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ANP Content in Rat Myocardium During and After Swimming

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Abstract

SUDA, K., KATO, J., NOZAWA, M., KOMABAYASHI, T., IMAI, H. and ERA, S., ANP Content in Rat Myocardium During and After Swimming. Adv. Exerc. Sports Physiol., Vol.12, No.4 pp.121-125, 2006. It is known that plasma ANP concentration increases during exercise. ANP plays a role in maintaining animals' body condition by diuresis and distension in blood vessels. But it is not known how much ANP contents in the myocardium reduce during exercise and how long it takes for the myocardium to recover to the basal level. We investigated ANP content during and after swimming. Plasma ANP concentration increased during swimming. ANP content in right atria significantly reduced during exercise and did not recover four hours after swimming. 24 hours after swimming, ANP content in right atria did not differ from the rat without exercise. There was no clear change in ANP content in the other myocardium. These results suggest that increased plasma ANP during swimming is mainly released from right atria and ANP content in right atria recovers in about one day in this study.

Keywords: ANP, rat, recovery, atria, ventricles

Introduction

It is known that plasma atrial natriuretic peptide (ANP) concentration increase during exercise. Increment of intra-heart pressure facilitates secretion of ANP. ANP dilates vessels and induces natriuresis (2), which reduces the intra-heart pressure. Venous return increase during exercise and this causes the heart secrete more ANP (11). Swimming is a good exercise for researching ANP action during exercise because hydraulic pressure is applied to the body, which increases intra-heart pressure together with enhanced venous return and then make the heart secrete more ANP. ANP plays a role in maintaining animals' body condition during exercise by the actions on the target organs, such as diuresis and distension of blood vessels. It is important for animals to have enough ability to secrete ANP when they exercise. ANP is released primarily from the heart. However, it is not known how much ANP content of the myocardium reduce during exercise and how long it takes for the myocardium to recover to the basal level. This knowledge is necessary to make a rational training frequency and intensity. In this study, we examined the contents of ANP in atria and ventricles of rats during and after swimming to make it clear which myocardium is important during exercise and how long it takes for the heart to recover the ANP contents.

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Materials and methods

Animal care and swimming program

8-week-old male Wistar rats were used for the experiment (Japan SLC). Rats began to swim in groups of seven. One rat was picked up after one hour of swimming for the experiment. Another rat was also picked up at the end of two hours. The rest of the rats were picked up at the end of three hours. These rats were sacrificed at the following times: immediately after exercise, 1, 2, 4, 24 hours after a 3-hour-swim (Fig. 1). Rats that didn't swim were also used for the experiment and considered in basal condition. Seven rats were used for each exercise or recovery condition. Rats were anethetized with pentbarbital (50 mg/kg i.p.). All experimental procedures were carried out in accordance with the guideline for the care and use of experimental animals in Tokyo Institute of Technology.

Measurement of ANP

Blood was collected for the determination of plasma ANP level. The heart was removed and cut into right and left atria and right ventricle. The rest of the heart was used as left ventricle. All parts were weighed and frozen with liquid nitrogen. They were kept at −80 ℃ until used for the ANP measurement. Each part of the heart was boiled for 5 min in 1 M acetic acid and 20 mM HCl to inactivate

intrinsic proteases which degrade ANP. After cooling, they were homogenized with Polytron mixer and centrifuged at 12,000 g for 10 min at 4 ℃. The supernatant was collected and weighed. After extraction of ANP from the supernatant with SepPak C18 (Waters), ANP was measured with an enzyme immunoassay kit (Peninsula). ANP was also extracted from the plasma with SepPak C18 (Waters) and measured with a radioimmunoassay kit (Peninsula).

Statistical method

All data are expressed as means ± S.E. One way ANOVA was used for statistic comparison of the data. Differences were considered significant when $P<0.05$.

Chemicals

All chemicals were commercially available and of the highest degree of purity.

Results

Plasma ANP concentration

The time course of plasma ANP concentration is shown in Fig. 2. Plasma ANP concentrations increased about threefold at the first, 2nd, 3rd hour of swimming and the concentrations were 28.4 ± 6.2, 30.7 ± 7.6 and 26.4 ± 6.0 pg/ml, respectively. All of them were significantly higher than the basal level (8.8 ± 1.2pg/ml). Plasma ANP concentration began to decrease after swimming. Although there was significant difference in plasma ANP concentration between 4 hours of recovery (21.0 ± 3.0 pg/ml) and the basal level, plasma ANP concentrations were not significantly different at 1, 2, 24 hours of recovery compared to the basal level.

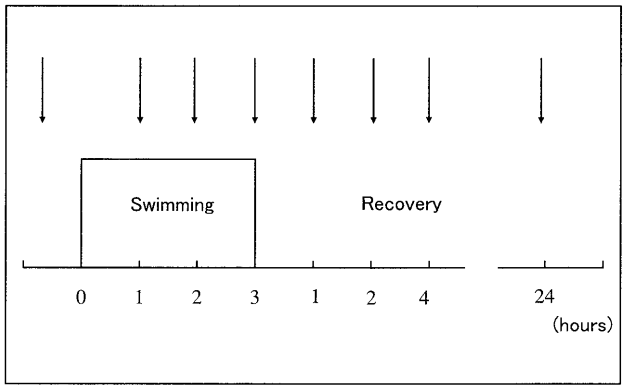

Fig. 1 Timing of data sampling.
The arrows denote time for data sampling.

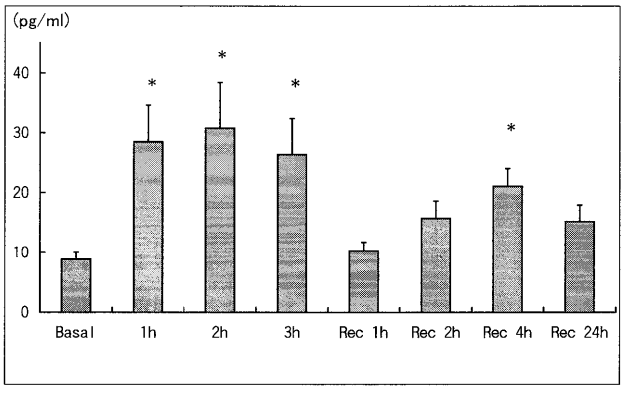

Fig. 2 Plasma ANP concentration during and after swimming. *denotes statistically significant difference from the basal value.

ANP contents in the myocardium

To exclude the effect of the rats' heart weight, ANP contents in the myocardium were expressed in weight per gram tissue.

Right atria

ANP contents in right atria immediately after 1, 2, 3 hours of swimming and 1, 2, 4 hours of recovery were 175 ± 16, 131 ± 22, 208 ± 29, 143 ± 29, 206 ± 24, 208 ± 33 μg/g, respectively. They were significantly lower than the basal level (312 ± 46 μg/g) (Fig. 3). There was no significant difference between the value from 24 hours recovery rats and the one from the rats without exercise.

Right ventricles

ANP contents in right ventricles expressed per gram tissue were generally about one tenth of the values from right atria (Fig. 4). There were no differences in ANP content in right ventricles immediately after 1, 2, 3 hours of swimming and 1, 4, 24 hours of recovery when compared with the basal level. The value was significantly higher at 2 hours of recovery (17.8 ± 1.7 μg/g) compared with the basal level (11.0 ± 2.5 μg/g).

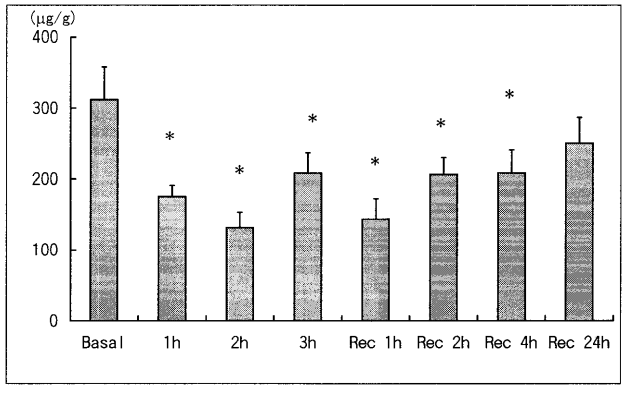

Fig. 3 ANP content in right atria during and after swimming. *denotes statistically significant difference from the basal value.

Left atria

ANP contents in left atria were comparable with the value from right atria (Fig. 5). But ANP contents in left atria were not significantly different from the basal level at any time after swimming or recovery.

Left ventricles

ANP contents in left ventricles were generally about one hundredths the values from left atria (Fig. 6). There were no significant differences in ANP contents in left ventricles between the values from rats at any time points of exercise or recovery and the one from rats without exercise.

Discussion

Basal plasma ANP concentration was about 10 times higher compared to our previous study (14). This difference might arise from the pureness of the sample for radioimmunoassay in the present study. Because we purified the plasma with SepPak C18 before the assay in this study.

Plasma ANP concentration increased threefold during the first hour of swimming and was kept at that level for three hours of the exercise. ANP release is known to be facilitated by volume expansion of the heart. Venous return increase during exercise, which results in volume expansion of the heart (1). Additionally, the muscle pumping effect during exercise and contraction of the vein may contribute to the increment of venous return (1). ANP reduces intra-heart pressure by diuresis and dilation of blood vessels and plays a protective role for the heart. We previously reported that rat plasma ANP concentration increases about twofold by treadmill running (14). On the other hand, Shiraishi et al. (13) reported that plasma ANP concentration increased by water immersion in human. During water immersion the blood and fluid are redistributed from the caudal to the cephalic portions of the body, leading to increment of venous return. This makes distension of the cardiac atria and increases plasma ANP concentration. In this study both water pressure and exercise are the factors which make cardiac atria distend, eventually raise plasma ANP concentration higher than when treadmill exercise was done by rats. Ruskoaho et al. (12) also reported that plasma ANP concentration rose threefold to fourfold when WKY, SHR swam. In this experiment four to seven rats swam simultaneously. They pulled each other into the water, which added more water pressure to their bodies. These results show that exercise used in this experiment was good to secrete much ANP into the blood.

Plasma ANP concentrations during exercise have been reported (12,14). However ANP contents in the myocardium are not often referred. Kinnunen et al. (6) measured ANP contents in the atria and the ventricles of Wistar Kyoto rats and spontaneously hypertensive rats. They mixed right and left atria and also they mixed right and left ventricles. The ratio of ANP content in the atria to the one in the ventricle was about three hundred in their report. The ratio of ANP content in right atria to left ventricle was also about three hundred in this study. The ratio is compa-

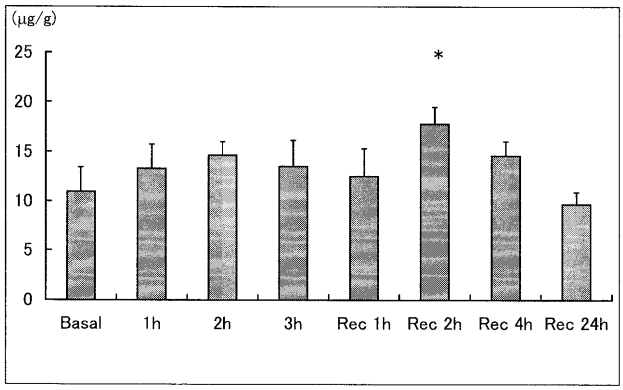

Fig. 4 ANP content in right ventricles during and after swimming. *denotes statistically significant difference from the basal value.

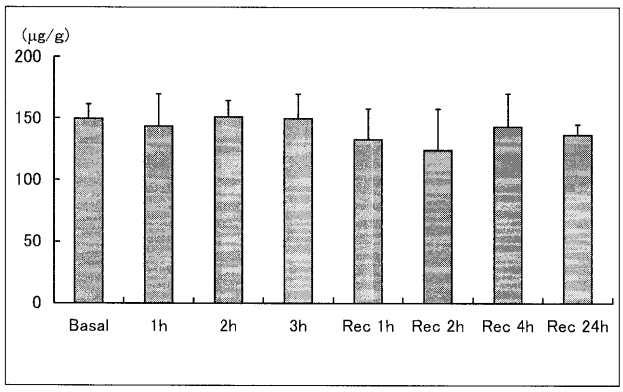

Fig. 5 ANP content in left atria during and after swimming. There is no statistically significant difference from the basal value.

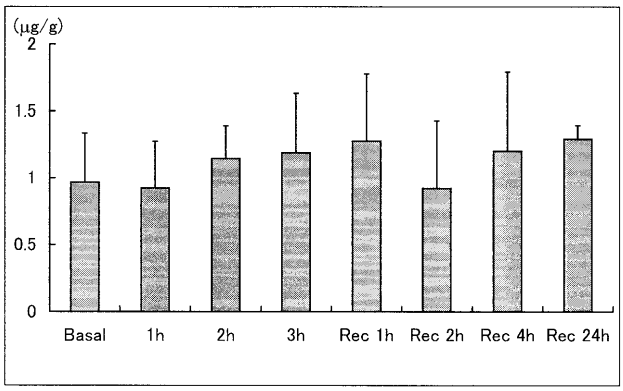

Fig. 6 ANP content in left ventricles during and after swimming. There is no statistically significant difference from the basal value.

rable between the two studies. The basal ANP contents in their study were one thirds to one fifths of this study. This discrepancy again might arise from the difference of preparation of the samples for the assay between the two studies because they did not extract ANP from the samples with SepPak C18.

Increased plasma ANP acts to the circulation system, which contributes to body homeostasis (2). If ANP exhausted, homeostasis of the circulation system may fail. It is important to know the time course of ANP storage during long time exercise. In this study, we investigated the time course of ANP content in the myocardium. And to know from which part of the heart ANP is primarily released during exercise, we measured ANP contents in the myocardium from four chambers.

ANP content in right atria reduced from the time the rats started swimming. Conversely, plasma ANP concentration increased from the time the rat started swimming. Rise in plasma ANP concentration and decrement of heart ANP content was also reported by Imada et al. (4). They thought the lowering of ANP content in the atria might be due to increased secretion of ANP. Also Kato et al. (5) reported increment of plasma ANP concentration was associated with decrement of atrial ANP content in sodium loaded rats. And they reported this might be due to decrement of ANP storage (5). Mangat et al. (8) and Dowsley et al. (3) reported that a portion of ANP is immediately and preferentially released upon synthesis. If quantity of released ANP is not much, stored ANP does not decrease. Left atria, left and right ventricles ANP content did not change significantly during exercise in this study, indicating that ANP release from these myocardium were relatively small. Although contents in these myocardium did not reduce significantly during exercise, it was possible that de novo ANP were released from these myocardium and contributed to the increment of plasma ANP concentration. On the other hand, ANP content in right atria decreased significantly. This suggests that a large part of stored ANP were released beside de novo ANP in right atria.

Plasma ANP concentration four hours after swimming and right ventricular ANP content two hours after swimming were significantly higher than the value from the rats without exercise. These results might occur from miss match between synthesis and secretion of right ventricle.

Klinger et al. (7) investigated ANP secretion from neonatal rat atria and ventricles induced by hypoxic condition. They reported that reduced oxygen tension increased release of ANP from atrial, but not ventricular cardiocytes. Imada et al. (4) reported plasma concentration of ANP increased with the progression of hypertension in spontaneous hypertensive rats. On the other hand, ANP content decreased in the atria and they reported that the lowering of ANP content in the atria might be due to increased secretion of ANP in response to increased blood pressure. They also reported that the pronounced reduction in left atria compared to right atria reflecting hypertension in arterial circulation. In contrast, the reduction was observed in right atria in this experiment. In human studies, Muller et al. (9) reported that right atrial plasma ANP correlated with right atrial pressure during bicycle ergometry. They also reported that reducing venous return by bilateral thigh-cuff occlusion decreased right atrial ANP. These studies support that enhanced plasma ANP during exercise was mainly released from right atria in the present study.

Although decreased content in right atria during swimming did not recover four hours after swimming, it was not significantly different from that of the rat without exercise 24 hours after swimming. This suggests ANP content in right atria recovers in about one day. If the exercise load is the same as this study, daily swimming is tolerable for rats from the view point of ANP secretion. In human study, Pastene et al. (10) reported time course of plasma ANP during and after marathon running for four days. They reported that plasma ANP concentration 30 minutes after marathon running was not different from the pre-exercise level. Similarly, plasma ANP concentration at one hour after swimming was not different in our study. From our results, ANP content in some myocardium may be low level at early recovery period even plasma ANP concentration seems to recover to the basal level. If ANP content does not recover one day after exercise, this work load should be considered too tough to do daily. To our knowledge, there is no research which reported time course of ANP content during and after exercise even in animals. Time course of ANP content is worth knowing when one considers training frequency. We studied only one exercise load in the present research, various exercise conditions remains to be examined.

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