

# Xanthine Oxidase and Adenosine Deaminase Activities of Renal Tissue in Rats with Hypertension Induced by N Sup Omega Nitro-L-Arginine Methyl Ester<sup>+</sup>

Ersin Fadillioglu\*, Hasan Erdogan\*, Memet Hanifi Emre\*

\*Department of Physiology, Inönü University School of Medicine, Malatya

Hypertension may alter kidney functions negatively. Purine metabolism may be affected from constricted blood vessels and increased cardiac output, because of hypertension. Nitric oxide synthase (NOS) inhibition by N sup omega Nitro-L- Arginine Methyl Ester (L-NAME) produces hypertension in rats. The aim of this study was to investigate adenosine deaminase (AD) and xanthine oxidase (XO) activities of renal tissue in rats after NOS inhibition. Rats were divided into three groups; one of them was control group and the others were study groups treated with 100 or 500 mg per liter L-NAME in drinking tap water for 15 days. After above-mentioned treatment, arterial blood pressure was measured via carotid artery cannula on anaesthetised rats. Activities of AD and XO were carried out in the renal tissue.

Systolic blood pressures showed significant increase in both L-NAME groups. XO activities of L-NAME groups were increased significantly compared to control group. Increased AD activities were also observed in L-NAME groups but this increase did not reach statistical significance. There was no significant difference in XO and AD activities between 100 mg and 500 mg L-NAME groups.

In conclusion, we speculated that hypertension induced by L-NAME causes increased purine nucleotides. Then, accumulated purine nucleotides may be removed from the renal tissue by increased XO and AD enzyme activities.

**Key Words:** Kidney, Hypertension, L-NAME, Xanthine oxidase, Adenosine deaminase

## **N Sup Omega Nitro-L-Arjinin Metil Ester İle İndüklenen Hipertansiyonlu Siçanların Renal Doku Ksantin Oksidaz Ve Adenozin Deaminaz Aktiviteleri**

Hipertansiyon böbrek fonksiyonlarını negatif yönde değiştirebilir. Pürin metabolizması hipertansiyondan dolayı olan kan damarlarındaki daralmadan ve artan kalp atım hacminden etkilenebilir. Nitrik oksit sentazın (NOS) N sup omega Nitro-L-Arjinin Metil Ester (L-NAME) ile inhibisyonu siçanlarda hipertansiyona yol açar. Bu çalışmanın amacı NOS inhibisyonu sonrası siçanlarda renal doku adenozin deaminaz (AD) ve ksantin oksidaz (XO) aktivitelerini araştırmaktır. Siçanlar üç gruba ayrıldı; biri kontrol ve diğerleri 15 gün süreyle içme sularında 100 veya 500 mg/L L-NAME uygulanan çalışma gruplarıydı. Yukarıda açıklanan uygulamanın sonunda, anestezide edilen siçanlarda karotid arter kanülasyonu yoluyla arteriyel kan basınçları ölçüldü. Renal dokuda AD ve XO aktiviteleri ölçüldü.

Sistolik kan basınçları L-NAME gruplarında anlamlı artış gösterdi. L-NAME gruplarındaki XO aktivitesi, kontrol grubuyla karşılaştırıldığında anlamlı olarak arttı. AD aktiviteleri de L-NAME gruplarında artmış olmasına karşın, bu artış anlamlı bulunmadı. 100 mg ve 500 mg L-NAME grupları arasında XO ve AD aktiviteleri açısından anlamlı fark yoktu.

Sonuçta, L-NAME ile indüklenen hipertansiyonun pürin nükleotidlerinde artışa neden olduğu kanısına vardık.

Böylece, artmış XO ve AD enzim aktiviteleri sonucu birikmiş pürin nükleotidleri renal dokudan uzaklaştırılabilir.

**Anahtar Kelimeler:** Böbrek, Hipertansiyon, L-NAME, Ksantin oksidaz, Adenozin deaminaz

<sup>+</sup> *This study was supported by grants from the Inönü University (No: 2001/31) and presented at "Türk Fizyolojik Bilimler Derneği 27. Ulusal Fizyoloji Kongresi, 8-12 Ekim 2001".*

Xanthine oxidase (XO) and adenosine deaminase (AD) are important enzymes in purine metabolism. XO also has a role in free radical metabolism and catalyses the conversion reaction of hypoxanthine to xanthine. If tissues are exposed to metabolic stress, xanthine dehydrogenase is converted to XO. In the presence of adequate purine substrate and oxygen, XO generates free oxygen radical such as superoxide anion ( $O_2^-$ ), hydrogen peroxide and hydroxyl radical. Xanthine oxidase has been implicated as a key oxidative enzyme in disease such as hypertension.<sup>1,2</sup> AD has an important role in DNA metabolism and catalyses the conversion reaction of adenosine to inosine and deoxyadenosine to deoxyinosine.<sup>3</sup>

Endothelial nitric oxide (NO) is generated by conversion of Larginine (L-Arg) to L-citrulline and modulates a variety of tissue specific events, such as vasodilation, prevention of superoxide radical accumulation.<sup>4</sup> Nitric oxide synthase can be inhibited by L-Arg analogous such as N sup omega nitro-L-arginine methyl ester (L-NAME). Relaxation of vascular smooth muscle is failed when NO production is inhibited.<sup>5</sup> Pharmacological inhibition of NO synthesis produces hypertension in many animal species and it has been used as hypertension model.<sup>6</sup> Long-term administration of L-NAME resulted in hypertension, intrarenal vascular, tubular and glomerular lesions and reduction in renal function.<sup>7</sup>

Assessment of the activities of AD and XO enzymes in the renal tissue may help to better understand the pathophysiological changes in the kidney of rats with hypertension. The aim of this study, thus, was to investigate purine nucleotide metabolism of renal tissue after inhibition of NO synthesis that causes hypertension.

## MATERIAL AND METHODS

### Animals and measurement of blood pressure

Experiments were performed on 30 male Wistar Albino rats (average weight, 250 g) purchased from İnönü University Animal Laboratory. The rats were housed in quiet rooms with 12:12-h light-dark cycle (7 AM to 7 PM) and the experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals, DHEW Publication No. (NIH) 85-23, 1985. The rats were divided into three groups; 1) control group: rats treated with only tap water, 2) rats treated with 100 mg per liter L-NAME in drinking tap water for 15 days, and 3) rats treated with 500 mg per liter L-NAME in drinking tap water for 15 days.

After 15 days of treatment, the rats were anaesthetized with urethane (Sigma Chemical Co) at an intraperitoneal dose of 1.2 g/kg. A catheter was introduced into the right carotid artery for measurement of arterial pressure. The arterial blood pressure was monitored by a Harvard model 50-8952 transducer and displayed on a Harvard Universal pen-recorder (Harvard Apparatus Ltd., Kent, UK).

After weighing the kidney, homogenate, supernatant and extracted samples were prepared as described elsewhere.<sup>8</sup> The kidney was taken out, tissue samples were washed with ice-cold saline to remove blood and then stored at  $-70^\circ\text{C}$  until analyses. Tissues were homogenised in Tris-HCl buffer (pH 7.4, 0.2 mM) with a homogenisator (IKA-WERKE Ultra-Turrax T25 basic homogenizer, Germany). The homogenate was then centrifuged at  $5000 \times g$  for 60 minutes to remove debris. Clear upper supernatant fluid was taken, and XO and ADA activities were determined in there. All procedures were performed at  $+4^\circ\text{C}$ . Protein concentrations of kidney supernatant were determined according to Lowry's method.<sup>9</sup>

### Adenosine deaminase activity determination

Tissue adenosine deaminase activities (AD; E.C. 3.5.4.4) were estimated spectrophotometrically (Shimadzu UV-1601 UV-visible spectrophotometer, Kyoto, Japan) by the method of Giusti, which is based on the direct measurements of the formation of ammonia, produced when AD acts in excess of adenosine.<sup>10</sup> Results were expressed as units per milligram protein (U/mg protein).

### Xanthine oxidase activity determination

Xanthine oxidase activity (XO; E.C. 1.2.3.2) was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbancy at 293 nm, according to Prajda and Weber's method (11). A calibration curve was constructed by using 10-50 milli units/mL concentrations of standard XO solutions (Sigma X-1875). One unit of activity was defined as 1  $\mu\text{mol}$  of uric acid formed per minute at  $37^\circ\text{C}$ , pH 7.5, and expressed in units per milligram protein (U/mg protein).

### Statistical analysis

Data were analysed by using a commercially available statistics software package (SPSS® for Windows v. 9.0, Chicago, USA). Distributions of the groups were analysed with one sample Kolmogorov-Smirnov test. All three groups showed normal distribution, so that parametric statistical methods were used to analyse

## Xanthine Oxidase and Adenosine Deaminase Activities of Renal Tissue in Rats with Hypertension Induced by N Sup Omega Nitro-L-Arginine Methyl Ester

the data. One-way ANOVA test was performed. Post Hoc multiple comparisons were done with LSD. Results are presented as means  $\pm$  SD. *P* values  $<0.05$  were regarded as statistically significant.

### RESULTS AND DISCUSSION

Mean arterial pressures of all groups were illustrated in Table 1. L-NAME groups showed increased mean arterial blood pressure compared to control group in anaesthetized rats ( $p < 0.05$ ). Enzyme activities were summarized in Table 2. XO of 100 and 500 mg L-NAME groups were higher than control group ( $p < 0.05$ ). There were no significant differences in the activities of XO between 100 and 500 mg L-NAME groups. ADA activities were not significantly different between the groups.

**Table 1.** Mean arterial pressures of all groups.

	<i>Mean arterial pressure (mmHg)</i>
Control Group (n=10)	$75.4 \pm 5.8$
100 mg L-NAME Group(n=10)	$106.4 \pm 6.4^*$
500 mg L-NAME Group (n=10)	$105.8 \pm 5.3^*$

Mean  $\pm$  SD, \* $p < 0.05$  compared to control.

**Table 2.** Activities of xanthine oxidase (XO) and adenosine deaminase (AD) in renal tissue of rats with hypertension induced by N sup omega nitro-L-arginine methyl ester (L-NAME) and normotensive rats.

	<i>XO (U/mg protein)</i>	<i>AD (U/mg protein)</i>
Control Group (n=10)	$0.730 \pm 0.163$	$134.79 \pm 53.12$
100 mg L-NAME Group (n=10)	$0.922 \pm 0.234^*$	$149.15 \pm 68.67$
500 mg L-NAME Group (n=10)	$0.967 \pm 0.199^*$	$157.34 \pm 73.68$

Mean  $\pm$  SD, \* $p < 0.05$  compared to control.

Nitric oxide is an important mediator to control vascular resistance. In hypertension pathophysiology increased peripheral resistance has a crucial role. Endothelial mediators, such as NO and endothelin-1, control this resistance. Nitric oxide is responsible from vasodilatation while endothelin-1 is responsible from vasoconstriction. If the production of them is disturbed, vascular resistance and thereby blood pressure were affected.<sup>4,12</sup> Inhibition of NO production causes the lack of vasodilatation and thus increased vascular resistance and increased arterial blood pressure. In this study chronically inhibited NO production caused higher mean arterial blood pressure. This result is confirmed by the literature.<sup>13,14</sup>

The kidney function plays a crucial role in the hypertension. Any change in the renal tissue may

cause an important result in the whole body. In the present study XO activities in hypertensive groups were increased significantly. Xanthine oxidase has been implicated as an important source of  $O_2^-$ . It has also a role in the production of uric acid during purine metabolism.<sup>2</sup> Laakso et al demonstrated that renal xanthine oxidoreductase (XOR) activity is increased in hypertension-prone rats. They also determined renal XOR activity after treating spontaneously hypertensive rats (SHRs) for three weeks with L-NAME and demonstrated the induced renal XOR activity after L-NAME treatment. However, it was shown that increased salt intake was no more able to aggravate L-NAME induced hypertension, but it did further increase the renal XOR activity.<sup>15</sup> In the light of these results, it was thought that increased activity of XO in our study may be due to lack of NO in the medium. In the present study we used normotensive rats and also analysed AD activities. The AD activities were increased in the L-NAME groups, although not significant. It seems that there is an increased purine metabolism in L-NAME groups. It was explained by the findings that endogenous adenosine had a possible regulator role on rennin release in renovascular hypertension.<sup>16</sup> It was demonstrated that endogenous adenosine negatively modulates rennin release by a direct effect on juxtaglomerular cells.<sup>17</sup> On the other hand, Tofovic et al. demonstrated that AD inhibition produced a marked fall in arterial blood pressure in older SHR.<sup>18</sup> Taken together, purine metabolism seems to be an important modulator in hypertension as seen in literature.

Higushi et al investigated the effects of Shichimotsukoka-to (SKT), which was prescribed to treat patients with essential hypertension, on renal lesions in stroke-prone spontaneously hypertensive rats (SHRSPs). Treatment with SKT strongly decreased the XO activity and increased the superoxide dismutase (SOD) activity in the kidney of SHRSPs; these values became close to those in normotensive rats.<sup>19</sup> The activity of XO may be increased during endothelial dysfunction in renal tissue, and higher XO activity causes increased  $O_2^-$ . It is observed that the endothelial dysfunction is associated with increased oxidative stress.

We concluded that XO activities of renal tissue were increased significantly in rats with hypertension induced by LNAME, but AD activities were non-significantly increased with L-NAME administration. Nitric oxide inhibition may cause increased purine metabolism and thus XO and AD activities are

increased in NOS inhibited groups. Further investigations with other *in vivo* hypertension models and with other specific NOS inhibitors are needed to provide definitive data about purine metabolites of kidney in hypertension and its relation with NO.

## REFERENCES

1. Lacy F, Gough DA, Schmid-Schonbein GW. Role of xanthine oxidase in hydrogen peroxide production. *Free Radic Biol Med* 1998; 25: 720-727.
2. Parks DA, Granger DN. Xanthine oxidase: Biochemistry, distribution and physiology. *Acta Physiol Scand* 1986; 548: 87-99.
3. Cristalli G, Costanzi S, Lambertucci C, Lupidi G, Vittori S, Volpini R, Camaioni E. Adenosine deaminase: functional implications and different classes of inhibitors. *Med Res Rev* 2001; 21: 105-128.
4. Moncada S. Nitric oxide in the vasculature: physiology and pathophysiology. *Ann N Y Acad Sci* 1997; 811: 60-69.
5. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988; 333: 664-666.
6. Doggrell SA, Brown L. Rat models of hypertension, cardiac hypertrophy and failure. *Cardiovasc Res* 1998; 39: 89-105.
7. Jover B, Mirran A. Nitric oxide inhibition and renal alterations. *J Cardiovasc Pharmacol* 2001; 38 Suppl 2: S65-70.
8. Irmak MK, Koltuksuz U, Kutlu NO, Yagmurca M, Ozyurt H, Karaman A, Akyol O. The effect of caffeic acid phenethyl ester on ischemia-reperfusion injury in comparison with alpha-tocopherol in rat kidneys. *Urol Res* 2001; 29: 190-193.
9. Lowry O, Rosenbraugh N, Farr L, Rondall R. Protein measurement with the folin-phenol reagent. *J Biol Chem* 1951; 183: 265-275.
10. Giusti G. Adenosine deaminase; in Bergmeyer MV (ed): *Methods of Enzymatic Analysis*, 2<sup>nd</sup> ed. New York, Academic Press 1974: 1092-1098.
11. Prajda N, Weber C. Malign transformation-linked imbalance: decreased XO activity in hepatomas. *FEBS Lett* 1975; 59: 245-249.
12. Vural P. Nitric oxide/endothelin-1 in preeclampsia. *Clin Chim Acta* 2002; 317: 65-70.
13. Bergamaschi CT, Biancardi VC, Lopes OU, Campos RR. Effects of angiotensin blockade in the rostral ventrolateral medulla on maintenance of hypertension induced by chronic L-NAME treatment. *Brain Res* 2002; 927: 195-199.
14. Yoneyama T, Ohkawa S, Watanabe T, Odamaki M, Kumagai H, Kimura M, Hishida A. The contribution of nitric oxide to renal vascular wall thickening in rats with L-NAME-induced hypertension. *Virchows Arch* 1998; 433: 549-57.
15. Laakso J, Vaskonen T, Mervaala E, Vapaatalo H, Lapatto R. Inhibition of nitric oxide synthase induces renal xanthine oxidoreductase activity in spontaneously hypertensive rats. *Life Sci* 1999; 65: 2679-2685.
16. Jackson EK, Ohnishi A. Development and application of a simple microassay for adenosine in rat plasma. *Hypertension* 1987; 10: 189-197.
17. Kuan CJ, Wells JN, Jackson EK. Endogenous adenosine restrains renin release in conscious rats. *Circ Res* 1990; 66: 637-646.
18. Tofovic SP, Kusaka H, Li P, Jackson EK. Effects of adenosine deaminase inhibition on blood pressure in old spontaneously hypertensive rats. *Clin Exp Hypertens* 1998; 20: 329-344.
19. Higuchi Y, Ono K, Sekita S, Onodera H, Mitsumori K, Nara Y, Satake M. Preventive effects of Shichimotsu-koka-to on renal lesions in stroke-prone spontaneously hypertensive rats. *Biol Pharm Bull* 1998; 21: 914-918.

## Yazisma Adresi :

Dr. Ersin FADILLIOĞLU  
İnönü Üniversitesi Tıp Fakültesi  
Fizyoloji AD, Malatya  
Tel : 422 341 0660-1413  
Fax : 422 341 0036  
E-mail : efadillioğlu@yahoo.com