Angiotensin-(1–7) attenuates hypertension in exercise-trained renal hypertensive rats

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1Department of Physiology, Research Center for Endocrine Sciences, Chonbuk National University Medical University, Jeonju, Korea; 2Department of Internal Medicine, Research Center for Endocrine Sciences, Chonbuk National University Medical University, Jeonju, Korea; and 3Department of Anatomy, Research Center for Endocrine Sciences, Chonbuk National University Medical University, Jeonju, Korea

Submitted 26 August 2011; accepted in final form 23 March 2012

Shah A, Oh YB, Lee SH, Lim JM, Kim SH. Angiotensin-(1–7) attenuates hypertension in exercise-trained renal hypertensive rats. Am J Physiol Heart Circ Physiol 302: H2372–H2380, 2012. First published March 30, 2012; doi:10.1152/ajpheart.00846.2011.—Angiotensin-(1–7) [ANG-(1–7)] plays a counterregulatory role to angiotensin II in the renin-angiotensin system. In trained spontaneous hypertensive rats, Mas expression and protein are upregulated in ventricular tissue. Therefore, we examined the role of ANG-(1–7) on cardiac hemodynamics, cardiac functions, and cardiac remodeling in trained two-kidney one-clip hypertensive (2K1C) rats. For this purpose, rats were divided into sedentary and trained groups. Each group consists of sham and 2K1C rats with and without ANG-(1–7) infusion. Swimming training was performed for 1 h/day, 5 days/wk for 4 wk following 1 wk of swimming training for acclimatization. 2K1C rats showed moderate hypertension and left ventricular hypertrophy without changing left ventricular function. Chronic infusion of ANG-(1–7) attenuated hypertension and cardiac hypertrophy only in trained 2K1C rats but not in sedentary 2K1C rats. Chronic ANG-(1–7) treatment significantly attenuated increases in myocyte diameter and cardiac fibrosis induced by hypertension in only trained 2K1C rats. The Mas receptor, ANG II type 2 receptor protein, and endothelial nitric oxide synthase phosphorylation in ventricles were upregulated in trained 2K1C rats. In conclusion, chronic infusion of ANG-(1–7) attenuates hypertension in trained 2K1C rats.

swimming exercise training; atrial natriuretic peptide; cardiac hypertrophy; Mas receptor

THE NEWLY DISCOVERED renin-angiotensin system (RAS) axis, angiotensin-converting enzyme 2 (ACE2)/angiotensin-(1–7) [ANG-(1–7)]/Mas receptor, coined as a counteregulatory axis to ACE/ANG II/angiotensin type 1 receptor (AT1R), is considered as an important core factor in cardiovascular pathophysiology (8, 16). ACE2 metabolizes ANG II into ANG-(1–7), one of the major pathways for ANG-(1–7) production among others (8, 11, 16, 19). The action of ANG-(1–7) includes vasorelaxation (46, 49), antihypertension (48, 55), antihypertrophy (25, 36, 52), and antifibrosis (20, 25), which are opposite to ANG II actions. At the receptor level, Mas receptor, ANG II type 2 receptor protein, and endothelial nitric oxide synthase phosphorylation in ventricles were upregulated in trained 2K1C rats. In conclusion, chronic infusion of ANG-(1–7) attenuates hypertension in trained 2K1C rats.

swimming exercise training; atrial natriuretic peptide; cardiac hypertrophy; Mas receptor

Angiotensin-(1–7) has been shown to decrease blood pressure (BP) in spontaneously hypertensive rats (SHR; Refs. 2, 40) and high fructose diet-fed rats (20). Chronic infusion of Mas receptor antagonist or treatment with the ACE2 inhibitor worsens the course of hypertension accompanied with a decreased renal hemodynamics (5). Both Mas receptor and ACE2 knockout animals show an impaired cardiac function (22, 50, 55), and cardiovascular symptoms during cardiovascular pathophysiology are more aggravated in these animals (7, 28, 44). In contrast, ANG-(1–7) does not prevent hypertension induced by ANG II infusion (9, 21) and in two-kidney, one-cliped (2K1C) hypertension (5). Therefore, it is possible that antihypertensive effect of ANG-(1–7) depends on type of hypertension and cardiovascular disease model used.

On the other hand, exercise training has many beneficial cardiovascular effects such as an improvement of cardiac function, an enhancement of left ventricular (LV) diastolic function, and a decreased heart rate (6, 42, 51). Exercise training also improves the course of diseases in myocardial infarction and chronic heart failure (14, 37, 41). One of the mechanisms by which exercise training causes beneficial effects is the modulation of RAS. For instance, exercise training reduces AT1R expression, ACE mRNA, and protein level and increases ACE2 expression in animals with chronic heart failure (27, 38). Additionally, aerobic exercise also increases ACE2, ANG-(1–7), and AT2R protein expression in the heart (15). Filho et al. (18) observed that Mas receptor mRNA and protein expression in ventricular tissue are increased significantly in swimming exercise-trained SHR, indicating the relationship among exercise, hypertension, and ANG-(1–7)/Mas axis. Therefore, we tested whether exercise training potentiates antihypertensive and cardioprotective effects of ANG-(1–7) in 2K1C rats.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley (SD) rats, obtained from the Orientbio (Seoungnam, Korea), were kept in a temperature-controlled room with a 12:12-h light-dark cycle. The animals were provided with free access to standard laboratory chow (SL79 Purina rat and mouse chow 18% chow; Charles River Laboratories, Wilmington, MA) and water. All experimental protocols conformed to the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (NIH publication No. 85–23, Revised 1996) and were approved by Chonbuk National University Medical University. SD rats, weighing 150–170 g, were anesthesized by intraperitoneal injection of a mixture of ketamine and xylazine (9:1, 2 ml/kg). After a laparotomy, the left renal artery was carefully separated from renal vein. A U-shaped silver clip having a diameter of 0.2 mm was inserted in the renal artery so that the renal artery was constricted partially. The abdominal muscle layer and skin layer were closed with black silk (1). Sham rats received a similar procedure to 2K1C rats without an application of silver clip to left renal artery. After 1 wk of surgery, systolic BP was measured by tail cuff plethysmography.
Society of Echocardiography recommendations (47) as following: thickness measured from the M-mode images according to American shortening (FS) were determined from the ventricular dimensions and ventricular septal thickness (IVST), and diastolic and systolic LV speed of 100 mm/s. The parameters measured were end-diastolic and of the papillary muscles. An M-mode image was obtained at a sweep to the interventricular septum and posterior wall of the LV at the level cm. From this view, an M-mode cursor was positioned perpendicular dimensional mode in the parasternal short axis view with a depth of 2 xylazine (9:1, 2 ml/kg), and echocardiography was performed in two-dimensional guided M-mode. The heart was imaged in the two- and Echocardiography. LV morphology and function were assessed using a noninvasive transthoracic echocardiography on 2 days before death. The echocardiography was performed in supine or semi-left lateral decubitus position using a Vivid 4 echocardiography machine equipped with a 10-MHz linear array transducer system (General Electric, Waukesha, WI). Animals were anesthetized using ketamine and xylazine (9:1, 2 ml/kg), and echocardiography was performed in two-dimensional guided M-mode. The heart was imaged in the two-dimensional mode in the parasternal short axis view with a depth of 2 cm. From this view, an M-mode cursor was positioned perpendicular to the interventricular septum and posterior wall of the LV at the level of the papillary muscles. An M-mode image was obtained at a sweep speed of 100 mm/s. The parameters measured were end-diastolic and end-systolic dimension (EDD and ESD), diastolic and systolic interventricular septal thickness (IVST), and diastolic and systolic LV posterior wall thickness (PWT). Ejection fraction (EF) and fractional shortening (FS) were determined from the ventricular dimensions and thickness measured from the M-mode images according to American Society of Echocardiography recommendations (47) as following:

\[
\text{EF} (%) = \frac{\text{LVEDV-LVESV}}{\text{LVEDV}} \times 100,
\]

\[
\text{FS} (%) = \frac{\text{LVDD-LVESV}}{\text{LVEDV}} \times 100.
\]

where LVEDV is LV end diastolic volume and LVESV is LV end systolic volume. LV mass was also automatically calculated using measurements from M-mode images by the uncorrected cube function formula:

\[
\text{LV mass (g)} = \left\{1.04 \left[\left(\text{LVEDD} + \text{PWT} + \text{IVST}\right)^3 - \text{LVEDD}^3\right]\right\}.
\]

where 1.04 is the specific gravity of the myocardium.

Histological analysis. For the determination of LV hypertrophy and fibrosis, the middle part of LV tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. The tissue was sectioned at 5 μm, rehydrated with ethanol, stained with hematoxylin and eosin, dehydrated, and mounted on slide for light microscopic analysis to evaluate the myocyte diameter. To detect LV fibrosis, the sections were stained with Masson’s trichrome. Briefly, tissue was deparfinitized with xylene two times for 2 min and then rehydrated with ethanol for 2 min. It was fixed with Bouin solution (mordant) at 60°C for 1 h and removed the picric acid with 70% ethanol at room temperature for 30 s. It was stained with weigert iron hematoxylin for 10 min, washed with tap water for 5 min, stained with biebrich scarlet-acid fuchsine solution for cytoplasm staining for 10 min, and washed with tap water again for 5 min. The tissue section was stained with phosphomolybdic-phosphotungstic acid for 20 min, stained again with aniline blue solution for collagen fiber for 5 min, washed with tap water for 5 min, dehydrated, and mounted on slide for observation. The hematoxylin and eosin-stained slides were examined with a light microscope under ×200 magnification. To ensure that the sections were perpendicular, only myocytes with centrally located nucleus were used. The tissue’s images were taken with the digital camera (Nikon digital sight DS-U1). The myocyte diameter was analyzed using image analysis software (Analysis prover. 3.2, Soft Imaging System). The cardiac fibrosis was quantified using the above-mentioned image analysis software at ×200 magnification under light microscope.

Measurement of atrial natriuretic peptide concentration. Atrial natriuretic peptide (ANP) in plasma was extracted using a Sep-Pak C$_{18}$ cartridge (Waters Associates, Milford, MA; Ref. 54). The concentration of ANP in plasma extracts was measured using radioimmunoassay, as described previously (54).

Western blot analysis. After death, ventricular tissues were frozen in liquid nitrogen and stored at −80°C. Frozen ventricular tissues were homogenized in lysis buffer with protease and phosphatase inhibitors. In lysates, the protein concentration was determined using the Bradford method. Thirty micrograms of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% polyacrylamide gel and transferred to a hydrobond-PVDF membrane using a Western blot apparatus. The membrane was incubated with 5% skim milk in Tris-buffered saline with Tween for 2 h at 4°C for blocking nonspecific binding sites. After being washed in Tris-buffered saline solution, the membrane was incubated with primary antibodies for Mas receptor (cat no. AAR-013, lot no. AAR013AN0502; Almone Labs), AT2R (cat no. sc-9040, lot no. D2811; Santa Cruz Biochemicals, Santa Cruz, CA), endothelial nitric oxide synthase (eNOS; cat no. 9572s, lot no. 13; Cell

Fig. 1. Experimental protocols for swimming exercise training. Systolic blood pressure (BP) was measured after 1 wk of surgery and hypertensive rats (BP >140 mmHg) were selected for the study. ANG-(1–7), angiotensin-(1–7); 2K1C, two-kidney one-clip hypertensive rats.
Signaling), p-eNOS (cat no. 9571s, lot no. 12; Cell Signaling), or β-actin (Santa Cruz Biochemicals; 1:1000) overnight at 4°C. Horseradish peroxidase-conjugated IgG was used as a secondary antibody (Santa Cruz Biochemicals). The protein expression levels were determined by analyzing the signals captured on the PVDF membranes using an image analyzer (Las-1000, Fuji-film; Ref. 39).

**Statistical analysis.** Results are presented as means ± SE. Statistical significance of differences was assessed using two-way ANOVA followed by the Bonferroni multiple comparison test. Student’s t-test was also used. The critical level of significance was set at P < 0.05.

**RESULTS**

*Effects of ANG-(1–7) on BP.* To evaluate the effect of chronic treatment of ANG-(1–7) on hemodynamics in sedentary and trained 2K1C rats, BP was recorded using tail cuff method and heart rate was recorded at the end of training period by echocardiography. The basal BPs on the seventh day after surgery in sedentary sham, sham ANG-(1–7), 2K1C, and 2K1C + ANG-(1–7) rats were 123.75 ± 1.75, 122.45 ± 1.85, 164.80 ± 3.32, and 135.12 ± 2.54 mm Hg, respectively.

**Fig. 2.** Effect of ANG-(1–7) infusion on systolic blood pressure in sedentary (A; n = 6–9) and trained (B; n = 6–9) 2K1C rats. ANG-(1–7) was infused via miniosmotic pumps, at a dose of 576 μg·kg⁻¹·day⁻¹ from day 14 after the surgery as indicated by horizontal shade. Rats were simultaneously subjected to swimming training exercise for 4 wk from day 14 onwards. *P < 0.05; **P < 0.01 vs. 2K1C group without ANG-(1–7) infusion.

**Fig. 3.** Changes in body weight and cardiac chambers by chronic infusion of ANG-(1–7) in sedentary (n = 6–9) and trained 2K1C rats (n = 6–9). Values are normalized to 100 g of body weight (BW). A: BW. B: heart wt. C: left atrium (LA) wt. D: right atrium (RA) wt. E: left ventricle (LV) wt. F: right ventricle (RV) wt. *P < 0.05, **P < 0.01 vs. sham group; #P < 0.05 vs. 2K1C group.
and 156.50 ± 3.88 mmHg, respectively (Fig. 2A). In sedentary 2KIC rats, BP maintained significantly higher than sham rats throughout the experiment. Chronic treatment of ANG-(1–7) did not reduce BP in sedentary sham and 2KIC rats (Fig. 2A). The basal BPs on the seventh day after surgery in trained sham, sham + ANG-(1–7), 2KIC, and 2KIC + ANG-(1–7)-infused rats were 122.45 ± 1.35, 123.45 ± 1.75, 158.60 ± 5.94, and 150.29 ± 4.24 mmHg, respectively. Exercise training did not decrease BP in sham and 2KIC rats (Fig. 2B). However, chronic ANG-(1–7) infusion decreased BP significantly from 2 wk onwards in trained 2KIC rats (Fig. 2B). Heart rates were not different among all the study groups (see Fig. 4A).

Effects of ANG-(1–7) on cardiac morphometry. In sedentary group, body weight (BW) was not different among sham, sham + ANG-(1–7), 2KIC, and 2KIC + ANG-(1–7) groups. As expected, 2KIC rats showed increases in heart weight (wt; Fig. 3B), left atrial (LA) wt (Fig. 3C), and LV-wt to-100 g BW ratio (Fig. 3E) indicating the LV hypertrophy. ANG-(1–7) infusion in sedentary 2KIC rats did not modify heart wt, LA wt, and LV wt-to-100 g BW ratio (Fig. 3). In exercise-training group, 2KIC rats also showed LV hypertrophy. Interestingly, ANG-(1–7) infusion in trained 2KIC rats significantly attenuated increases in heart wt, LA wt, and LV wt compared with trained 2KIC rats (Fig. 3).

Effect of ANG-(1–7) on LV remodeling and LV function. LV remodeling and LV function among the study groups were assessed by echocardiography. In agreement with autopsy data, sedentary 2KIC rats showed a significant increase in LV mass (1.68 ± 0.05 mg vs. 1.24 ± 0.03 mg, Fig. 4B). Similarly, there was a tendency to increase the posterior wall thickness and septal wall thickness without significance in 2KIC rats (Fig. 4, C–E). Infusion of ANG-(1–7) in sedentary 2KIC rats did not modify LV mass (Fig. 4B), posterior wall thickness (Fig. 4, C and D), and septal wall thickness (Fig. 4, E and F). In trained 2KIC rats, LV mass was significantly higher than trained sham rats (Fig. 4B). Systolic and diastolic LV posterior wall thickness were 3.21 ± 0.10 and 2.25 ± 0.13 mm, respectively, in trained 2KIC rats, which were higher compared with trained sham rats (2.67 ± 0.09 and 1.8 ± 0.09 mm; respectively; Fig. 4, C and D). Similar results were obtained regarding a septal wall thickness (Fig. 4, E and F). Interestingly, the LV remodeling was significantly blocked by chronic infusion with ANG-(1–7) in trained 2KIC rats (Fig. 4, B–F). LV systolic functions, as indicated by EF and FS, were not impaired by 2KIC hypertension in sedentary group but were increased in trained 2KIC groups. In addition, chronic treatment of ANG-(1–7) had no effect on these parameters (Table 1).

Effect of ANG-(1–7) on cardiac myocyte diameter and fibrosis. To further validate the finding at a cellular level, the histological analysis of the LV tissue was performed and cardiac myocyte diameter was measured. Interestingly, LV myocyte diameter was increased in sedentary 2KIC rats but ANG-(1–7)
Table 1. Effect of angiotensin-(1−7) on left ventricular systolic function in sedentary and trained 2K1C rats

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
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<th>2K1C</th>
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<tr>
<td>Ejection fraction, %</td>
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<td>Trained</td>
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<td>72.50 ± 2.66</td>
<td>84.50 ± 1.99*</td>
<td>77.71 ± 1.84</td>
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<td>Fractional shortening, %</td>
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<tr>
<td>Sedentary</td>
<td>42.25 ± 0.75</td>
<td>41.25 ± 0.65</td>
<td>42.00 ± 1.73</td>
<td>41.50 ± 3.52</td>
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<td>Trained</td>
<td>37.75 ± 1.75</td>
<td>35.75 ± 1.82</td>
<td>49.16 ± 2.52†</td>
<td>41.71 ± 1.67</td>
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<td>Diastolic LV volume</td>
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<tr>
<td>Sedentary</td>
<td>0.89 ± 0.09</td>
<td>0.90 ± 0.10</td>
<td>0.94 ± 0.10</td>
<td>1.10 ± 0.14</td>
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<td>Trained</td>
<td>0.98 ± 0.07</td>
<td>0.99 ± 0.11</td>
<td>0.90 ± 0.12</td>
<td>1.03 ± 0.11</td>
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<tr>
<td>Systolic LV volume</td>
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<tr>
<td>Sedentary</td>
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<td>0.20 ± 0.03</td>
<td>0.21 ± 0.04</td>
<td>0.26 ± 0.06</td>
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<tr>
<td>Trained</td>
<td>0.26 ± 0.03</td>
<td>0.28 ± 0.05</td>
<td>0.17 ± 0.03</td>
<td>0.27 ± 0.06</td>
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</table>

Values are expressed as means ± SE (n = 4–9). Transthoracic echocardiography was performed in sham, sham + angiotensin-(1–7) [ANG-(1–7)], trained two-kidney one-clip hypertensive (2K1C), and 2K1C + ANG-(1–7) groups. LV, left ventricular. *P < 0.01, †P < 0.001 vs. sedentary sham rats.

Chronic infusion of ANG-(1–7) in 2K1C rats tended to decrease plasma ANP level (89.84 ± 7.30 pg/ml) in trained 2K1C rats (Fig. 7). In trained groups, plasma ANP level was higher in 2K1C rats (165.33 ± 20.37 pg/ml) compared with sham rats (68.35 ± 7.83 pg/ml). Chronic infusion of ANG-(1–7) tended to reduce plasma ANP in trained 2K1C rats (107.03 ± 13.41 pg/ml; Fig. 7).

**Effect of ANG-(1–7) on plasma ANP level.** In sedentary groups, plasma ANP level in 2K1C rats was higher compared with sham rats (131.25 ± 19.41 pg/ml vs. 58.59 ± 5.69 pg/ml). Chronic infusion of ANG-(1–7) in 2K1C rats tended to decrease plasma ANP level (89.84 ± 7.30 pg/ml in sedentary 2K1C rats). Plasma ANP level in trained 2K1C rats (Fig. 7). In trained groups, plasma ANP level was higher in 2K1C rats (165.33 ± 18.37 pg/ml) compared with trained sham rats (68.35 ± 7.83 pg/ml). Chronic infusion of ANG-(1–7) tended to reduce plasma ANP in trained 2K1C rats (107.03 ± 13.41 pg/ml; Fig. 7).

**Change of Mas receptor, AT2R, and eNOS protein level.** Western blotting result showed that Mas receptor and AT2R protein level in ventricular tissue were up-regulated in trained 2K1C rats more than in sedentary rats (Fig. 8). Especially, exposure to ANG-(1–7) may enhance Mas receptor and AT2R and phosphorylation of eNOS in trained 2K1C rats.

**DISCUSSION**

Several studies have demonstrated that ANG-(1–7) has antihypertensive (48, 55), antihypertrophic (25, 36, 52) and antiinflammatory (20, 25) functions depending on experimental conditions. The present study showed that ANG-(1–7) decreased high BP predominantly with decreased cardiac hypertrophy and fibrosis only in trained 2K1C rats but not in sedentary 2K1C rats. We suggest that exercise improves the BP and fibrotic response to ANG-(1–7).

A number of studies have been reported about the antihypertensive effect of ANG-(1–7) in different hypertensive models using high fructose diet-fed rats (20), SHR (10, 40), SHR treated with N-nitro-L-arginine methyl ester (2), and 2K1C rats (5). In contrast, ANG-(1–7) does not prevent ANG II-induced hypertension (9, 21) and neither chronic infusion of ANG-(1–7) nor overproduction of ANG-(1–7) in transgenic rats modifies the course of 2K1C hypertension (5). In the present study, chronic infusion of ANG-(1–7) for 4 wk did not cause any significant changes in BP and cardiac remodeling in 2K1C rats. These results are consistent with others (5, 9, 21). Exercise training causes beneficial effects on cardiovascular system through the modulation of RAS (15, 27, 38). Interestingly,

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Fig. 5. Light microscopic images of cross section of cardiac muscle stained with hematoxylin-eosin (A and B). Myocyte diameter (μm > 100 myocytes) was analyzed using image analysis software (C). **P < 0.01 vs. sham group; #P < 0.01 vs. trained 2K1C group.
SHR who received swimming exercise showed an increased ventricular Mas receptor mRNA and protein expression (18). Therefore, we tested whether swimming exercise improves the BP and fibrotic response to ANG-(1–7) in 2K1C rats. Surprisingly, ANG-(1–7) attenuated hypertension in 2K1C rats that were subjected to exercise swimming. Despite several studies (14, 24, 35) showing that exercise training caused a slight decrease in BP in hypertensive animals, we did not observe the similar result in trained 2K1C rats without ANG-(1–7) infusion. These data are partly consistent with others (16, 18). The differential regulation of ACE and ACE2 in both the clipped kidney and nonclipped kidney in 2K1C rats suggests their contribution of intrarenal ANG II and ANG 1–7 content (43). Therefore, we could not underestimate the role of other components of RAS such as AT1R, ACE, and ACE2 as they could be modulated by exercise training. In addition, it has been also reported that ANG-(1–7) upregulates ventricular NOS activity and eNOS phosphorylation in SHR through AT2R (10) and modulates ANG II-dependent reactive oxygen species formation (22). In this way, ANG-(1–7) may elicit its cardioprotective action and contribute to some of the counter-regulatory AT2R effects opposite to AT1R effects. In the present study, we found that increased eNOS phosphorylation was similar to both in sedentary and trained 2K1C rats but was higher only in trained 2K1C rats receiving ANG-(1–7). These data support the results that ANG-(1–7) attenuates hypertension in trained 2K1C rats. To identify the mechanism involved in the regulation of Mas receptor and AT2R protein expression by swimming exercise in 2K1C rats requires further investigation.

Fig. 6. Light microscopic images of cross section of cardiac muscle stained with trichrome (A and B). Cardiac fibrosis was quantified using the image analysis software (C). *P < 0.05 vs. sham group; **P < 0.01 vs. trained 2K1C group.

It is well established that chronic hypertension in both human and experimental animal models such as SHR, 2K1C rats and myocardiac infarction leads to cardiac remodeling and dysfunction (40, 53) ultimately leading to cardiac failure. Besides pharmacological intervention, the common practice to treat these pathological cardiovascular changes is the exercise training (34, 37). Swimming exercise training attenuates LV chronic heart failure. Additionally, aerobic exercise increases expression of ACE2, ANG-(1–7), and AT2R protein in the heart (15). Interestingly, Filho et al. (18) found that swimming training increased LV ANG-(1–7) level and the expression of Mas receptor mRNA and protein only in SHR but not in Wistar-Kyoto rats and hence suggested that the beneficial effects induced by swimming training in hypertensive rats might involve an augmentation of ANG-(1–7) and its receptor in the heart. This speculation could be attributed to our finding that infusion of exogenous ANG-(1–7) in only 2K1C rats during swimming training attenuated the hypertension. In fact, Mas receptor and AT2R protein levels were increased in trained 2K1C rats and further increased by ANG-(1–7) infusion in trained 2K1C rats. These data are partly consistent with others (16, 18). The differential regulation of ACE and ACE2 in both the clipped kidney and nonclipped kidney in 2K1C rats suggests their contribution of intrarenal ANG II and ANG 1–7 content (43). Therefore, we could not underestimate the role of other components of RAS such as AT1R, ACE, and ACE2 as they could be modulated by exercise training. In addition, it has been also reported that ANG-(1–7) upregulates ventricular NOS activity and eNOS phosphorylation in SHR through AT2R (10) and modulates ANG II-dependent reactive oxygen species formation (22). In this way, ANG-(1–7) may elicit its cardioprotective action and contribute to some of the counter-regulatory AT2R effects opposite to AT1R effects. In the present study, we found that increased eNOS phosphorylation was similar to both in sedentary and trained 2K1C rats but was higher only in trained 2K1C rats receiving ANG-(1–7). These data support the results that ANG-(1–7) attenuates hypertension in trained 2K1C rats. To identify the mechanism involved in the regulation of Mas receptor and AT2R protein expression by swimming exercise in 2K1C rats requires further investigation.

Fig. 7. Changes in plasma atrial natriuretic peptide (ANP) level in sedentary (n = 6–9) and trained groups (n = 6–9). **P < 0.01 vs. sham group.
remodeling and improves LV function in rats with myocardial infarction (12). Exercise training also improves cardiac function, increases vascular compliance, and reduces arterial stiffness (6). ANG-(1–7) has numerous antiremodeling functions, including inhibition of cardiomyocytes growth (52), prevention of cardiac fibrosis (25), and reduction of cardiac hypertrophy (36). ANG-(1–7) also attenuates hypertension (13, 26) and improves cardiovascular dysfunction as observed in different disease conditions (3, 33). However, in some studies, exercise training failed to exhibit the cardioprotective effects (4) or ANG-(1–7) did not prevent hypertrophy induced by isoproterenol in transgenic rats overexpressing ANG-(1–7) (17). In 2K1C rats, chronic infusion of ANG-(1–7) or overexpression of ANG-(1–7) did not attenuate hypertension and cardiac hypertrophy and did not alter renal function (5). These divergent observations might be due to differences in intensity, duration and type of exercise, training protocol (29), animal disease model, or type of anesthetics used in the experiments. Interestingly, chronic infusion of ANG-(1–7) attenuated cardiac hypertrophy in trained 2K1C rats confirming the antihypertrophic effect of ANG-(1–7) during exercise training. The in vivo imaging of the heart by echocardiography also demonstrated the similar results. Using histological technique, we also confirmed the antihypertrophic and antifibrotic effect of ANG-(1–7) in trained 2K1C rats. Based on these observations, it could be speculated that ANG-(1–7) attenuates cardiac remodeling during exercise training in 2K1C animal models.

One of the characteristics of cardiovascular pathophysiology is the alteration in LV systolic and/or diastolic function. Chronic hypertension is a known cause for the impairment of LV function. To combat the workload during hypertension, LV undergoes remodeling and ultimately leads to cardiac dysfunction. Therefore, the therapeutic strategy for a decrease of cardiac hypertrophy is the prevention of cardiac remodeling and the improvement of cardiac dysfunction (30). Mice with 4 wk postmyocardial infarction exhibited LV dilatation and systolic dysfunction and decreased EF and FS. Alternatively, in 2K1C mice (53) and rats (4), LV dimensions, FS, and EF were not altered. In agreement with these findings, the LV function was not altered in 2K1C rats in this study. However, swimming training improved cardiac systolic function in 2K1C rats, which could be because of the normalization or increase of the contractile protein enzymatic activity and of myosin isoenzyme (29, 48). In the present study, ANG-(1–7) infusion did not show any effect on LV functions. In fact, the 2K1C hypertensive model may not be appropriate to evaluate the effect of ANG-(1–7) on LV functions as cardiac functions were intact in 2K1C rats as observed by us and other studies (4, 53).

In conclusion, ANG-(1–7) prevented hypertension with cardiac hypertrophy and fibrosis in trained 2K1C rats. These observations suggest that ANG-(1–7) may be important to prevent cardiac remodeling in the early course of hypertension in trained 2K1C rats and could be implied as future therapeutic agents in combination with moderate exercise training.
ANGIOTENSIN-(1–7) ATTENUATES HYPERTENSION

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GRANTS

This work was supported by a National Research Foundation of Korea grant funded by the Korea Government (2010-0029496).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.S., Y.-B.O., S.H.L., and J.M.L. performed experiments; A.S. analyzed data; A.S. drafted manuscript; S.H.K. conception and design of research; S.H.K. interpreted results of experiments; S.H.K. edited and revised manuscript; S.H.K. approved final version of manuscript.

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