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The Effect of Aerobic and Resistance Training on Gene Expression and Protein Levels of ANP in Male Wistar Rats

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Abstract

Introduction: Hypertension is one of the common diseases in modern societies. ANP hormone is one of the factors regulating blood pressure. Apart from conventional clinical treatments, recent attention has been paid to the impact of exercise on ANP. But there is still debate around the type of exercise that is being used. Therefore, the aim of this study was to investigate the effect of aerobic and resistance training on gene expression and protein levels of ANP in male Wistar rats.

Methods: In this experimental study, 15 male Wistar rats were divided into 3 groups of 5 subjects including (1) control, (2) aerobic training, and (3) resistance training. Aerobic training included running on treadmill at 20 m/min (5 sessions, each session 60 minutes per week), while resistance training included climbing a ladder of one meter with 85-degree slope. For analyzing the findings, one-way ANOVA with Tukey's post hoc test were run using SPSS version 19 ($p<0.05$).

Results: aerobic training had a significant effect on the increased levels of the ANP gene expression ($p = 0.001$) and ANP tissue concentration ($p = 0.001$) in the heart tissue of rats. Resistance training had a significant effect on the increased levels of ANP gene expression ($p = 0.001$) and ANP tissue concentration ($p = 0.001$) in the heart tissue of rats. Also, aerobic training had a greater effect on the ANP gene expression and ANP tissue concentration in the heart tissue of rats compared to resistance training ($p = 0.001$).

Conclusion: It seems that aerobic and resistance training with a significant increase in ANP gene expression and ANP concentration, elevate heart health, however, the effect of aerobic training in this field was more than resistance training.

Keywords: Training, Heart, ANP

Introduction

One of the risk factors for cardiovascular health is hypertension (1, 2). There are several reasons for this, in particular the sedentary lifestyle. In contrast, physical activity plays a protective role against hypertension (3).

Reduced blood pressure has been confirmed in response to resistance and endurance trainings (4- 6), but there is still debate about the best way and intensity of physical activity to regulate blood pressure. Among all the physiological mechanisms that influence blood pressure regulation, the atrial natriuretic peptide (ANP) hormone plays a role as a highway (7). ANP hormone is synthesized in myocardial cells of atrial wall and in part in ventricles and responds to the tension of the heart wall (7- 9). ANP hormones affect the cardiac function, affect the structure and diameter of the vessels, especially coronary arteries, and reduce the sympathetic nervous activity and nervous system neural mediators, and hence are an important factor in lowering blood pressure (10- 12). ANP peptide hormone also affects the endocrine mechanism and so regulates body fluids and subsequently blood

pressure (13, 14). Although several studies have evaluated the function of this hormone and its effect on sympathetic and parasympathetic nerves by measuring its levels in plasma (ANP) and brain tissue (BNP) (8). Several studies have investigated the effect of resistance or aerobic training on plasma ANP changes and reported contradictory results. Earlier it was shown that aerobic training did not have an effect on the resting ANP concentration (15- 17). However, some studies have shown that in response to aerobic training with different intensities, the plasma ANP concentration in resting state has increased (8, 10, 13, 18, 19). Of course, as noted, some studies have measured BNP levels due to the effects of ANP on the nervous system (8, 19).
It has also been pointed out that resistance training can enhance the effect of aerobic training (20, 21), although cardiovascular effects of endurance training are more noticed (20). However, limited studies have been conducted on the effect of resistance training. In addition to some of the contradictions observed in previous reports, although several studies have focused on the effects of different trainings on ANP, limited studies have evaluated gene expression and tissue concentrations of this hormone in the heart tissue and simultaneously using different training patterns. Therefore, considering the importance of ANP and its deep impact on blood pressure regulation as well as some contradictions in the results of various studies and training methods, the aim of this study was to determine the effect of aerobic and resistance training on ANP gene expression and ANP protein levels in heart tissue of male Wistar rats.

Methods

In this experimental study, 15 male Wistar rats were purchased from Animals Breed and reproduction Center of Ferdowsi Medical Sciences University of Mashhad within 10- 12 weeks of age and transferred to the Animals Laboratory of Ferdowsi University of Mashhad, Faculty of Physical Education and Sport Sciences. Rats were maintained in the ambient temperature 22 to 24 ° C, humidity 50 to 55%, and 12:12 hours of darkness/light cycle with free access to water and food in shelves of transparent polycarbonate for rodents with a metal cap, which was covered in floor with clean wooden chips. After 7 days of adaptation, the laboratory rats were randomly divided into 3 groups of 5 subjects including (1) control, (2) aerobic training, and (3) resistance training. Rats in aerobic training group (on a special treadmill for Wistar rats) and resistance training (a ladder with a height of one meter and 85-degree gradient) did trainings for eight weeks. Before implementing the protocol, the rats got familiar with the way of running on treadmill for a week. The acquaintance program for aerobic training group included 5 sessions of running on treadmill at speed of 15 m/min and a zero-degree gradient for 20 to 40 minutes. The acquaintance program for resistance training groups included 5 sessions of climbing up a ladder of one meter and a slope of 60 to 80 degrees, after closing the clamp and the weight attached to the tail of the animal. The aerobic training program included eight weeks (5 days a week) running on a treadmill at a speed of 20 m/min and 60 minutes per session, so that the 10 minutes of the onset of training included warm-up and the 10 minutes of the end of the training was dedicated to cooling at a speed of 15 meters per minute. Resistance training included eight-weeks of climbing up a one-meter ladder with a gradient of 85 degrees. Each session consisted of three sets with five repetitions; with one minute of interval rest for each repetition and two minutes of interval rest for each set. The training was performed after attaching weight to the rat's tail. During the first week, the weight attached to the tail was 50 % of the rat's body weight, which gradually increased by 10 % per week up to 120 % of the rat's body weight in the final week. The control group was also available to experience all the conditions at the training site. At the end of the eighth week, all groups in exactly identical manner and at baseline conditions (72 hours after the last training session) were sacrificed (ketamine (30-50 mg/kg) and xylazin (3-5 mg/kg). The heart tissue immediately after isolation was introduced into special tubes and transferred to the liquid nitrogen and then stored in the refrigerator at -80 ° C until the given index was measured. To measure the levels of ANP gene expression in the heart tissue the Real Time-PCR method was employed using Primix syber green II (USA Applied Biosystems Step One). The sequence of primers used is reported in Table 1; in the mentime, GAPDH was used as the reference gene. The expression of the given genes was also measured by CTΔΔ-2. In order to measure the tissue concentration of ANP, tissue samples were placed in special tubes after washing with saline and immediately stored in liquid nitrogen. ANP tissue concentration was measured by ELISA method (R & D Systems) and special measurement kit (Minneapolis, MN, USA) in accordance with the manufacturer's instruction. In order to analyze the data, data distribution was first examined using the Kolmogrov-Smirnov test. Afterwards, one-way analysis of variance and Tukey's post hoc test were used to analyze the inferential statistics in SPSS software version 19 ($P< 0.05$).

Results

The results of one-way ANOVA in Table 2 showed that there was a significant difference in the levels of gene expression ($F= 237.86$, $p = 0.001$) and protein concentration ($F = 27.36$, $p = 0.001$) of ANP in the heart tissue of rats. The results of Tukey's post-hoc test in Table 3 showed that aerobic training ($M= -0.93$, $p= 0.001$) and resistance training ($M = -0.42$, $p=0.001$) had a significant effect on the increase of ANP gene expression in the heart tissue of rats. In addition, aerobic training had an increased effect on ANP gene expression in the heart tissue of rats compared to resistance training ($M= 0.51$, $p= 0.001$); Also the results of Tukey's post-hoc test in Table 3 showed that aerobic training ($M= -108.53$, $p=0.001$) and resistance training ($M =-0.55$, $p = 0.002$) had a significant effect on the increase of ANP protein concentration in the heart tissue of rats; also, aerobic training had a significant effect on increasing the ANP protein concentration in the heart tissue of the rats compared to resistance training ($M = 0.52$, $p = 0.003$).

Table 1. Sequence of forward-reverse primers to determine the level of ANP gene expression

Gene	Forward (5´- 3´)	Reverse (5´- 3´)	Product Size (bp)
ANP	CATACGCTGACCCTAGCCTG	TTTCTTCAATCCGGTGGCGA	135

Table 2. Results of one-way analysis of variance analysis to examine changes in gene expression and protein concentration in the heart tissue of rats in three groups of research

Variable	Factor	Sum of Squares	df	Mean Square	F	P
ANP Gene Expression	Between Groups	4.42	2	2.21	237.86	0.001
	Within Groups	0.25	12	0.009		
	Total	4.67	14			
ANP Protein Concentration	Between Groups	58915.81	2	29457.90	27.36	.001
	Within Groups	29064.15	12	1076.45		
	Total	87979.96	14			

Table 3. The results of Tukey's post hoc test to determine the difference between the research groups at the levels of gene expression and protein concentration of ANP in the heart tissue of rats

Variable	Group	Aerobic Training	Resistance Training
ANP Gene Expression	Control	M=-0.93, P=0.001	M=-0.42, P=0.001
	Aerobic Training		M=0.51, P=0.001
ANP Protein Concentration	Control	M=-108.53, P=0.001	M=-55.93, P=0.002
	Aerobic Training		M=52.85, P=0.003

Discussion

The results of this study showed that, in response to both aerobic and resistance trainings, the ANP gene expression increased, which was grater in aerobic training group. These results were consistent with many previous studies (10, 18, 22). In contrast, it was different from some studies that there was no significant change in ANP (15, 16, 20, 23, 24). As noted, many environmental and physiological factors contribute to the release and re-synthesis of ANP (9- 12, 14). However, since research on lab animals has been performed in the same conditions for all, many of these interfering variables have been controlled. However, some differences in methods such as the simultaneous use of resistance and aerobic trainings make it difficult to measure plasma concentrations rather than measuring gene expression or study on different species of these comparisons. Even the development of science and the application of different laboratory methods are also effective in this regard, which does not fit into this scope. One of the most important physiological points that can justify the difference in the results of studies with each other is the principle of compatibility. Indeed, in some studies, the duration taken may improve the function of the hormonal receptors and increase their sensitivity, which may have reduced the resting levels of ANP (25). This phenomenon was observed in a study examining the effect of 22 weeks of training (24). One of the most important reasons for an increase in ANP hormone in response to physical activity is the increase in the diameter of the heart wall (6, 26, 27), which probably plays an important role in increasing the gene expression of the hormone as the origin of ANP synthesis. The most likely mechanism for further elevation of the ANP in response to endurance training is the increase in cardiac preload and increased diastolic end-length and subsequently traction of the walls of the heart (6, 20, 26, 27). Remember that the last cycle of stimulating the secretion of the ANP is an increase in the traction of the walls of the heart. It is likely that more ANP secretion in compatibility with exercise will increase gene expression and increase the tissue concentration of this hormone, which was also observed in this study. Endurance training has a greater effect on the diameter of the left ventricle wall and the return of venous blood, both of which increase preload (6, 20, 27). However, this adaptation in resistance training reveals itself as increases in post-load and blood pressure (13, 28). On the other hand, since increase in preload can cause microscopic damage to myocytes through eccentric hypertrophy, this damage may be associated with the release of ANP. Because there is an association between the release of ANP and the presence of troponin due to transient myocyte damage (11, 14). Long-term compatibility with endurance physical activity results in the synthesis and storage of ANP in the myocytes of the heart and adaptation changes in the structure of myocytes. However, resistance trainings lead to the execution of Valsalva maneuver and muscle contraction, which will reduce venous return and cardiac preload. Perhaps for this reason, in the present study, the ANP response to adaptation to resistance training was less witnessed than endurance training. Endothelin-1 is also a potent vasoconstrictor, and researchers have shown that there is a significant relationship between ANP increase in response to physical activity and endothelin-1 reduction (29, 30). In fact, aging leads to an increase in endothelin-1 secretion, which was reduced by aerobic training (30). Resistance trainings have also been able to reduce endothelin-1 concentrations (29). It was shown earlier that, in response to prolonged training, ANP increased and endothelin-1 decreased (31). However, the focus of the present study was on the gene expression of ANP, which had received less attention in the previous studies. Nonetheless, the effects of endothelin-1 and angiotensin-2 which cause calcium ions to enter the cardiac muscles and the smooth muscles of the vascular wall (13, 28, 32, 33) should be more closely examined, which requires a separate study. Note that apart from hemodynamic factors, one of the factors of increased post-load in response to resistance training is the role of endothelin-1 and angiotensin-2 in reducing the effects of ANP (13, 28). Apelin changes should also be mentioned as another possible mechanism. Apelin is secreted from adipose tissue and ANP is also effective in lipolysis of adipose tissue. Since the ANP peptide hormone can facilitate lipolysis, further elevation of it in endurance training is justifiable (34, 35). It has been reported that ANP stimulates lipolysis through the stimulation of the receptor NPrA and the cGMP second messenger pathway, which is also a stimulant for urinary production (35, 36). Therefore, another possible pathway of hypotension due to an increase in ANP after exercise may be this point. However, the most remarkable impacts of ANP in response to exercise are mentioned in cardiovascular effects, because during the exercise, the secretion of ADH and aldestrone inhibits urinary excretion and the effects of ANP will be more likely to be seen on the balance of internal body fluids, such as increased vascular filtration and edema incidence (14), which is particularly visible in athletics' resistance trainings. Apelin, in turn, through connection to its receiver, namely, inositide, activates the Akt signal pathways and the eNOS hydrolysis, and consequently L-arginine releases Nitric oxide (NO). Nitric oxide, in turn, also increases cGMP and vascular dilatation (37).

The findings of this study showed that probably aerobic training is the best way to increase the gene expression and ANP concentration in the heart tissue of male Wistar rats. However, resistance training also significantly increased these indices compared to the control group, but the effect of aerobic training was very significant. Therefore, it is suggested that people who are involved in resistance training for various reasons, should perform aerobic trainings along with resistance trainings to control the factors affecting blood pressure. In this vein, in the following studies, it is better to investigate the effects of changes in blood pressure factors in response to aerobic and resistance trainings at different intensities, taking into account the function of the ANP hormone receptors and the measurement of the diameter of the walls and the volume of the heart. Also, considering the fact that the clinical emergence of results such as cardiac waves by electrocardiogram and measurements of systolic blood pressure are important for researchers, the limitations of this research include the lack of examination of cardiac waves and blood pressure in rats; therefore, it is suggested that in future studies, the results of cardiovascular and blood pressure studies as well as its association with ANP should be reported.

Conclusion

Regarding the results of this study, it seems that aerobic and resistance trainings have a significant effect on the increase of gene expression levels and ANP protein concentrations in the heart tissue of rats, but comparing the two types of training, it can be said that aerobic training has a more favorable effect on the increase of levels of gene expression and protein concentration of ANP in the heart tissue of rats.

Ethical issues

Not applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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