The Korean Journal of Internal Medicine
Vol. 12, No. 1, January, 1997

Changes of the Plasma Endothelin in Adaptation to Increased Salt Intake in Rats

Hyun-Sook Oh, M.D.*, Kwangjay Yoo, M.D., Miwon Kim, Ph.D.
Ki Chul Choi, M.D.** and Jong-Un Lee, M.D.

*Departments of Physiology and **Internal Medicine, Chonnam University Medical School, Kwangju; *Department of Internal Medicine, Kang-Nam General Hospital, Seoul*

Objectives: *Roles for vascular endothelial hormones in body fluid balance have been variously suggested. The present study was aimed at investigating whether the plasma endothelin is altered in responses to acute and chronic perturbations in body fluid balance.*

Methods: *Effects of intravenous infusion of N^G-nitro-L-arginine methyl ester (L-NAME), a competitive inhibitor of endothelium-derived nitric oxide (NO) synthesis, on urinary excretion, blood pressure and plasma levels of endothelin were examined in rats kept on either normal or high-salt diet for two weeks. The plasma endothelin levels in response to an acute extracellular volume expansion (VE) were also determined in normal and 2-kidney, 1 clip (2K1C) hypertensive rats.*

Results: *L-NAME (20 and 200μg · kg^{-1} per min) elicited diuretic and natriuretic effects in association with increased blood pressure both in normal and high-salt rats. In high-salt rats, however, the urinary response to L-NAME was attenuated and the pressor response was augmented compared with the control. High-salt intake per se caused a small, but significant, increase of the plasma endothelin. L-NAME(200μg · kg^{-1} per min) markedly increased the plasma endothelin, which was not, however, affected by high-salt intake. The plasma endothelin was also marginally increased following VE, the magnitude of which did not differ between the normal and 2K1C rats.*

Conclusion: *These results suggest that the endothelin system takes part in adaptation to increased salt-intake. Another evidence indicating a negative modulation of NO on the release of endothelin is also provided.*

Key Words : *Nitric oxide, High-salt intake, 2-Kidney, 1 clip hypertension, Endothelin.*

INTRODUCTION

It has been widely suggested that the endothelium-derived nitric oxide (NO) takes part in the regulation of arterial pressure[1, 2]. Its synthesis is inhibited by L-arginine analogues such as N^G-nitro-L-arginine methyl ester (L-NAME)[3] and acute intravenous or long-term oral administration of these agents results in a dose-dependent increase of systemic blood pressure[4-7]. Roles for NO in regulating renal hemodynamics and excretory functions[8, 9] and in adaptation to increased dietary salt loads have been also suggested[10, 11].

On the other hand, it has been known that NO can modulate the release of endothelin, among other hormones. An endothelial activation leading to increases in NO production could exert a feedback control on endothelin release[12, 13]. NO also functions as a physiological antagonist of endothelin-induced contractions[14]. Although these findings suggest an interaction between NO and endothe-

Address reprint requests to: Jong-un Lee, M.D. Department of Physiology, Chonnam University Medica School, Kwangju 501-190. *This work was supported in part by Research Grants from Hormone Research Center, Chonnam National University (1996).*

lin, little information has been available on that in adaptation to an altered body fluid balance.

The present study was aimed at exploring roles of NO and endothelin in regulating extracellular fluid homeostasis in salt-loaded conditions. Urinary excretion and plasma endothelin responses to an inhibited NO synthesis were examined in normal and sodium-loaded rats. To delineate whether the endothelin response is related with the arterial pressure, the plasma endothelin was also determined in normotensive and hypertensive rats.

MATERIALS AND METHODS

1. Materials

Male Sprague-Dawley rats (220-260g) were kept on either normal or high-salt diet for two weeks, in which the latter was achieved by giving 0.9% saline as a drinking solution before the experiment. Two-kidney, one clip (2K1C) hypertension was made using rats weighing 160-190g by constricting the left renal artery with a silver clip having an internal gap of 0.25mm under ketamine anesthesia. They were used 4 weeks after clipping the artery. Mean arterial pressure was higher in 2K1C rats (155±8mmHg) than in the control (115±5mmHg).

On the experimental day, under thiopental anesthesia(50mg/kg, i.p.), the left femoral artery was cannulated to measure arterial pressure and the vein to serve as an infusion route. A bladder catheter was implanted to collect urine samples.

2. Experimental Protocols

Following the surgical preparation, a 30 to 60-min equilibration period was allowed to elapse. Urine was collected every 15min by flushing the bladder with 1mL of distilled water followed by 1mL of air. Basal urinary data were obtained by averaging values of three consecutive periods before L-NAME was started. L-NAME (Sigma, St. Louis, MO) was infused for 60min at a rate of 5-200μg · kg^{-1} per min(16μL/min) in normal and high-salt rats. Volume-expansion (VE) was induced in control and 2K1C rats by intravenous infusion of saline (0.9% NaCl) over 45min, amounting to 5% of the body weight.

Blood samples were taken from the femoral artery upon termination of the protocol. The plasma was extracted with Sep-Pak C18 cartridges (Waters Associates, Milford, MA) and lyophilized. The lyophilized samples were reconstituted with assay buffer, and concentrations of endothelin in the aliquots were determined using endothelin-1 radioimmunoassay kit (Peninsula Laboratories, Belmont, CA).

Results were expressed as means±SEM. To determine the statistical significance, ANOVA with repeated measures or nonpaired t-test was used.

RESULTS

1. Urinary Responses to L-NAME

A low dose of L-NAME (5μg · kg^{-1} per min) was without effect on urinary excretion and blood

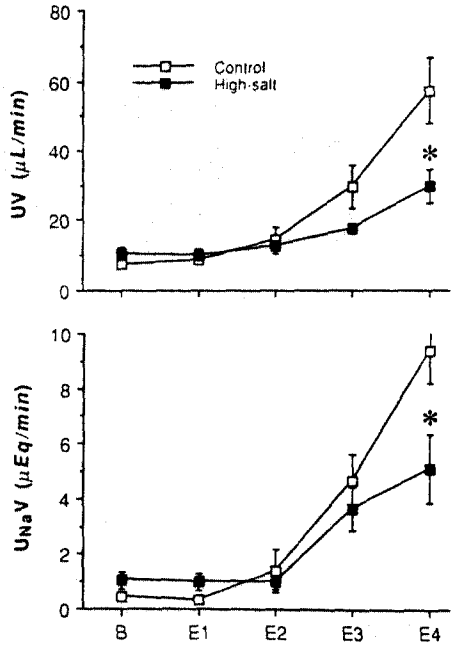

Fig. 1. Urinary volume and sodium excretion before and during infusion of L-NAME (200μg · kg^{-1} per min) in control and high-salt rats. L-NAME was infused during the periods of [E1]-[E4], 15 min each. [B] represents basal period. Number of rats in each group was 7. High-salt intake attenuated the urinary responses to L-NAME infusion (*: $p<0.05$, compared with control).

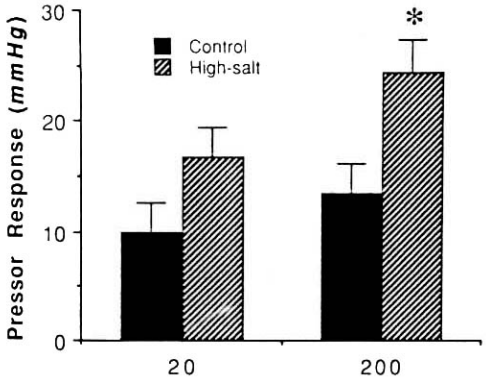

Fig. 2. Maximum blood pressure increases during L-NAME infusion (20 and 200μg · kg^{-1} per min). High-salt intake augmented the pressor effect. *: $p<0.05$, compared with control.

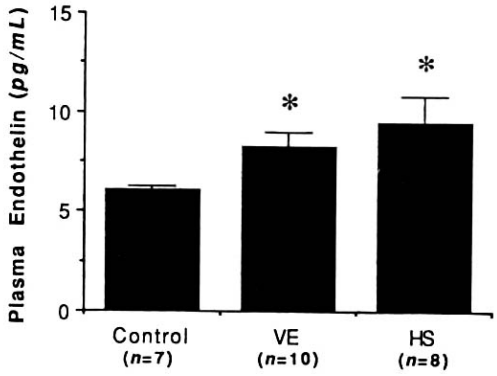

Fig. 3. Plasma endothelin levels following acute extracellular volume expansion (VE) and prolonged high-salt intake (HS). n=Number of rats in each group. *: $p<0.05$, compared with control.

pressure. The higher doses of L-NAME (20-200μg · kg^{-1} per min) elicited diuretic and natriuretic responses, which were significantly attenuated in high-salt rats compared with the control (Fig. 1). The maximum pressor effect caused by L-NAME was augmented in high-salt rats (Fig. 2).

2. Plasma Concentrations of Endothelin

VE as well as prolonged high-salt intake caused a significant, albeit marginal, increase of the plasma endothelin (Fig. 3). VE increased the plasma endothelin in 2K1C rats (9.34±0.75, n=5; vs. basal 6.12±0.73pg/mL, n=5), by a similar magnitude to that in control (8.25±0.76, n=11; vs. basal 6.13±0.28pg/mL, n=6). L-NAME (200μg · kg^{-1} per min) markedly increased the plasma endothelin, the degree of which was not affected by high-salt intake (Table 1).

Table 1. The Plasma Concentrations(pg/mL) of Endothelin

Control (n=8)	5.27±0.42
L-NAME (n=5)	21.43±5.23*
L-NAME/HS (n=5)	22.69±4.80*

[L-NAME] and [L-NAME/HS] denote the groups infused with L-NAME(200μg · kg^{-1} per min) in normal and high-salt rats, respectively. n=Number of animals. *: $p<0.01$, compared with control.

DISCUSSION

It has been found in rats with increased salt-intake that an enhanced renal NO synthesis induces diuresis/natriuresis and allows maintenance of normal blood pressure, unless renal injury with concomitant massive salt overload and chronic NO inhibition is present[11]. The present study provides another evidence indicating an adaptive role for endogenous NO in renal sodium and water handling in salt-loaded conditions, in which the urinary response to intravenous infusion of L-NAME was attenuated in association with an enhanced pressor response. These findings would support the hypothesis that blockade of NO synthesis induces a sodium-dependent hypertension[15]. A defect in NO synthesis has been indeed suggested to underlie the salt-sensitive hypertension[10]. If an increased salt load were improperly handled, the ensuing positive sodium balance may result in hypertension.

However, previous studies are not consistent with the concept that altered salt intake modifies the time course and the final blood pressure level of L-NAME-hypertension[16-18]. The activity of NO synthase to generate NO may not be fully inhibited to cause an augmentation of the pressor response to L-NAME in some experimental setups.

On the other hand, the plasma endothelin was

markedly increased by L-NAME. This is in accord with previous studies which showed increases in plasma endothelin levels following injection of L-NAME in vivo and endothelin concentrations in cultured endothelial cells in vitro[12]. Conversely, an inhibited release of endothelin has been observed by NO[13]. In addition, a link between the high-salt intake and development of hypertension has been hypothesized in salt-sensitive Dahl rats, in that ouabain-like factor(s) is thought to trigger production of endothelin[18]. Other investigators also found an enhanced endothelin-1 gene expression in the aorta and mesenteric arteries of deoxycorticosterone acetate-salt hypertension and a contribution of endothelin to the adaptive modulation of sodium excretion[19, 20]. The pressor effect of L-NAME may therefore result not only from the removal of vasodilatory influence of NO, but also from an enhanced vasoconstrictor effect of the elevated endothelin.

Evidence in favor of a renal interaction of endothelin and NO in L-NAME hypertension has also been suggested, in which the urinary excretion of immunoreactive endothelin increased in rats treated with L-NAME[17]. Several lines of evidence have pointed to an antinatriuretic effect of endothelin in association with reduced filtered load and increased circulating aldosterone[21, 22]. The increased endothelin is, in part, responsible for the attenuated diuretic/natriuretic response to L-NAME.

The plasma endothelin was also increased following acute VE or prolonged high-salt intake. A positive body fluid balance may stimulate the release of endothelin, since among stimuli capable of eliciting endothelin release is low shear stress[23]. Endothelin may be, in turn, causally related to the high blood pressure through its renal as well as vascular action in salt-sensitive model of hypertension. Alternatively, however, the elevated levels may simply reflect a diffuse endothelial injury as a consequence of the high blood pressure, since endothelin is a sensitive marker of endothelial injury[24].

Nevertheless, the plasma endothelin levels following L-NAME infusion were comparable between the high-salt and control rats, despite the higher pressor effect in the former. In addition, no significant difference was noted in either VE-stimulated or basal endothelin level between the normotensive and 2K1C hypertensive rats. In fact, in experimental animal and human hypertension, endothelin levels are not consistently elevated. The plasma endothelin has been reported as only slightly increased or normal in different models of hypertension[25-27]. Other studies also showed that plasma endothelin-1 concentration remained unchanged after 5 weeks of high-salt intake[28]. The precise role of endothelin in body fluid homeostasis and possible implication in hypertension needs further clarification.

In summary, chronically salt-loaded rats demonstrated an attenuated urinary excretion and an increased pressor response to NO synthase inhibition. It is suggested that the endothelin system participates in the renal adaptation to increased salt intake and perturbations in body fluid balance.

REFERENCES

1. Rees DD, Palmer RMJ, Moncada S. *Role of endothelium-derived nitric oxide in the regulation o blood pressure. Proc Natl Acad Sci USA 1989; 86 3375.*
2. Moon SH, Yang MJ, Oh SH, Kim M, Yoo K, Lee J, Jun JY, Yeum CH, Yoon PJ. *A central pressor response to endogenous nitric oxide synthesis inhi bition in anesthetized rats. Kor J Physiol 1994; 28 197.*
3. Rees DD, Palmer RM, Schulz R, Hodgson HF, Moncada S. *Characterization of three inhibitors o endothelial nitric oxide synthase in vitro and in vivo Br J Pharmacol 1990; 101:746.*
4. Gardiner SM, Compton AM, Bennett T, Palmer RMJ, Moncada S. *Regional haemodynamic changes during oral ingestion of N^G-monomethyl-L-arginine or N^G-nitro-L-arginine methyl ester in conscious Brattleboro rats. Br J Pharmacol 1990; 101:10.*
5. Wang YX, Pang CC. *Pressor effect of N^G-nitro1-L-arginine in pentobabital-anesthetized rats. Life Sc 1990; 47:2217.*
6. Arnal JF, Warin L, Michel JB. *Determinants of aortic cyclic guanosine monophosphate in hypertension induced by chronic inhibition of nitric oxide synthase. J Clin Invest 1992; 90:647.*
7. Ribeiro MO, Antunes E, De Nucci G, Lovisolo SM, Zatz R. *Chronic inhibition of nitric oxide synthesis: a new model of arterial hypertension. Hypertension 1992; 20:298.*
8. Bayliss C, Harton P, Engels K. *Endothelial-derived relaxing factor controls renal hemodynamics in the*

normal rat kidney. J Am Soc Nephrol 1990; 1:875.

9. Lahera V, Salom MG, Miranda F, Moncada S, Romero JC. *Effects of N^G-nitro-L-arginine methylester on renal function and blood pressure. Am J Physiol 1991; 261:F1033.*
10. Chen PY, Sanders PW. *L-Arginine abrogates salt-sensitive hypertension in Dahl/Rapp rats. J Clin Invest 1991; 88:1559.*
11. Schultz PJ, Tolins JP. *Adaptation to increased dietary salt intake in the rat. J Clin Invest 1993; 91 642.*
12. Cao WB, Zeng ZP, Zhu YJ, Luo WC, Cai BQ. *Inhibition of nitric oxide synthesis increases the secretion of endothelin-1 in vivo and in cultured endothelial cells. Chinese Med J 1994; 107:822.*
13. Boulanger C, Lüscher TF. *Release of endothelin from the porcine aorta: Inhibition by endothelium derived nitric oxide. J Clin Invest 1990; 85:587.*
14. Vanhoutte PM, Lüscher TF, Gräser T. *Endothelium-dependent contractions. Blood Vessels 1991; 28:74.*
15. Salazar FJ, Alberola A, Pinilla JM, Romero JC, Quesada T. *Salt-induced increase in arterial pressure during nitric oxide synthesis inhibition. Hyper tension 1993; 22:45.*
16. Jover B, Herizi A, Ventre F, Dupont M, Mimran A. *Sodium and angiotensin in hypertension induced by long-term nitric oxide blockade. Hypertension 1993 21:944.*
17. Rivas AF, Estan JG, Vargas F. *Effects of chronic increased salt intake on nitric oxide synthesis inhibition-induced hypertension. J Hypertension 1995 13:123.*
18. Goligorsky MS, Iijima K, Morgan M, Yanagisawa M, Masaki T, Lin L, Nasjletti A, Kaskel F, Frazer M, Badr KF. *Role of endothelin in the development of Dahl hypertension. J Cardiovas Pharmacol S484, 1991*
19. Lariviere R, Day R, Schiffrin EL. *Increased expression of endothelin-1 gene in blood vessels o deoxycorticosterone acetate-salt hypertensive rats Hypertension 1993; 21:916.*
20. Schiffrin EL, Lariviere R, Li JS, Sventek P, Touyz RM. *Deoxycorticosterone acetate plus salt induces overexpression of vascular endothelin-1 and severe vascular hypertrophy in spontaneously hypertensive rats. Hypertension 1995; 25:769.*
21. Goetz KL, Wang BC, Madwed JB, Zhu JL, Leadley RJ. *Cardiovascular, renal, and endocrine responses to intravenous endothelin in conscious dogs. Am J Physiol 1988; 255:R1064.*
22. Miller WL, Redfield MM, Burnett JC. *Integrated cardiac, renal, and endocrine actions of endothelin J Clin Invest 1989; 83:317.*
23. Yoshizumi M, Kurihara H, Sugiyama T, Takaku F, Yanagisawa M, Masaki T, Yasako Y. *Hemodynamic shear stress stimulates endothelin production by cultured endothelial cells. Biochem Biophys Res Commun 1989; 161:859.*
24. Neild GH. *Endothelin plasma levels in hypertensive patients with vascular disease. J Hypertens 1994; 12(Suppl 1):S17.*
25. Suzuki N, Miyauchi T, Tomobe Y, Matsumoto H, Goto K, Masaki T, Fujino M. *Plasma concentrations of endothelin-1 in spontaneously hypertensive rats and DOCA-salt hypertensive rats. Biochem Biophys Res Commun 1990; 167:941.*
26. Kohno M, Yasumari K, Murakawa KI, Yokokawa K, Horio T, Fukui T, Takeda T. *Plasma immunoreactive endothelin in essential hypertension. Am J Med 1990; 88:614.*
27. Shichiri M, Hirata Y, Ando K, Emori T, Ohta K, Kimoto S, Ogura M, Inoue A, Marumo F. *Plasma endothelin levels in hypertension and chronic rena failure. Hypertension 1990; 15:493.*
28. Michel H, Backer A, Meyer-Lehnert H, Migas I, Kramer HJ. *Rat renal, aortic and endothelin-I receptors: Effect of changes in sodium and water intake. Clin Sci 1993; 85:593.*