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The Effect of Swimming Training on Adrenomedullin mRNA Levels in the Aorta, Kidney, and Adrenal Gland of L-NAME-induced Hypertensive Rats

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Abstract: Adrenomedullin, which is produced by the vascular endothelial and smooth muscle cells and cardiomyocytes, is considered to be a local factor controlling vascular tone, cardiac contractility, and renal sodium excretion. Hypertension is the most common cause of cardiovascular disorders and diseases. Exercise has beneficial effects on hypertension, but pathophysiological factors involved in exercise-mediated amelioration of hypertension are yet to be elucidated. We hypothesized that adrenomedullin produced through exercise could play an essential role in the protection from hypertension. For this study rats were subjected to swimming training for six weeks (1-h per day and five times each week). Meanwhile, hypertension was induced by the oral administration of L-NAME (60 mg/kg). Here, we show that L-NAME administration *per se* leads to a significant increase in mean arterial blood pressure. Notably, the 6-week swimming exercise causes a protective effect from the development of hypertension. In addition, the rats rescued from hypertension have high mRNA levels of renal adrenomedullin while they have low levels of adrenomedullin mRNA in the aorta. The obtained data indicate that a 6-week exercise intervention rescues rats from high blood pressure by leading to changes in adrenomedullin levels in the aorta and kidney. The increased expression of adrenomedullin in the aorta might have been a result that compensates for the hypertensive effect of L-NAME. On the other hand, exercise probably exerts its protective effects on hypertension by increasing adrenomedullin in the kidney. A more extended exercise period may give more apparent results regarding the level of adrenomedullin in different organs.
Keywords: *Adrenomedullin; hypertension; L-NAME; mRNA; Swimming training.*

L-NAME İndüksiyonu ile Oluşturulan Hipertansiyon Modelinde Yüzme Egzersizinin Aorta, Böbrek ve Adrenal Bez Adrenomedüllin mRNA Düzeylerine Etkisi

Özet: Adrenomedullin, vasküler gerim, kardiyak kontraktilite ve renal sodyum atılımında rolü olduğu ileri sürülen, vasküler endotelyal ve düz kas hücreleri ile kardiyomiyositlerden eksprese olan bir hormondur. Hipertansiyon, kardiyovasküler hastalıkların en yaygın nedenidir. Egzersizin hipertansiyon üzerinde yararlı etkileri bulunmaktadır ancak egzersiz-aracılı düzelmeyi sağlayan halen daha açıklanmamış patofizyolojik mekanizmalar da bulunmaktadır. Bu çalışmada, egzersizin hipertansiyon üzerindeki etkisinde adrenomedullinin aracılık ettiği düşünülmektedir. Bu amaçla sıçanlara 6 hafta boyunca yüzme egzersizi (haftada 5 gün, 1 saat süreyle) uygulandı. Hipertansiyon, L-NAME'in oral yolla (60 mg/kg) verilmesiyle indüklendi. L-NAME uygulaması hipertansif grupta ortalama kan basıncını belirgin olarak artırdı. Ayrıca, egzersiz L-NAME indüksiyonu yapılan sıçanlarda renal adrenomedullin mRNA düzeyinde artışa neden olarak koruma sağlarken, aortada adrenomedullin mRNA ekspresyonunu düşürdü. Elde edilen bu veriler 6-haftalık yüzme egzersizin aorta ve böbrekte adrenomedullin ekspresyonunu değiştirerek hipertansiyona karşı koruma sağladığına işaret etmektedir. Aortadaki adrenomeduülin artışının hipertansiyonu kompanze etmek için artmış olabileceği düşünülmektedir. Bunun yanında, yüzme egzersizi muhtemelen böbrek adrenomedüllin düzeyini artırarak hipertansiyon üzerindeki koruyucu etkisini göstermektedir. Daha uzun bir egzersiz süresi, farklı organlardaki adrenomedüllin düzeyi hakkında daha net sonuçlar verebilir.
Anahtar kelimeler: *Adrenomedullin; Hipertansiyon, L-NAME; mRNA; Yüzme egzersizi.*

Introduction

Adrenomedullin is a hormone with a potent vasodilator effect, consisting of 52-aminoacid discovered in human pheochromocytoma tissue (Kitamura et al., 1993a). It has since been reported that adrenomedullin can be synthesized by numerous tissues, such as the heart, adrenal medulla, kidney, smooth muscle and vascular endothelial cells, neurons, and fibroblasts (Kitamura et al., 1993a; Kitamura et al., 1993b; Satoh et al., 1995; Sugo et al., 1994a; Sugo et al., 1994b). The most well-known function of adrenomedullin is vasodilation in vascular resistance and capacitance. Adrenomedullin lowers blood pressure; however, it interestingly increases blood flow (Kitamura et al., 1993a). Besides its vasodilatory effects, adrenomedullin is thought to have an essential role in protecting endothelial integrity (Voors et al., 2019).

Hypertension is one of the significant risk factors for cardiovascular morbidity (Boutcher and Boutcher, 2017), which causes drastic changes in various organs such as the kidneys, heart, vessels, eyes and eventually increases the risk of health complications. It is estimated that more than one billion of the world population is hypertensive (Moraes-Silva et al., 2017). Not only does hypertension treatment fail to result in a fast response, but also it costs substantially. Therefore, non-pharmacological approaches, including reducing salt intake and alcohol consumption, quitting smoking, losing weight, changing diet, and doing regular exercise, are encouraged (Mancia et al., 2014). Exercise is a crucial component of lifestyle therapy for the primary prevention and treatment of hypertension. The effects of exercise on hypertension could be seen due to alterations in different physiopathological mechanisms, such as the reduced peripheral vascular resistance (Rueckert et al., 1996), increased vasodilator bioavailability (Nyberg et al., 2012), and improved the countenance between antioxidant systems and reactive oxygen species formation (Higashi and Yoshizumi, 2004). Exercise also causes beneficial changes in some vasoconstrictor peptides such as reductions in serum endothelin-1 levels (Nyberg et al., 2013). In addition, exercise may have crucial effects on hypertension by increasing known circulating peptides (e.g., atrial natriuretic peptide) (Sarzani et al., 2017), but the involvement of adrenomedullin remains unclear.

Materials and Methods

Experimental Animals and Design: The present study was approved by Aydin Adnan Menderes University Animal Care and Ethical Committee (ADÜ-HADYEK-Approval No: 64583101/2016/189).

A total of twenty-eight male Sprague Dawley, eight weeks of age, rats were given *ad libitum* food and tap water, housed at 22±2 ºC temperature, 50-70% humidity and a 12 / 12 hours' light / dark cycle throughout the experiment. The rats were divided randomly into four groups (7 animals/each group); control (C), exercise (E), hypertensive (L), and hypertensive + exercise (LE). The oral administration of L-NAME at a dose of 60 mg/kg every other day for six weeks induced hypertension (Koc Yildirim et al., 2021). The C and E groups were applied orally to drinking water to induce similar stress.

Training Protocol: All rats were adapted to water before starting the experiment. For this, rats were kept in shallow water for 1 hour 5 times a week (Souza et al., 2009). After that, the rats were floated five times a week for 1 hour for six weeks. The workload was bound to the rats' tails as 2% of the body weight from the second week of the exercise and 5% of the body weight in the following weeks. Rats in control and hypertensive groups were kept in shallow water for similar periods.

Determination of Hemodynamic Parameters: Tail-cuff plethysmograph (NIBP200A, Commat Ltd, Turkey) used to record systolic and diastolic blood pressures (SP and DP) from the tail in the awake rats once a week. SP and DP were measured as described earlier (Koc Yildirim et al., 2021). A standard method used to estimate the MAP was calculated (DeMers and Wachs, 2020): MAP = DP + 1/3(SP-DP).

qRT-PCR: The adrenal gland, thoracic aorta, and kidney samples were collected after the rats were sacrificed by cervical dislocation under deep anesthesia. Total RNA of kidney, adrenal and thoracic aorta were extracted exploiting the TRIzol® Reagent (Thermo Fisher Scientific, USA) concerningthe producer's directives. 1 µg total RNA was made to use to be reverse-transcribed cDNA from using the GeneAll HyperScript™ Reverse Transcriptase (GeneAll, Korea) with oligo dT and random hexamer primers in reaction volume as described by the manufacturer. SYBR Green PCR Master Mix was used to carry out qRT-PCR (GeneAll, Korea). GAPDH was used as an endogenous control. StepOne™Real-Time PCR System (Thermo Fisher Scientific, USA) was used to analyze each sample in triplicate. The PCR circumstances were performed as follows: 10 min at 95 °C, 40 cycles of 95 °C for 15 s, 60 °C for 60 s, respectively. Gene copy number data was determined by the $2^{-\Delta\Delta Ct}$ method (Livak

and Schmittgen, 2001) reference to the GAPDH expression compared with the control. Primers sequences for adrenomedullin and GAPDH were respectively: forward 5' - CAG GAC AAG CAG AGC ACG TC - 3', reverse 5' - TCT GGC GGT AGC GTT TGA C - 3', 82bp (Li et al., 2010); forward 5' - TGC ACC ACC AAC TGC TTA GC - 3', reverse 5' - GGC ATG GAC TGT GGT CAT GAG - 3', 87bp (Sun et al., 2012).

Statistical Analyses: The SPSS software (22.0) was used to perform statistical analyses. Shapiro-Wilk's test was used to detect whether or not the dependent variables were normally distributed. Repeated-measures 2-way ANOVA commented the change in mean arterial pressures by the time. The general linear model (GLM) procedures were used to do post hoc analyses when the effect of the intervention and interaction is significant. Correlations were assessed using Pearson's correlation analyses according to the distribution of the data. The results are shown as means ± SEM. P ≤ 0.05 was considered significant.

Results

In the present study, the administration of L-NAME caused a significant increase in MAP (P_{group}<0.001 and $P_{interaction}$=0.001) compared with the rats that did not receive L-NAME, which shows that hypertension was hypertension successfully induced. Furthermore, during the 6-week experimental period, the MAP of L-NAME-treated rats significantly (P<0.001) increased over time. On week 6, there was no difference between control rats and the rats subjected to both L-NAME administration and swimming training, suggesting that the 6-week swimming training returned blood pressure to normal (Table 1).

Table 1. Mean arterial pressure (MAP) (mmHg).

Group	MAP0	MAP1	MAP2	MAP3	MAP4	MAP5	MAP6
Control	92±5	95±6	95±2	92±1^{b}	96±4	92±5^{b}	101±5^{bc}
Exercise	87±2	94±5	92±3	103±2^{b}	100±4	95±4^{b}	91±2^{c}
L-NAME+Exercise	87±6	103±6	99±5	123±4^{a}	98±7	105±4^{ab}	112±3^{b}
L-NAME	84±9	107±6	102±4	127±6^{a}	112±6	113±7^{a}	127±5^{a}
Significance	NS	NS	NS	P<0.001	NS	P<0.01	P<0.001

$^{a, b, c}$ Means bearing different superscripts within the same week differ significantly. NS: not significant. Data are shown as means ± SEM.

Adrenomedullin mRNA levels of the thoracic aorta in hypertensive rats were found higher than the other groups (P<0.001) (Figure 1A). Adrenomedullin mRNA in the aorta was marked lower in the LE group than that of the other groups (P<0.001) (Figure 1A). Interestingly, renal adrenomedullin mRNA levels were significantly increased in the LE group (P<0.001) (Figure 1B). The unexpected result of the adrenomedullin mRNA concentrations might be due to a compensatory effect. Adrenal adrenomedullin mRNA expressions did not exhibit to be different between groups of (Figure 1C). Correlation analysis also indicated that there was no relationship between adrenomedullin mRNA expression of selected organs (aorta, kidney, and adrenal gland) and MAP on week 6 (r_p=0.334; r_p=0.125; r_p=-0.058, respectively, P>0.05).

Discussion and Conclusion

Exercise has reduced blood pressure in humans and experimental animals (Diaz and Shimbo, 2013). In this study, 6-week regular swimming exercises significantly decreased MAP in hypertensive rats with induced LNAME, consistent with other studies using rats with spontaneous hypertension Rodrigues et al., 2018) and with hypertension induced by L-NAME (Cardoso et al., 2014).

It is thought that adrenomedullin, a product of the cardiovascular system, may also have essential roles in regulating of blood pressure (Wong et al., 2012). In hypertension and organ protection, the infusion of chronic adrenomedullin and endogenous adrenomedullin could have beneficial effects (Nagaya et al., 2000; Wong et al., 2012). In the

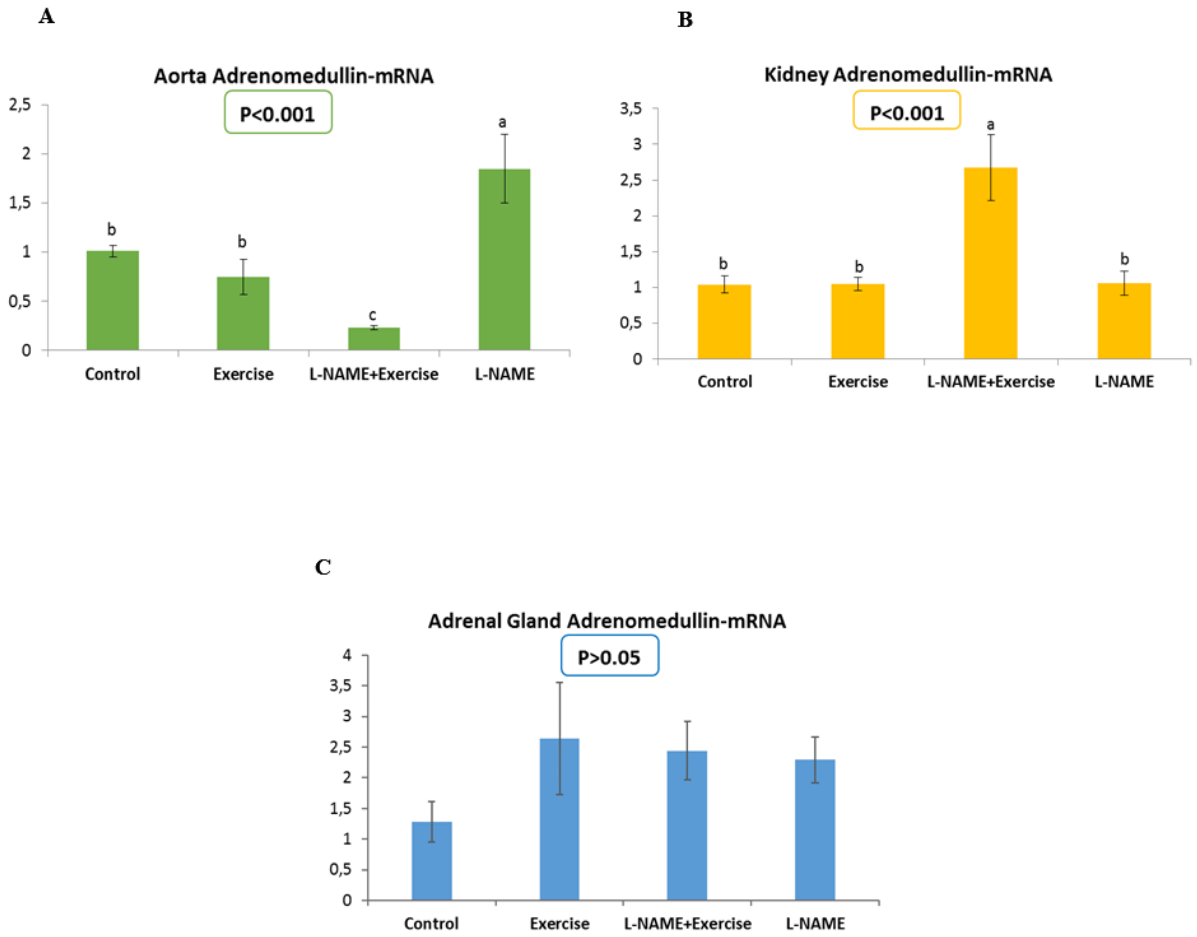

Figure 1. Adrenomedullin mRNA expression in the aorta **(A)**, kidney **(B)**, and adrenal gland **(C)**. [a, b, c]Means bearing different superscripts between the groups differ significantly.

present study, adrenomedullin mRNA expression in the aorta was distinctly increased in the L-NAME-induced hypertensive rats. This result confirms previous studies (Pan et al., 2006; Qi et al., 2003), which suggest that the increased adrenomedullin mRNA might have been a result that compensates the hypertensive effect of L-NAME via its hypotensive, natriuretic, and diuretic actions. Since adrenomedullin has a vasodilatory effect, it is possible that swimming training might induce adrenomedullin levels in the aorta to decrease high blood pressure. However, the results were opposite to this direction which suggest that adrenomedullin in the aorta may not directly be involved in anti-hypertensive actions of swimming training. The reason that adrenomedullin mRNA levels decreased in the aorta could be due to shear stress in the hypertensive vessel (Shinoki et al., 1998). In addition, exercise lowers adrenomedullin mRNA levels of the heart which further confirms that the reduction in adrenomedullin of the circulatory tissues responsds to hypertensive stress (Iemitsu et al., 2001).

On the other hand, unlike the aorta, exercise increased in renal adrenomedullin mRNA expression in the rats who received L-NAME, indicating that swimming exercise probably exerts its protective effects on hypertension by increasing adrenomedullin in the kidney. Further studies should confirm this by measuring blood and renal adrenomedullin levels. Another explanation of why renal adrenomedullin mRNA levels increased might be renal vasculature alterations in L-NAME-induced hypertension states (Raine, 1994). Glomerular capillary hypertension and glomerular damage are also induced by chronic inhibition of NO synthesis, compared to systemic hypertension (Baylis et al., 1992) that might cause an increase in adrenomedullin levels in the kidney, which is an opposite response to that of the aorta. Even though studies suggesting that exercise plays a protective role against renal damage in hypertension (Peeri et al., 2013), some studies that suggest that exercise increases glomerulosclerosis (Kuru et al., 2005) because of elevated renal sympathetic activity and endothelin-1 levels during training and the activation of the rennin-angiotensin system (Kuru et

al., 2005) which might eventually result in renal damage leading an increase in ADM expression in the kidney (Kohno et al., 1995; Yamaguchi et al., 1996).

The findings indicate that L-NAME-induced hypertension is prevented, and blood pressure is decreased by exercise, likely by changing adrenomedullin levels in different tissues. Prolonging the exercise period may give a more effective result on the expression of adrenomedullin in different organs. Further studies will elucidate whether the possibility that adrenomedullin might have antihypertensive effects could lead to a promising target for novel anti-hypertensive medicines. In addition, our results are thought to promote the continuous search intensively for the mechanisms of non-pharmacological antihypertensive treatment.

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Conflicts of Interest

The authors declare no conflict of interest. The sponsors had no role in the design, execution, interpretation, or writing of the study.

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