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ORIGINAL RESEARCH

The effects of renal dopaminergic system on the development of hypertension with high salt diet and L-NNA administration

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Abstract

Objective: *We aimed to investigate the intrarenal dopamine synthesis efficiency, blood pressure changes and the effects of this system on hypertension developed by NOS inhibition and high salt diet.*

Method: *Wistar Albino male rats were administered water containing 50mg/L or 100mg/L concentrations of L-NNA, standard rat feed containing 0.8%salt, or 4%high salt alone or with L-NNA for 7days. Blood pressure measurements were made with the tail-cuff method. 24-hour water intake and urine volume were also measured.*

Results: *Administration of L-NNA or high-salt diet alone for 7days did not cause a change in blood pressure, while their combined administration resulted in a significant increase in blood pressure. Blood pressures were found to be higher in the L-NNA100+HS group compared to the other groups. While the amount of water intake in 24hours did not change, the amount of 24-hour urine was reduced. 24-hour urinary sodium excretion, sodium clearance and GFR was decreased, and 24-hour urine dopamine concentrations were increased.*

Conclusion: *Co-administration of nitric-oxide inhibitor and high-salt diet failed to prevent renal dopaminergic system blood pressure increase. Despite the increase in dopamine synthesis, intrarenal dopamine activity could not be realized by receptor interaction and it is thought that the increase in blood pressure is caused by the development of renal oxidative stress.*

Keywords: Dopamine, Hypertension, L-NNA, Salt.

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Introduction

Essential hypertension may develop because of functional increase and/or decrease in the activities of the systems involved in the regulation of blood pressure at different levels. Factors such as renin angiotensin aldosterone system imbalances, peripheral arterial changes, ion transport changes, endothelial dysfunction, oxidative stress, excessive sodium intake and renal sodium retention are contributors to the development of essential hypertension[1,2]. Progressive insufficiency developing in the activities of endogenous natriuretic and vasodilating agents such as atrial natriuretic peptide (ANP), nitric oxide (NO), prostacyclin (PGI_2) and intrarenal dopaminergic system rather than hyperactivity of endogenous vasoconstrictor and antinatriuretic mechanisms are involved in the development and maintenance of essential hypertension[3,4].

Basal release of nitric oxide (NO) from endothelial cells changes with various stimuli such as blood pressure changes and shear stress and contributes to the regulation of local blood flow. It is known that NO causes strong vasodilation, suppresses platelet adhesion and aggregation and proliferation of vascular smooth muscle cells, and contributes to the regulation of arterial blood pressure[5,6]. In addition, NO, which is synthesized tonically in the kidneys, regulates glomerular filtration rate, total renal and medullary blood flow, pressure natriuresis, epithelial Na^+ transport and the synthesis of vasoactive agents such as renin, and plays an important role in sodium excretion[7]. NO is synthesized from L-arginine by nitric oxide synthase (NOS) enzymes, and in hypertension developed by the inhibition of this enzyme with L-nitro-N-arginine (L-NNA), water and salt retention, increased vascular tone and oxidative stress occur[3,6,8–12]. When the primary role of the kidneys in regulating long-term blood pressure levels was investigated, it was found that renal oxidative stress and NO deficiency mediate the development of hypertension[13–18]. Administration of reactive oxygen species scavengers and antioxidants in experimental hypertension models resulted in improvement in blood pressure levels[19].

Not only does a high salt diet always cause hypertension, but if there is a concomitant deterioration in renal sodium excretion, the harmful effects of high salt occur. Increased sodium intake is a necessary but not sufficient factor alone in the development of hypertension. While a high-salt diet alone can be compensated for in salt-tolerant organisms, it may contribute to the development of hypertension by causing deficiencies in salt-sensitive organisms[2,20]. Approximately 50% of patients with essential hypertension are salt-sensitive individuals[21]. In salt sensitivity, excess salt is not excreted at a sufficient rate and ratio due to the insufficiency of the endogenous natriuretic systems[22]. A high-salt diet is known to cause progressive damage to blood vessels, kidneys, and heart[23]. Treatment of arterial hypertension is necessary to prevent target organ damage

in patients with essential hypertension. Salt restriction is important in terms of preventing target organ damage as well as blood pressure control[22,24].

The intrarenal dopaminergic system, which spontaneously regulates the salt balance of the organism against excessive salt intake, is one of the most important endogenous natriuretic systems[25,26]. L-dopa is converted to dopamine in the kidney by the activity of dopa decarboxylase. The increase in the amount of Na^+ filtered by excessive salt intake increases the amount of L-dopa co-transported with Na^+ in the renal tubule cells. Dopamine synthesized in the tubule cell with increased L-dopa influx stimulates its specific D_1 and D_2-like receptors, inhibiting the Na^+/H^+ exchange pump and Na-K ATPase, thereby reducing Na^+ absorption and removing excess salt. Intrarenal dopamine is the most important system for excretion of excessive salt[27]. In individuals with essential hypertension, abnormal signals were observed in renal dopamine receptors such as D_1 and D_2, and insufficiency in the intrarenal dopaminergic system, and this situation was thought to be related to salt sensitivity and essential hypertension[4,14,25,26,28]. Physiological concentrations of dopamine in the kidney have protective effects against oxidative stress, and dopamine receptors maintain redox balance by inhibiting the production of free oxygen radicals. When any of the dopamine receptor subtypes are missing, an increase in blood pressure is observed, regardless of oxidative stress[29].

The aim of this study is to determine the intrarenal dopamine synthesis ability and efficiency changes that occur because of NOS inhibition and high salt diet, and to investigate the effects of this system on hypertension that develops with these interventions. For this purpose, blood pressure, water and salt balance, glomerular filtration rate, urine flow rate, sodium clearance, fractional sodium excretion, tubular sodium rejection fraction and urinary dopamine levels were measured in subjects who were administered L-NNA and high-salt diet separately and together at doses that would create partial NOS inhibition.

Material and Methods

This study was carried out at Çanakkale Onsekiz Mart University Experimental Research Application and Research Center (ÇOMÜDAM). The experimental protocols were approved by Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee (Project No: 2014-125).

Materiel: In this study, a total of 36 8-week-old Wistar Kyoto male rats with an average weight of 205±15gr were used. The rats used in the experiment were obtained from ÇOMÜDAM Animal Laboratory. Rats were housed in standard light (12 hours daylight/12 hours dark, ventilated,

constant temperature) in standard rat cages with an equal number of rats in each cage. The rats were fed with sufficient (ad libitium) water (tap water or water containing L-NNA) and feed (0.8% salt-containing standard rat feed or 4% salt-containing salty feed) for a total of 7 days. On the last day of the experiment, rats were placed in individual metabolic cages.

L-NNA administrations: L-NNA was administered with drinking water and water intake was not restricted. The L-NNA dose taken by the rats was calculated by considering the amount of water they drink[12]. Every day, 50 mg/L and 100 mg/L concentrations of L-NNA were prepared freshly and administered to rats for 7 days.

High salt applications: For 7 days, the rats were fed with high salt diet with 4% salt or one of the standard rat diets with 0.8% salt and their feeding was free of restrictions. Rats were divided into six groups with 6 rats in each group (n=36). Tap water and standard rat feed were given to control group (n=6). High salt (HS) group (n=6): The rats were fed with rat feed containing 4% high salt. L-NNA50 group (n=6): The rats were given 50 mg/L L-NNA in their drinking water. L-NNA100 group (n=6): L-NNA was given to the rats at a concentration of 100 mg/L in their drinking water. L-NNA50+HS group (n=6): The rats were given 50 mg/L L-NNA and rat feed containing 4% high salt. L-NNA100+HS group (n=6): The rats were given 100 mg/L L-NNA in their drinking water and rat feed containing 4% high salt.

Blood pressure measurements: On day 0 and day 7, systolic blood pressure measurements were made using the tail cuff method (MAY BPHR 9610-PC TAIL-CUFF Indirect Blood Pressure Recorder, Ankara, Turkey). Measurements were made in a quiet laboratory environment, after a resting period of approximately 20 minutes, at the time of the regular signal tone obtained. The blood pressure values obtained were recorded on the computer. The mean systolic blood pressure was calculated by taking 3 measurements from each rat.

Collection of urine samples: Rats were placed in metabolic cages on the last day of the experimental protocol. The amount of water they drink and urine output during 24 hours were recorded. 0.1 ml of 6 N HCL was added to the containers for 24-hour urine collection and the urines were collected protected from sunlight. Urine samples were placed in 1.5 ml Eppendorf tubes and stored at -800ºC (SANYO ULTRA LOW TEMPERATURE FREEZER MDF-U4086S) for biochemical measurements.

Na^+, creatinine and urea measurements in serum and urine: Na^+, urea and creatinine levels in serum and urine samples were measured using an autoanalyzer (ROCHE COBAS 6000).

Measurement of urine dopamine levels: For the measurement of urinary excretion of dopamine, 24-hour urine samples were collected, and dopamine levels were measured by Düzen Laboratories group using a high-pressure liquid chromatography device (HPLC, Shimadsu, Japan).

Calculation of water balance, sodium clearance, glomerular filtration rate, fractional sodium excretion: The water balance of the rats (water intake (ml/day)- urine amount(ml/day) = water balance(ml/day)) was calculated by measuring the 24-hour water intake and urine amount of the rats taken into metabolic cages. Using the data obtained from collected urine and blood samples.

Sodium clearance (CNa) (ml/min) = Urine sodium(mEq/L) x Urine flow rate (ml/min) / Plasma sodium(mEq/L)

Glomerular filtration rate (GFR) (creatinine clearance) (ml/min) = (Urine creatinine(mg/dL) / Plasma creatinine(mg/dL)) x Urine flow rate(ml/min)

Fractional sodium excretion (%FENa) = (Plasma creatinine (mg/dL)) x Urine sodium(mEq/L)) / (Plasma sodium (mEq/L) x Urine creatinine(mg/dL))) X 100

Statistical analysis: Data were expressed as mean±standard error of mean (SEM). Statistical differences were calculated with independent student-t tests in independent groups. Paired student-t test was used to evaluate the difference between the values of the same group on days 0 and 7. Student's t test was used to interpret the results obtained, and a P value of <0.05 was considered statistically significant.

Results

Blood Pressures: There was no significant difference between the groups in the baseline blood pressures of the rats (Table 1). In both doses applied in the experimental protocol, L-NNA did not cause a rise in blood pressure by providing partial inhibition of NOS. However, it was observed that the application of both doses of L-NNA together with a high salt diet for 7 days caused a significant increase in the blood pressures of the subjects compared to the baseline blood pressures within the group. In addition, the blood pressures of the L-NNA100+HS group (n=6; 121.2±2.76: 147±3.37) were found to be higher than the L-NNA50+HS group (n=6; 124.3±0.96: 136.9±2.15) (Table 1, $P<0.05$ L-NNA100+HS vs. L-NNA50+HS rats). Partial NOS inhibition increased blood pressure in a dose-dependent manner when co-administered with a high-salt diet.

Table 1. *Baseline (day 0) and final (day 7) blood pressure values of groups*

Groups	Baseline BP (mmHg)	Final BP (mmHg)
Control (n=6)	122,5 ± 1,24	122,7 ± 1,28
HS (n=6)	121,2 ± 1,43	123,1 ± 1,55
L-NNA50 (n=6)	124,2 ± 1,46	127,8 ± 1,89
L-NNA100 (n=6)	122 ± 3,43	128,1 ± 1,89
L-NNA50+HS (n=6)	124,3 ± 0,96	136,9 ± 2,15 *
L-NNA100+HS (n=6)	121,2 ± 2,76	147 ± 3,37 * $^{\alpha}$

Data were presented as mean±SE. *P<0.05 final BP vs. baseline BP within the groups. $^{\alpha}$ P<0.05 final BP of a group compared to final BP of other groups.

Effects on Water Intake, Urine Volume and Water Balances: L-NNA100 (n=6; 47±1.83) or L-NNA50 (n=6; 50±1.15) applications significantly reduced 24-hour water intake compared to the control group (n=6; 56.1±1.47). While the high salt diet alone did not cause a significant change in the 24-hour water intake of the subjects, it was determined that the co-administration of L-NNA50+HS significantly reduced the 24-hour water intake compared to the control group. L-NNA100 administration alone significantly reduced 24-hour water intake compared to both control and L-NNA100+HS administration (Table 2, P<0.05).

Table 2. *24-hour water intake, urine amount and water balance values*

Groups	24-hour water intake (ml/day)	24-hour urine volume (ml/day)	Water balance (ml/day)
Control (n=6)	56,1 ± 1,47	14,6 ± 0,66	41,1 ± 2,01
HS (n=6)	56,8 ± 2,94	10,8 ± 0,79**	46 ± 2,84
L-NNA50 (n=6)	50 ± 1,15**	10 ± 0,82**	40 ± 1,75
L-NNA100 (n=6)	47 ± 1,83**	11 ± 1,15**	36 ± 1,18**
L-NNA50+HS (n=6)	48,8 ± 2,59**	10,3 ± 1,05**	38,5 ± 2,31
L-NNA100+HS (n=6)	53,1 ± 1,16	6,1 ± 0,91**$^{\# \beta}$	46,8 ± 1,38**$^{\beta}$

Data were presented as mean±SE. ** P<0.05 vs. control. $^{\#}$ P<0.05 vs. HS. $^{\Omega}$ P<0.05 vs. LNNA50. $^{\beta}$P<0.05 vs. LNNA100.

Administration of either dose of L-NNA or high salt alone or in combination significantly reduced 24-hour urine output compared to the control group. In addition, the group administered L-NNA100+HS significantly decreased the 24-hour urine volume compared to the groups treated with both high salt and L-NNA100 and L-NNA50+HS (Table 3, P<0.05).

Table 3. *Serum sodium concentration and sodium concentration in 24-hour urine, calculated sodium clearance (Cl_{Na}), glomerular filtration rate (GFR), fractional sodium excretion (%FeNa)*

Groups	Serum sodium concentration (mEq/l)	Urine sodium concentration (mEq/l)	Cl_{Na}	GFR	%FeNa
Control (n=6)	136 ± 2,4	1,87 ± 0,21	0,095 ± 0,01	17,8 ± 0,13	0,53 ± 0,04
HS (n=6)	138,4 ± 1,4	1,66 ± 0,31	0,083 ± 0,01	13,7 ± 0,12	0,59 ± 0,08
L-NNA50 (n=6)	142,2 ± 0,5**	1,41 ± 0,2	0,069 ± 0,02	15,6 ± 0,14	0,45 ± 0,01
L-NNA100 (n=6)	138,4 ± 1,4	1,53 ± 0,21	0,076 ± 0,01	15,2 ± 0,25	0,52 ± 0,03
L-NNA50+HS (n=6)	143,4 ± 0,9**	1,72 ± 0,33	0,083 ± 0,1	13,3 ± 0,27	0,65 ± 0,01
L-NNA 100 +HS (n=6)	137,6 ± 1,4	0,87 ± 0,16**	0,05 ± 0,1** $^{\beta}$	10,5±0,18**	0,49 ± 0,06

Data were presented as mean±SE.** P<0.05 vs. control. $^{\beta}$ P<0.05 vs. LNNA100.

Administration of L-NNA50 or high salt diet alone or in combination did not affect water balance, while administration of L-NNA100 (n=6; 36±1.18) significantly decreased water balance compared to control group (n=6; 41.5±1.78) (P<0.05). However, co-administration of L-NNA100 with a high salt diet significantly increased the amount of water retained in the body. It was determined that L-NNA100+HS (n=6; 46.8±1.3) application did not change the amount of water consumed in 24 hours, but significantly decreased the amount of 24-hour urine compared to the other groups, thus increasing the water balance compared to the control group. Increased water balance indicates that there may be water-salt retention in the body (Table 2).

Serum sodium and urine sodium levels: L-NNA50 (n=6; 142.6±0.5) and L-NNA50+HS (n=6; 143.4±0.9) applications were found to increase serum sodium values (Table 4, P<0.05).

Table 4. Dopamine levels measured from 24-hour urine samples

Groups	Dopamine (µg/L)
Control (n=6)	9,36 ± 2,75
HS (n=6)	9,24 ± 0,71
L-NNA50 (n=6)	6,72 ± 1,11
L-NNA100 (n=6)	35,88 ± 6,28** $^{\Omega}$
L-NNA50+HS (n=6)	19,74 ± 7,26
L-NNA100+HS (n=6)	20,6 ± 3,33** $^{\#}$

Data were presented as mean±SE.** P<0.05 vs. control. $^{\#}$ P<0.05 vs. HS. $^{\Omega}$ P<0.05 vs. LNNA50.

L-NNA50, L-NNA100 or high salt application alone did not affect 24-hour urinary sodium excretion, while L-NNA100+HS (n=6; 0.87±0.16) administration significantly decreased 24-hour urinary sodium excretion compared to the control group (n=6; 1.87±0.21) (Table 4, P<0.05). Co-administration of a high-salt diet with L-NNA 100 reduced sodium excretion.

Sodium clearance, glomerular filtration rate and fractional sodium excretion calculation: High salt, L-NNA50 or L-NNA100 applications alone did not affect sodium clearance. The sodium clearance of the group administered with L-NNA100 and high salt was found to be significantly lower than the control group and the group administered with L-NNA100. It was determined that L-NNA100+HS application decreased sodium clearance (Table 3, P<0.05). The glomerular filtration rate of the L-NNA100+HS applied group was significantly decreased compared to the control group. However, no significant difference was found between the glomerular filtration rates of the other experimental groups (Table 3, P<0.05).

Urine dopamine values: Urinary dopamine concentrations were found to be significantly higher in groups where L-NNA100 was administered alone (n=6; 35.88±6.28) or in combination with a high-salt diet (n=6; 20.6±3.33) (Table 4, P<0.05).

Discussion and Conclusion

Essential hypertension is a strong risk factor for many diseases such as coronary disease, stroke, peripheral artery disease and heart failure, and the cause is still unknown. The effects of essential hypertension on human health and the fact that it is among the top preventable causes of death explains the fact that many studies have been conducted to elucidate the pathophysiology of the disease[30–32]. In this study, it was aimed to determine the changes in intrarenal dopamine synthesis and activity in blood pressure changes caused by NOS inhibition and high salt diet, and to investigate the effects of this system on developed hypertension.

In this study, 50 mg/L or 100 mg/L concentration of L-NNA or high salt diet (4%) was administered alone or in combination for 7 days. It was observed that L-NNA at both doses applied in the experimental protocol did not cause an increase in blood pressure alone by causing partial inhibition of NOS, but the application of L-NNA in both doses together with a high-salt diet caused a significant increase in the blood pressure of the subjects. In addition, the blood pressures of the L-NNA100+HS group were found to be higher than the L-NNA50+HS group (Table 1, P<0.05). Co-administration of partial NOS inhibition with a high-salt diet has been found to increase blood pressure in a dose-dependent manner.

Similar to this study, Tolins et al. concluded that the endogenous NO system directly modulates renal hemodynamics and sodium utilization, and high-salt diet administration also contributes to renal adaptation. Enhanced NO synthesis in response to increased salt intake facilitates sodium excretion, maintaining normal blood pressure. However, it has been shown that with the inhibition of NO synthesis with a high-salt diet, renal hemodynamics change, renal and

systemic vascular resistance increases, and sodium excretion decreases, resulting in increased blood pressure and sodium sensitivity in rats[33,34]. However, it has been reported that high-salt diet alone did not significantly affect water intake, diuresis and natriuresis. Similarly, it had no effect on renal dopamine synthesis, renal parameters and blood pressure[3,35]. Interestingly, another study reported that administration of high 1% salt in drinking water to rats increased natriuresis, diuresis, and urinary dopamine levels. In the related study, it was determined that the water intake, diuresis, and urinary sodium excretion of the control group were not similar to our study, and blood pressure measurement was not performed[36].

Administration of L-NNA alone in both doses resulted in a significant reduction in 24-hour water intake and decreased urine output. This decrease in the amount of urine may be related to the decrease in water intake, but the significant decrease in the amount of urine observed while the 24-hour water intake of the L-NNA100+HS group did not change, suggesting that the amount of water retained in the body increased (Table 2). A significant decrease was found in the 24-hour urine Na excretion of the L-NNA100 and high-salt diet group, while other treatments did not significantly affect sodium excretion. It is known that diuresis and natriuresis occur in the organism due to increased blood pressure[7,37]. However, in this study, natriuresis and diuresis, which were expected to increase blood pressure, did not occur as a result of the co-administration of L-NNA (100mg/L) and high salt. It was determined that in the L-NNA100+HS group, 24-hour urinary sodium excretion and calculated sodium clearance values were significantly reduced compared to the control group. When these data are evaluated, we can think that the pressure-natriuresis relationship was impaired in the L-NNA100+HS group, and that increased Na retention caused an increase in blood pressure. Impairment of pressure-natriuresis due to NOS inhibition is associated with hemodynamics and renal tubules. For example, reduction of NO synthesis directly causes an increase in basal renal vascular resistance or tubular reabsorption, or indirectly leads to a decrease in renal sodium excretion functions in the form of renin angiotensin activation or an increase in renal vascular response to vasoconstrictors[7,38]. In various studies, it has been reported that increased salt retention contributes to hypertension that occurs as a result of long-term low-dose NOS inhibition, and this application causes the development of salt sensitivity[33,34]. In another study, it was reported that long-term low-dose inhibition with L-NAME 16 mg/dL for 8 weeks and high salt for 4 weeks increased blood pressure by increasing the levels of noradrenaline and adrenaline excreted in 24-hour urine from the first week without changing the 24-hour urine amount and sodium excretion. In the development of NOS inhibition-mediated hypertension, the increase in salt sensitivity and sympathetic system activity has been emphasized[8].

In this study, when creatinine and urea levels measured in serum and urine are evaluated, it can be argued that these applications do not cause pathological changes in renal functions (Table 4). However, L-NNA100 and high salt application decreased glomerular filtration rate compared to other groups. In the study of Salazar et al. on dogs, it was shown that although there was no change in blood pressure administered with 50 ng/kg/min L-NAME intravenous infusion for 3 days, there was a decrease in GFR, water and salt retention, renal vasoconstriction, an increase in plasma renin activity and no change in aldosterone levels. It has been reported that NOS inhibition plays a role in the long-term regulation of renal hemodynamics and renal excretion functions as a result of the renal parameters reaching the levels in the control period after the administration is completed[13]. In another study, rats fed a high-salt diet for 2 weeks were infused with 50-125 mg/kg of L-NAME into the renal artery. It was found that only salt loading did not significantly affect glomerular filtration rate, renal perfusion pressure and renal vascular resistance, but adding L-NAME to salt loading dose-dependently decreased glomerular filtration rate, increased renal perfusion pressure and renal vascular resistance[33].

Reabsorption of sodium into the body takes place in the kidneys via Na^+/K^+ ATPase and Na^+/H^+ exchange pumps. The function of this pump is to ensure the reabsorption of Na^+ ions in the lumen into the renal tubules. Dopamine inhibits the activity of this pump in the proximal tubule. In the proximal tubule, this effect is mediated by dopamine receptors and the adenylate cyclase. Dopamine levels measured in 24-hour urine show intrarenal dopamine activity[3,26,27]. In this study, 4% high-salt diet alone for 7 days did not change the dopamine levels measured in 24-hour urine, while its co-administration with L-NNA increased urinary dopamine levels. Alexander et al. showed that increasing the amount of salt consumed for 8 days to 209-259 mEq in normal individuals who take 9 mEq of salt daily increased dopamine levels measured in 24-hour urine in individuals[39]. However, while the 25 times salt diet applied in this study increased the urinary dopamine levels, in our study, the 5 times more salt diet did not affect the urinary dopamine levels. In another study conducted in Dahl salt-sensitive (DS) and salt-resistant (DR) rats, a diet containing 0.4% or 8% salt was applied for 8 weeks, and hypertension developed in salt-sensitive rats, and hypertension did not develop in salt-resistant rats. 24-hour urine sodium values of DS and DR rats treated with high salt were found to be higher than those of DS and DR rats treated with low salt, but no difference was found between 24-hour urine dopamine levels. Changes in the mechanisms controlling renal vascular resistance rather than sodium excretion were thought to be responsible for the development of hypertension in DS rats[40]. To investigate the diuresis and natriuresis efficiency of intrarenal dopamine, normal salt (0.28%) or high salt (4%) was administered to rats under

anaesthesia for 5 days, and it was shown that water, sodium excretion and urinary dopamine concentrations increased in the high salt group[41]. In another study, increasing the amount of salt consumed for 8 days to 209-259 mEq in normal individuals who take 9 mEq of salt daily increased the sodium and dopamine levels measured in 24-hour urine[39].

Under normal physiological conditions, high salt intake increases renal dopamine production, which has been shown to be responsible for maintaining blood pressure with increased sodium excretion, but when oxidative stress is created, high salt intake causes renal dopamine oxidation, renal inflammation and dysfunction, and subsequent development of high blood pressure[4]. In hypertension, the interaction between dopamine receptors is absent or impaired, and natriuresis, diuresis and vasodilation, which are functions of renal dopamine, are impaired[42]. While it is not claimed that intrarenal dopamine is generally involved in the control of sodium excretion or arterial pressure in animals with normal sodium balance, a possible dependence of dopamine's tonic effect on the magnitude of sodium excess in the body has been demonstrated[43]. In this study, applying a high-salt diet alone for 7 days did not cause an increase in blood pressure, nor did it affect 24-hour urine dopamine levels. However, administration of a 4% salt diet with 100mg/L L-NNA increased 24-hour urine dopamine levels, which is an indicator of intrarenal dopamine synthesis. Despite this, while sodium excretion was expected to increase in 24-hour urine, salt excretion decreased and hypertension developed. It suggests that co-administration of L-NNA100 and high salt may cause oxidative stress and may fail to exert the natriuretic activity of intrarenal dopamine, although intrarenal dopamine production is increased as a result of disruption of dopamine G protein dopamine receptor coupling without affecting intrarenal dopamine synthesis ability.

Co-administration of L-NNA and high salt diet decreased 24-hour urinary sodium excretion, sodium clearance and GFR, and increased water salt retention and blood pressure in the body. This application increased 24-hour urine dopamine concentrations. However, the increased intrarenal dopamine natriuresis was insufficient to realize its effectiveness, and the increase in intrarenal dopamine production but its inability to function suggests that there may be a disorder in the G protein coupling of dopamine and D_1 and D_2-like receptor interaction. However, those interactions could not be detected within the scope of the study. Further studies are needed to elucidate the interactions at the dopamine receptor level and the effects of oxidative stress in the pathogenesis of hypertension.

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