to ANG II in control-exercising rats, but the same phenomenon was not observed in trained-exercising rats. However, an acute but not chronic endurance exercise enhanced ANG II-induced maximal tension in the absence of endothelium in both groups of rat aortae. This enhancement may be associated with an increase in endothelium-dependent relaxation factors in response to an acute exercise.

We are extremely grateful to T. Mineno for technical assistance. This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

1. Oltman CL, Parker JL, Adams HR, and Laughlin MH: Effects of exercise training on vasomotor reactivity of porcine coronary arteries. *Am J Physiol* **263** (Heart Circ Physiol **32**): H372-H382, 1992
2. Delp MD, McAllister RM, and Laughlin MH: Exercise training alters endothelium-dependent vasoreactivity of rat abdominal aorta. *J Appl Physiol* **75**: 1354-1363, 1993
3. Leon AS, Pettinger WA, and Saviano MA: Enhancement of serum renin activity by exercise in the rat. *Med Sci Sports* **5**: 40-43, 1973
4. Tidgren, B, Hjemdahl P, Theodorsson E, and Nussberger J: Renal neurohormonal and vascular responses to dynamic exercise in humans. *J Appl Physiol* **70**: 2279-2286, 1991
5. Stebbins CL and Symons JD: Role of angiotensin II in hemodynamic responses to dynamic exercise in miniswine. *J Appl Physiol* **78**: 185-190, 1995
6. Izawa T, Komabayashi T, Shinoda S, Suda K, Tsuboi M, and Koshimizu E: Possible mechanism of regulating adenylate cyclase activity in adipocyte membranes from exercise-trained male rats. *Biochem Biophys Res Commun* **151**: 1262-1268, 1988
7. Abe M, Morikawa M, Inoue M, Tsuboi M, Aoyagi Y, and Ohta A: Effects of mammalian lignan, 2,3-dibenzylbutane-1,4-diol, on contraction and Ca^{2+} mobilization induced by noradrenaline in rat aorta. *Gen Pharmacol* **22**: 663-668, 1991
8. Hashimoto M: Effects of exercise on plasma lipoprotein levels and endothelium-dependent vasodilation in young and old rats. *Eur J Appl Physiol* **61**: 440-445, 1990
9. Friedman DB, Ordway GA, and Williams RS: Exercise-induced functional desensitization of canine cardiac β -adrenergic receptors. *J Appl Physiol* **62**: 1721-1723, 1987
10. Howard MG, DiCarlo SE, and Stallone JN: Acute exercise attenuates phenylephrine-induced contraction of rabbit isolated aortic rings. *Med Sci Sports Exercise* **24**: 1102-

- 1107, 1992
11. Izawa T, Komabayashi T, Suda K, Shinoda S, and Tsuboi M: β -Adrenergic receptor adaptation after an acute exercise in rat myocardium. *Jpn J Physiol* **39**: 447–454, 1989
 12. Werle EO, Strobel G, and Weicker H: Decrease in rat cardiac β_1 - and β_2 -adrenoceptors by training and endurance exercise. *Life Sci* **46**: 9–17, 1990
 13. Holtz J, Forstermann U, Pohl U, Giesler M, and Bessenge E: Flow-dependent, endothelium-mediated dilation of epicardial coronary arteries: effects of cyclooxygenase inhibition. *J Cardiovasc Pharmacol* **6**: 1161–1169, 1984
 14. Miller VM and Vanhoutte PM: Endothelial α_2 adrenoreceptors in canine pulmonary and systemic blood vessels. *Eur J Pharmacol* **118**: 123–129, 1985
 15. Bullock GR, Taylor SG, and Weston AH: Influence of the vascular endothelium on agonist-induced contractions and relaxations in rat aorta. *Br J Pharmacol* **89**: 819–830, 1986