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Effect of Prolonged Exercise on BNP Content in the Myocardium from DOCA-salt Hypertensive Rats

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Abstract

SUDA, K., TAKARA, A. and KOMABAYASHI, T. Effect of Prolonged Exercise on BNP Content in the Myocardium from DOCA-salt Hypertensive Rats. Adv. Exerc. Sports Physiol., Vol.19, No.2 pp.33-37, 2013. To investigate the effect of exercise on plasma BNP and BNP content in the myocardium in hypertensive animals, we produced DOCA (deoxycorticosteron acetate) -salt hypertensive model rats, and examined plasma BNP concentration and BNP content in the myocardium at rest, and after 3 hours of swimming. Mean blood pressure of the hypertensive group was 131.3 ±2.3 mmHg, which was significantly higher than the normotensive group (97.9 mmHg) at 9 weeks old. Plasma BNP concentration was also significantly higher in the hypetensive group than the normotensive group (0.38 ± 0.09 ng/ml and 0.15 ± 0.02 ng/ml, respectively) in accordance with previous studies. Volume overload, wall stretch, and hemodynamics might cause BNP secretion. BNP content changed especially in the right ventricle. Exercise-induced wall stretch in the right ventricle might cause BNP synthesis in the myocardium.

Keywords: BNP, DOCA, hypertension, myocardium, swimming

Introduction

BNP (brain natriuretic peptide or B-type natriuretic peptide) is one of a family of natriuretic peptides that also includes ANP. ANP was found first in the atria, and was named atrial natriuretic peptide. BNP was found first in the brain, and was named brain natriuretic peptide. Although BNP was first discovered in the brain, it predominantly exists in the heart ventricles (8,7,14,18). Similar to ANP, BNP causes diuresis, natriuresis and vasodilation, and the main stimulus for secretion is the stretching of the myocardium, such as volume expansion and pressure overload (6). BNP is often used for heart failure diagnosis (9).

Exercise is recommended for moderate hypertensive patients. With the onset of exercise, cardiac output increases; blood circulation then increases because of sympathetic activity during exercise. Mean arterial pressure increases with a progressive increase in systolic blood pressure as workload increases (11). Natriuretic peptides must be important for the regulation of the circulatory system during exercise when blood pressure increases, because these peptides decrease blood pressure by vasodilation and the regulation of blood volume.

Studies have previously been performed concerning exercise and BNP (1,16) as well as exercise and ANP. Although some studies have been reported concerning exercise and ANP content in the myocardium, there have been few studies concerning exercise and BNP content in the myocardium. It is important to know how much BNP is secreted during exercise, and how much BNP content of the myocardium remains after exercise in hypertensive animals. Therefore we aimed to know plasma concentration and BNP content in the myocardium at rest, and after exercise in hypertensive animals. There are several kinds of hypertensive model rats. In this study, we used DOCA-salt hypertensive rats, a volume-dependent hypertensive model rat.

Methods

DOCA-salt hypertensive rats

Four-week-old male Wistar rats were purchased from SLC laboratory (Tokyo, Japan). They were randomly divided into two groups: the hypertensive group and the normotensive group. Each group consisted of 20 rats. DOCA (deoxycorticosterone acetate) dissolved in corn oil (20 mg/ml) was injected into the femoral muscle of the hypertensive group at four and five weeks of age. Corn oil was similarly injected into the normotensive rats. Water containing 1 % NaCl was given to the hypertensive group ad lib. And tap water was given to the normotensive group. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Tokyo Institute of Techonology.

Measurement of blood pressure

Blood pressure was measured with a tail cuff sphygmomanometer at 4, 5, 7, and 9 weeks old.

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Exercise

Rats were further divided into an exercise group and a rest group. Both subgroups consisted of 10 rats. Rats of the exercise group swam for three hours in water at 35 ℃. Immediately after exercise, the rats were anesthetized with pentobarbital (50mg/kg B.W.). Blood was withdrawn and cooled on ice and centrifuged at 10,000 g; the supernatant was collected and frozen with liquid nitrogen and kept at −80 ℃; and the heart was excised from each of the rats. The right and left atria were removed from the heart. Then the right ventricle was isolated from the heart. The rest of the heart was treated as the left ventricle. These tissues were also kept at −80 ℃ until the assay of BNP. Rats of the rest group were treated the same as the exercise group, but without performing any exercise.

BNP measurement

Tissues were cut into pieces and homogenated with a Polytron homogenizer. Then they were centrifuged at 10,000g at 4 ℃. The supernatant was collected and BNP was extracted with SepPak C18 (Waters) and then freeze dried. They were reconstituted and assayed with a BNP assay kit (Assaypro, MO).

Statistics

All data are expressed as mean ± S.E. Data were analyzed by two-way ANOVA followed by Fisher's PLSD. Differences were considered significant when $P < 0.05$.

Chemicals

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

Results

Mean blood pressures (MBP) are shown in Fig. 1. MBP in the hypertensive group and the normotensive group were similar at four weeks old. MBP in the hypertensive group were 99.9 ± 2.5, 136.2 ± 2.8, and 131.3 ± 2.3 mmHg at 5, 7, and 9 weeks old, respectively. MBP in the normotensive group were 84.2 ± 2.7, 94.3 ± 6.1 and 97.9 ± 6.4 mmHg at 5, 7, and 9 weeks old, respectively. There were significant differences between the two groups.

BNP contents in the myocardium at rest are shown in table 1. BNP content of the right ventricle in the normotensive group at rest was 13.7 ± 2.2 μg/g tissue, and 58.7 ± 16.2 μg/g tissue in the hypertensive rats. The value was significantly larger in the hypertensive group. At rest, there was no other significant difference in BNP contents of the other part of the myocardia between the normotensive and the hypertensive groups.

Plasma BNP concentrations are shown in Fig. 2. BNP concentration at rest in the normotensive group was 0.15 ± 0.02 ng/ml, and 0.38 ± 0.09 ng/ml in the hypetensive group. The value was significantly larger in the hypertensive group. Although a statistical difference was not found, plasma BNP concentration slightly increased after exercise in the normotensive group, but slightly decreased in the hypertensive group.

BNP content of the left and right atria and ventricles in the two groups at rest and after exercise are shown in figures 3 through 6. BNP content in the left atria in the normotensive group after exercise was not significantly different from the value at rest. On the other hand, it was 2.2 ± 0.3 μg/g tissue after exercise and 1.7 ± 0.2 μg/g tissue at rest in the hypertensive group. The value was significaltly

Table 1. BNP content in the myocardium

	Normotensive Group (μg/g tissue)	Hypertensive Group (μg/g tissue)
Left Atria	1.3 ± 0.2	1.7 ± 0.2
Right Atria	2.3 ± 0.4	1.7 ± 0.3
Left Ventricles	34.3 ± 4.8	34.2 ± 4.9
Right Ventricles	13.7 ± 2.2	58.7 ± 16.2 *

Values are mean ± s.e. * denotes statistical significant difference between the two groups.

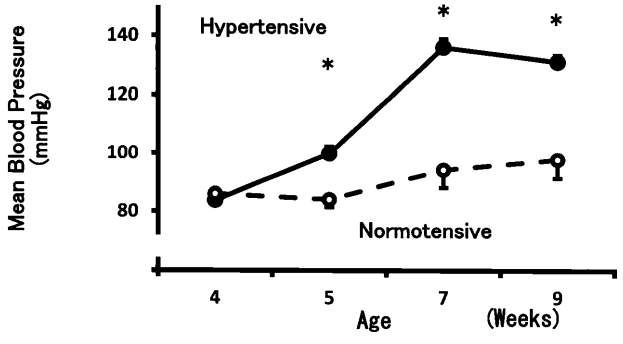

Fig. 1. Mean blood pressure of the normotensive (solid line) and the hypertensive group (dotted line). Error bars in some data are in the markers.
* denotes significant difference between the groups.

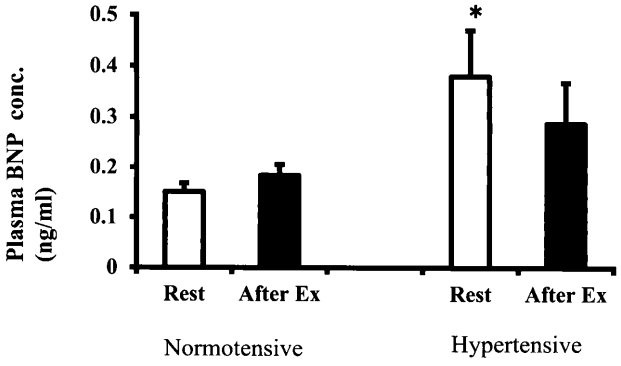

Fig. 2. Plasma BNP (Brain Natriuretic Peptide) concentrations at rest and after exercise.
* denotes significant difference between the groups.

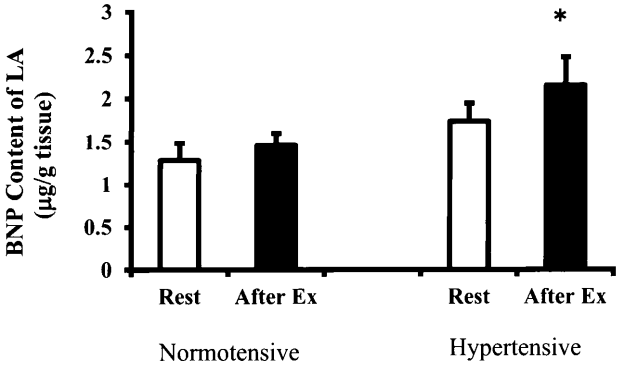

Fig. 3. BNP contents of the left atria (LA) at rest and after exercise from the normotensive and the hypertensive group.
* denotes significant difference between the value at rest and after exercise in the hypertensive group.

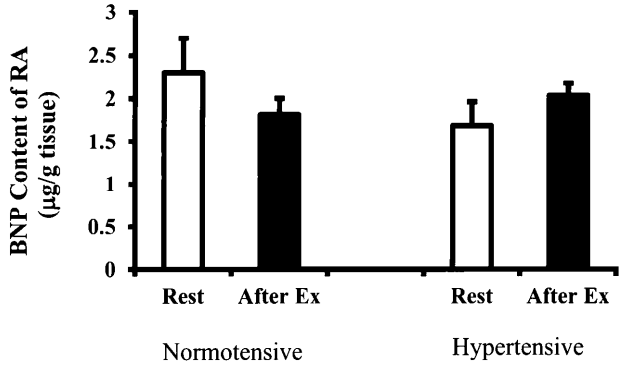

Fig. 4. BNP contents of the right atria (RA) at rest and after exercise from the normotensive and the hypertensive group.

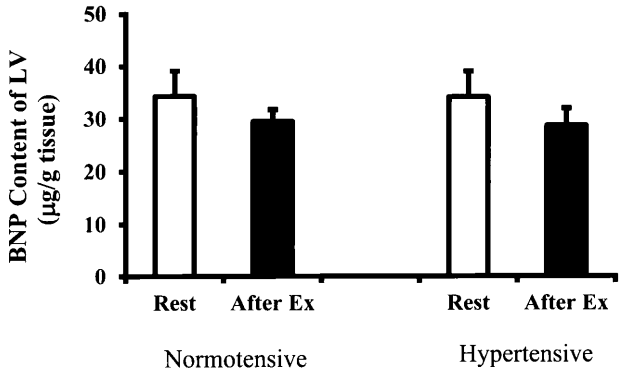

Fig. 5. BNP contents of the left ventricles (LV) at rest and after exercise from the normotensive and the hypertensive group.

larger after exercise compared with the value measured at rest. BNP contents in the right ventricle of the normotensive group at rest were 13.7 ± 2.2 μg/g tissue and 27.8 ± 2.9 μg/g tissue after exerice. There was a significant difference between the values. In the hypertensive group, BNP content in the right ventricle at rest was 58.7 ± 16.2, and 102.3 ± 13.0 μg/g tissue after exercise. The value significantly increased after exercise in the hypertensive group. There was no significant difference in the left ventricle between BNP content after exercise and BNP content at rest in both the normotensive group and the hypertensive group.

Discussion

Mean blood pressure at four weeks old was similar in the two groups. It increased gradually in the hypertensive group and the value did not increase very much in the normotensive group. The difference was significant at 5, 7 and 9 weeks old. This shows that we succeeded to prepare hypertensive model group.

BNP contents in the right ventricles were more than 10 μg/g tissue and it was more than 40 μg/g tissue in the left ventricles. On the other hand, BNP contents in the right and left atria were less than 2.5 μg/g tissue. These differences suggest that the main source of BNP is the ventricles. This is in agreement with previous reports (8,7,14,18).

Plasma BNP concentration slightly increased after exercise in the normotensive group. Although few studies have reported plasma BNP concentration immediately after exercise in rats (2), several studies have reported that exercise caused plasma BNP concentration increment in humans (4,5). Huang et al. (4) reported plasma BNP increased after exercise in healthy men and considered volume overload, wall stretch, and hemodynamics might contribute to exercise-induced increases in BNP. In this study these changes probably occurred during exercise. In addition, we used swimming as the form of exercise, so hemodynamic changes might be much larger because of hydrostatic pressure.

In hypertensive patients, Mottram et al. (10) reported that BNP concentration elevated with exercise concomitant with systolic blood pressure increments. They considered the left ventricle filling pressure facilitated BNP secretion. In this experiment, although significant difference was not found, plasma BNP declined after exercise in the hypertensive group. This suggests that blood pressure might not increase during exercise compared with the resting value in a hypertensive state. In fact, Ruskoaho et al. (15) reported that mean blood pressure of hypertensive rats during swimming increased at 10 min of swimming, but it decreased to the resting level at 30 min of swimming. When we previously measured plasma ANP concentration of hypertensive rats after 3 hours of swimming, the value was lower than the level at rest. This suggests that blood pressure might decrease during exercise (17).

BNP contents in the left atria significantly increased after exercise. But this may not affect plasma BNP concentration because BNP is mainly released from the ventricles, and the amount of BNP content is much larger in the ventricles (6)

There was no significant difference in BNP content between the value at rest and after exercise in the right atria, both in the normotensive and the hypertensive group. In contrast, ANP content of the right atria significantly de-

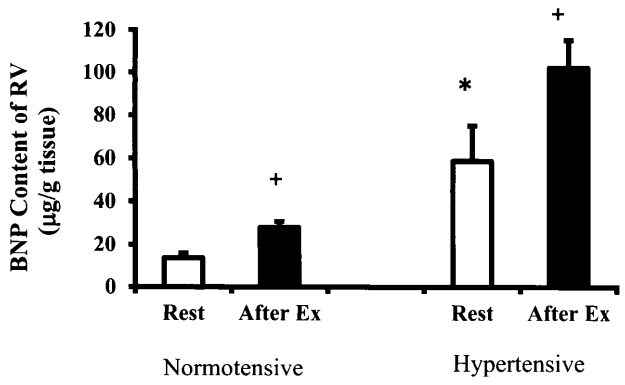

Fig. 6. BNP contents of the right ventricles (RV) at rest and after exercise from the normotensive and the hypertensive group.
+ denotes significant difference between the value at rest and after exercise in the same blood pressure group.
* denotes significant difference between the value at rest from the normotensive and the hypertensive group.

creased after exercise in the normotensive group in our previous report (17). As described previously, BNP content in the atria is small compared with the ventricles, so the value might change little even if the pressure changed enough to alter ANP content. There was no significant difference in BNP content between the value at rest and after exercise in the left ventricle either. From this result, it seems that exercise did not change blood pressure significantly in the left ventricles. In fact, in our previous report using the same type of model rat, ANP content did not change in the left ventricles, suggesting blood pressure did not change significantly.

On the other hand, BNP content in the right ventricles increased in the hypertensive group, both at rest and after exercise. Augmented BNP synthesis in the ventricle of hypertensive rats compared with normotensive rats had been reported (12,13). So it is natural that hypertension induced the increment of BNP content in this study. BNP content of the right ventricle increased after exercise in both the hypertensive group and the normotensive group. BNP is secreted by a constitutive mechanism (3). This implies cells are dependent on activation of the BNP gene when increased peptide secretion is called upon (3). So it was possible that exercise-induced wall stretching in the right ventricle caused activation of the BNP gene which increased BNP content of the myocardium.

In conclusion, plasma BNP concentration increased in the hypertensive group, and the BNP content of the ventricles increased in the hypertensive group in accordance with previous studies. Although plasma BNP concentration did not change significantly with exercise, BNP content changed after exercise especially in the right ventricle, suggesting the exercise affected BNP synthesis in the ventricles.

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