Chronic High-Sodium Diet Increases Aortic Wall Endothelin-1 Expression in a Blood Pressure–Independent Fashion in Rats

YU-HWAI TSAI,* MAMORU OHKITA,*,[1] AND CHERYL E. GARIEPY*,†,[2]

*Department of Pediatrics and Communicable Disease, University of Michigan, Ann Arbor, Michigan 48109; and †C. S. Mott Children's Hospital, Ann Arbor, Michigan 48109

Vascular endothelin (ET)-1 is upregulated in several forms of salt-induced hypertension. It is unclear to what extent these effects are primary or secondary to endothelial damage. We hypothesized that a high-sodium diet (HNa) increases vascular ET-1 production independent of arterial blood pressure changes. We investigated the effect of chronic HNa with and without ET_A blockade on circulating and aortic ET-1 protein levels as well as aortic expression of *ET-1* and ET_A messenger RNA (mRNA) in inbred Wistar-Kyoto (WKY) and congenic ET_B-deficient rats. Comparing WKY rats fed a low-sodium diet (LNa) with those fed HNa for 3 weeks, aortic wall ET-1 protein is significantly increased in response to HNa (331 ± 43 pg/g tissue for LNa vs. 557 ± 34 pg/gm tissue for HNa). HNa also increased aortic wall *ET-1* mRNA levels by 40%, as determined by quantitative reverse transcriptase polymerase chain reaction. We then compared rats chronically treated with the ET_A-selective antagonist, ABT-627, while receiving either LNa or HNa. There were no differences in arterial blood pressure (mean arterial pressure 89 ± 1 mm Hg for WKY on LNa; 90 ± 3 for WKY on HNa; 91 ± 2 for ET_B-deficient/ABT-627–treated on HNa) or heart rate. However, aortic wall ET-1 protein levels were 4-fold higher in the HNa group. Further, HNa increased aortic wall *ET-1* mRNA (~1.5- to 3-fold) and ET_A mRNA (~2- to 7-fold), independent of activation of ET_B. Therefore, the expression of *ET-1* mRNA by the aortic wall is increased in response to chronic high dietary sodium in WKY rats in the absence of changes in arterial blood pressure. Exp Biol Med 231:813–817, 2006

Key words: hypertension; salt-sensitive; endothelin-A receptor; antagonist; artery; expression

Introduction

The endothelin (ET) system is activated in most models of low-renin, salt-sensitive, and severe forms of hypertension, including deoxycorticosterone acetate–salt hypertensive rats, Dahl salt-sensitive rats, insulin-resistant (fructose-fed) rats, sensory-denervated rats, and stroke-prone spontaneously hypertensive rats (1–7). However, because elevated blood pressure (BP) may result in endothelial damage, altered ET-1 levels or expression may be secondary to hypertension rather than primary.

Arguing in favor of a primary role for the ET system in BP salt sensitivity is the induction of salt sensitivity through genetic and pharmacologic manipulation of system components. Salt-insensitive rats develop hypertension when chronically given a high-salt diet along with an ET_B-selective antagonist (8), and rats genetically deficient in ET_B demonstrate marked salt-induced hypertension (9). Nonhuman primates fed a standard diet also develop hypertension in response to ET_B-blockade (10). In addition, chronic elevation of circulating ET-1 produces salt-sensitive hypertension in rats (11). ET_B-deficient and ET-1 excess hypertension are both prevented or resolved through blockade of the ET_A receptor, suggesting that increased activation of this receptor is pathogenic (12, 13). Finally, mice deficient in renal collecting duct ET-1 exhibit hypertension exacerbated by dietary sodium (14).

To investigate whether the expression of the ET system is affected by dietary sodium in the absence of arterial BP changes, we examined salt sensitivity and expression of ET-1 and ET_A in the aorta in an inbred Wistar-Kyoto (WKY) rat strain with and without simultaneous ET_A blockade. We found significant changes in *ET*-1 mRNA and ET-1 protein levels in the aortic wall of salt-fed rats in the absence of hemodynamic changes. This suggests that the ET system may play a primary role in the development of salt-sensitive hypertension.

Materials and Methods

Animals. Inbred WKY (WKY/NHsd) rats were purchased from Harlan (Indianapolis, IN). ET_B-deficient

1 Current address: Department of Pharmacology, Osaka University of Pharmaceutical Sciences, Osaka 569-1094, Japan.

[2] To whom correspondence should be addressed at Rm A520B, 1150 W. Medical Center Drive, Medical Science Research Building I, Ann Arbor, MI 48108-0656. E-mail: cgariepy@med.umich.edu

Received September 9, 2005.
Accepted November 17, 2005.

1535-3702/06/2316-0813$15.00

rats are $ET_B^{sl/sl}$; *DBH-ET*$_B$ transgenic rats backcrossed onto the WKY/NHsd background (>6 generations; Ref. 13). The colonies are maintained by brother to sister matings. All animal procedures were approved the University Committee on Use and Care of Animals at The University of Michigan. All rats were housed as specific-pathogen free in temperature- and humidity-controlled environments, with a 12:12-hr light:dark cycle. Only male rats were used experimentally.

Chronic Dietary Treatment and ET_A Blockade. Diets were purchased from Harlan Teklad (Winfield, IA). Rats (age 10 wk) were fed a low-sodium diet (LNa, 0.008% NaCl) for 24 hrs, and then were started on a selective ET_A antagonist, ABT-627 (5 mg/kg twice daily by gavage; Abbott Laboratories, Abbott Park, IL), or vehicle treatment. We confirmed that this dose of ABT-627 effectively antagonizes the acute pressor effect of exogenous ET-1. ABT-627 was orally administered in a volume of 1 ml/kg. One day after the start of ABT-627 treatment, the chow of half of the rats was changed to a high-sodium diet (HNa; 8% NaCl). Sodium intake was measure for a 24-hr period once weekly during the subsequent 3 wks. Three weeks after starting the drug plus diet treatment, rats underwent catheterization with blood sample collection.

Arterial Catheterization and BP Measurement. A catheter was placed in the right femoral artery using standard surgical techniques as described elsewhere (9), under isoflurane inhalation anesthesia. Twenty-four hours later, in the afternoon, the externalized arterial catheter was connected to a calibrated BP transducer and the rats were acclimated to the measurement environment for 1 hr. Rats were unrestrained and allowed free access to food and water while attached to the transducer. Pulsatile BP was recorded during 1 h, using the PowerLab system (AD Instruments, Colorado Springs, CO).

ET-1 Concentrations. ET-1 was extracted as described elsewhere (15, 16). Measurement of immunoreactive ET-1 concentrations was performed using a commercially available kit (Assay Designs, Inc. Ann Arbor, MI). The kit has a low level of cross-reactivity with ET-2 but does not detect ET-3 or big ET-1.

Quantitative Reverse Transcriptase (RT) Polymerase Chain Reaction (PCR). Total RNA was extracted with TRIZOL Reagent (Invitrogen, Carlsbad, CA). Copy DNAs were synthesized from 1 μg total RNA with oligo-dT primers using Omniscript RT (Qiagen, Valencia, CA). *ET*-1 and ET_A complementary DNAs (cDNAs) were subjected to 50 cycles of quantitative real-time PCR using SYBR Green I (BioRad, Hercules, CA) as the detection reagent. The control was β-actin. Primer sequences were gatatcgctgcgctcgtcgtc/cctcggggcatcggaacc for β-actin, gaggccatcagcaacagcatca/tccgaggccatccccagac for *ET*-1, and cagcctggcccttggagaccttat/ttctgtgctgctcgcccttgtatt for ET_A cDNA fragment amplification. Single PCR products of the appropriate size were confirmed by melting curve and agarose gel electrophoresis.

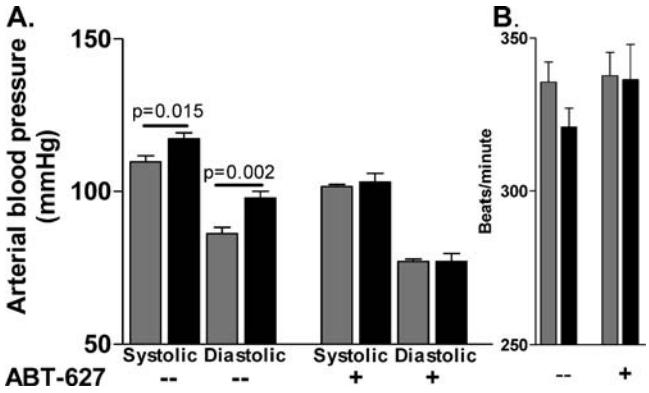

Figure 1. Chronic ET_A blockade lowers BP and prevents salt sensitivity in WKY/NHsd rats. Male rats were given the ET_A selective antagonist, ABT-627, or vehicle along with either the LNa (0.008%NaCl, gray bars) or the HNa (8%NaCl, black bars) diet for 3 weeks before direct BP measurement under conscious and unrestrained conditions. ABT-627 or vehicle was started 1 day before the HNa diet. (A) Systolic and diastolic pressures were slightly but significantly higher in vehicle-treated animals on the HNa diet. Systolic and diastolic pressures are significantly lower in ABT-627–treated rats than vehicle-treated rats on either diet. There was no difference in BP between ABT-627–treated animals on the LNa diet vs. the HNa diet. (B) Diet and ABT-627 treatment did not affect heart rate. Each group contained nine animals.

Results

WKY Rats Are Mildly Salt-Sensitive. We chose to study the WKY rat because it is the original strain of the spontaneously hypertensive rat (SHR). WKY rats are used as the control for SHR and are generally thought to be normotensive and not salt-sensitive. We found, however, that when male WKY rats are chronically subjected to extremes in dietary sodium intake, they exhibit small but significant changes in arterial BP (Fig. 1A). We began treating rats on the LNa for 1 week with vehicle 1 day before switching half of the rats to the HNa diet. Compared with rats on the LNa diet for 3 weeks, mean arterial BP increased in rats fed the HNa diet by approximately 10 mm Hg. We were unable to detect a significant difference in the pulse rate between the two groups (Fig. 1B).

Salt-Induced Increases in Arterial BP Are Blocked by Concurrent Administration of an ET_A Antagonist. We began treating rats on the LNa diet with the ET_A-selective antagonist, ABT-627, 1 day before switching half of the rats to the HNa diet. ABT-627 and the LNa or HNa diet were continued for the next 3 weeks. Arterial BPs were reduced in the rats treated with ABT-627 compared with the vehicle-treated rats, regardless of diet. The mean arterial pressure of LNa and vehicle-treated rats was 98 ± 2 mm Hg, whereas the mean arterial pressure of the LNa and ABT-627–treated rats was 89 ± 1 mm Hg; $P = 0.002$. ABT-627 prevented salt-induced increases in arterial BP (Fig. 1A), but had no affect on the total chow/salt intake (72 ± 2 g/kg/d for vehicle treated and 70 ± 4 g/kg/d for ABT-627 treated; $P = 0.74$). The mean arterial pressure of HNa and ABT-627–treated rats was 90 ± 3 mm Hg. ABT-

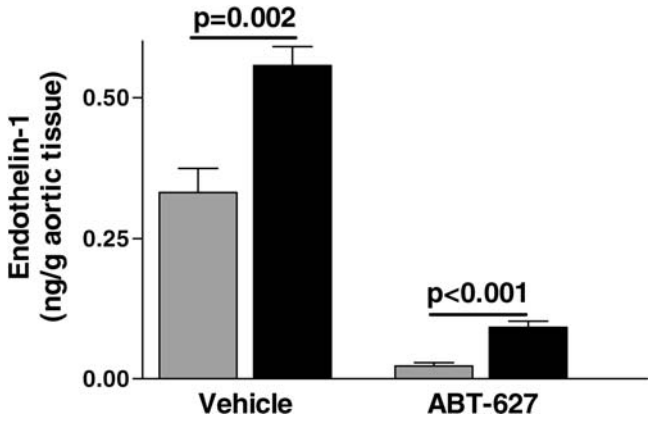

Figure 2. Chronic ET_A blockade does not prevent salt-induced increases in aortic wall ET-1 protein. ET-1 protein was measured from a segment of the abdominal aorta in rats fed either the LNa diet (gray) or the HNa diet (black) along with ABT-627 or vehicle treatment for 3 weeks. ET_A blockade was started 1 day before starting the HNa diet. ET_A blockade significantly reduced aortic wall ET-1 protein content on either diet. The amount of ET-1 protein per gram of aorta was higher in rats fed the HNa diet compared with rats fed the LNa diet, regardless of drug treatment. There were four animals in each group.

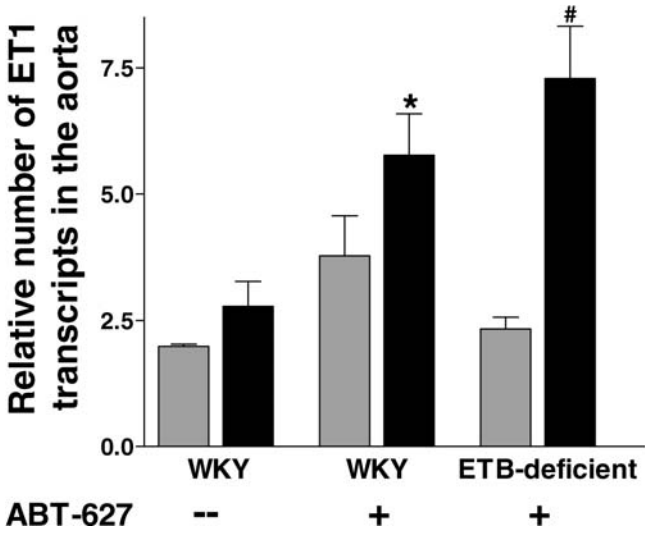

Figure 3. High dietary sodium is associated with an in increased number of ET-1 transcripts in the wall of the aorta in the absence of ET signaling or BP changes. Young, male WKY rats or congenic ET_B-deficient rats were fed either the LNa diet (gray) or the HNa diet (black) for 3 weeks before direct BP measurements under conscious and unrestrained conditions. ABT-627 was started 1 day before HNa diet and continued for 3 weeks. Prepro-ET-1 mRNA was relatively quantified using real-time RT-PCR. ET-1 transcripts were increased in rats fed HNa diet compared with those fed LNa diet, regardless of drug treatment or ET_B deficiency. *$P < 0.04$ compared with WKY vehicle-treated rats on HNa diet. #$P < 0.02$ compared with ET_B-deficient, ABT-627–treated rats on LNa diet. $P < 0.05$ for the effect of dietary sodium by ANOVA. $P < 0.005$ for the effect of ET_A blockade in WKY rats by ANOVA. There were four animals in each group.

627 treatment did not affect heart rate and we detected no difference in the heart rate between LNa- and HNa-fed, ABT-627–treated rats (Fig. 1B).

Aortic ET-1 Protein Is Increased When WKY Rats Are Chronically Fed the HNa Diet. We measured ET-1 protein levels in an isolated segment of the abdominal aorta after 3 weeks of vehicle treatment and either LNa or HNa diet. The HNa diet resulted in an increase in aortic ET-1 protein by approximately 1.7-fold. Because this difference may be secondary to the slight difference in the arterial pressures of these two groups of rats, we performed the same measurement in the rats treated with ABT-627. We found that chronic ET_A blockade did not prevent salt-induced increases in aortic ET-1 protein in WKY rats. Although chronic ET_A blockade significantly reduced aortic wall ET-1 protein in both LNa- and HNa-fed rats ($P < 0.001$), the HNa diet still produced an approximately 4-fold increase in aortic ET-1 levels (Fig. 2).

Aortic Prepro-ET-1 Transcripts Are Increased Under High-Salt and Chronic ET_A Blockade. To determine whether the increase in aortic wall ET-1 protein is related to an increase in production, we performed quantitative RT-PCR for prepro-ET-1 mRNA on copy DNA derived from isolated segments of the abdominal aorta of vehicle- or ABT-627–treated rats on either LNa or HNa diets. To examine the role of ET_B signaling in control of ET-1 production in the wall of the aorta, we included a group of ET_B-deficient rats in this experiment. ET_B-deficient rats exhibit severe salt-induced hypertension that is completely blocked by concomitant treatment with ABT-627. We have previously shown that arterial BPs are identical between salt-fed WKY and ET_B-deficient rats on the WKY genetic background if they are chronically treated with ABT-627 (13). We found that ET-1 transcripts are increase by the HNa diet, independent of ET_A and ET_B signaling (Fig. 3). By two-way analysis of variance (ANOVA), dietary sodium significantly increases aortic wall ET-1 transcripts in WKY rats ($P < 0.05$) and ET_B-deficient rats under ET_A blockade ($P < 0.02$). ET_A blockade increased aortic ET-1 transcripts in WKY rats ($P < 0.005$ by two-way ANOVA). Statistically, there was no significant interaction between dietary sodium and ET_A blockade in determining the number of aortic ET-1 transcripts.

High-Salt Diet Does Not Affect Circulating ET-1 Protein Levels. We next examined whether the salt-induced increase in ET-1 production resulted in increased circulating ET-1 protein levels. We measured circulating ET-1 in the aorta of vehicle- or ABT-627–treated rats on either LNa or HNa diets for 3 weeks. We found no significant effect of dietary sodium on circulating ET-1 levels (Fig. 4). ABT-627 treatment resulted in a significant increase ($P < 0.0001$ by two-way ANOVA) in circulating ET-1 levels on either diet.

Chronic ET_A Blockade Reduced ET_A Transcripts in the Aorta. Finally, we examined the effect of dietary sodium and ET_A blockade on ET_A expression in the aorta (Fig. 5). We found that chronic ET_A blockade reduced the relative number of transcripts in the aorta of WKY rats regardless of the level of dietary sodium ($P < 0.05$ by two-

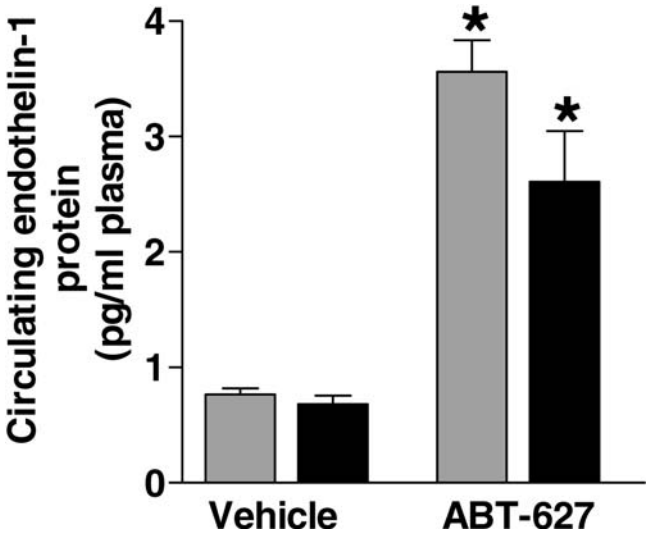

Figure 4. Dietary sodium does not affect circulating ET-1 levels. Young, male rats were fed either the LNa diet (gray) or the HNa diet (black) for 3 weeks with and without chronic ET_A blockade with ABT-627. Blood samples were collected from the abdominal aorta. ET_A blockade results in significantly higher circulating ET-1 levels. Circulating ET-1 levels were not different between the two diets. *$P < 0.001$ compared with vehicle-treated rats. There were five animals in each group.

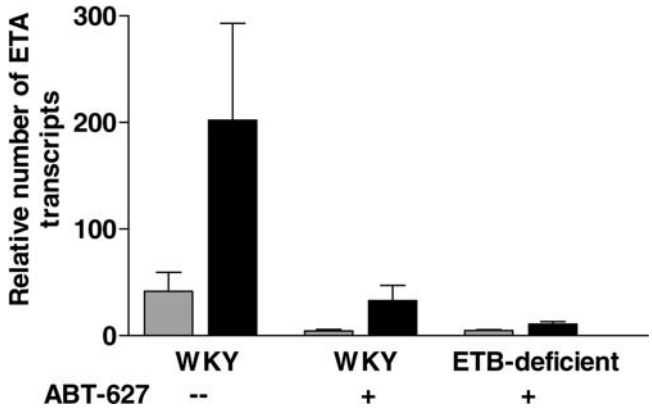

Figure 5. In the absence of BP changes and ET signaling, high dietary sodium is associated with increased ET_A transcription in the wall of the aorta. Young, male WKY rats or congenic ET_B-deficient rats were fed either the LNa diet (gray) or the HNa diet (black) for 3 weeks before direct BP measurements under conscious and unrestrained conditions. ABT-627 was started 1 day before HNa diet and continued for 3 weeks. ET_A mRNA was relatively quantified using real-time RT-PCR. ET-1 transcripts were increased in rats fed the HNa diet compared with those fed the LNa diet, $P < 0.04$ by ANOVA. Chronic ET_A blockade also reduced the relative number of transcripts in WKY rats on either diet, $P < 0.05$ by ANOVA. There were four animals in each group.

way ANOVA). ET_A transcripts were also increased by HNa ($P < 0.04$ by two-way ANOVA). Statistically, there was no significant interaction between dietary sodium and ET_A blockade in determining the aortic ET_A transcript levels.

Discussion

Alemayehu *et al*. (17) reported marked genetic diversity between inbred WKY strains. These authors provide a detailed description of conscious arterial BP, including diurnal variation and salt sensitivity, in WKY/lj-tf (an inbred substrain developed from rats obtained from Teconic Farms, Germantown, NY) and WKY/lj-cr (an inbred substrain developed from rats obtained from Charles River Laboratories, Wilmington, MA). Interestingly, they found significant salt sensitivity in arterial pressure not only in SHR but also in WKY/lj-tf, with systolic pressures increased approximately 20 mm Hg in both stains on HNa diets compared with the regular diet (0.7% NaCl). They described the WKY/lj-tf as a new, inbred hypertensive WKY substrain and the WKY/lj-cr as "normotensive," despite a small increase in BP in response to chronic high dietary sodium of similar magnitude to that which we observed in WKY/NHsd rats. Other reports of a complete absence of BP sensitivity to chronic HNa in WKY rats are difficult to interpret because measurements are made under anesthesia and/or the specific WKY substrain or vender are not listed (18, 19).

To minimize the possibility that the differences in ET-1 and ET_A expression we observed could be secondary to hypertension or irreversible vascular damage, we chose to study young rats and to begin treatment with the ET_A antagonist before exposure to HNa. This resulted in low-normal and equal BPs in rats on the LNa and the HNa diets. The mechanism by which ET-1 and ET_A expression is increased by chronic high dietary sodium *in vivo* remains unclear. Others have shown that high dietary sodium increases renal ET-1 production *in vivo*, in the absence of BP changes (20–24). Herrera and Gavin demonstrated that increased osmolality stimulates ET-1 release in primary cultures of thick ascending limbs (21). Vascular production of ET-1 in response to dietary sodium is less well studied. Increasing osmolality does not affect ET-1 production by cultured rat pulmonary endothelial cells (20), although plasma ET-1 levels increase in response to acute iso-osmolar volume expansion *in vivo*. This is generally thought to be the result of increased ET-1 release by the endothelium in response to increased vessel wall stretch (25). Although chronic intravascular volume expansion and vessel wall stretch may be responsible for the observed increase in aortic wall ET-1 production in the current study, small changes in osmolality of the plasma and/or vascular interstitial space may play a role. We have shown elsewhere that chronic high dietary sodium leads to expansion of intravascular volume with plasma solute dilution in WKY rats, independent of changes in BP and ET_A signaling. Similar volume expansion occurs in salt-fed ET_B-deficient rats treated with ABT-627 (26).

Chronic ET_A blockade lowered arterial BP even in rats fed LNa. This makes the observed effects of ET_A blockade on aortic ET-1 and ET_A transcript levels in WKY rats difficult to interpret. ABT-627 treatment, and, therefore, lower BP, seems to be correlated with a reduction in the level of ET_A mRNA and an increase in the level of ET-1

transcripts. Although ET-1 signaling within the wall of the aorta probably has little influence on arterial BP, these studies shed light on the control of ET-1 production *in vivo* in the vasculature under chronic high-salt conditions. Further investigation is needed to determine whether similar mechanisms are active in arterioles.

We thank Dr. Masashi Yanagisawa for very helpful discussions and the use of the transgenic ET_B-deficient rats. We are also very grateful to the Global Oncology Research & Development Group at Abbott Laboratories (Abbott Park, IL) for the use of ABT-627.

1. Lariviere R, Thibault G, Schiffrin EL. Increased endothelin-1 content in blood vessels of deoxycorticosterone acetate-salt hypertensive but not in spontaneously hypertensive rats. Hypertension 21:294–300, 1993.
2. 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 25:769–773, 1995.
3. Verma S, Bhanot S, McNeill JH. Effect of chronic endothelin blockade in hyperinsulinemic hypertensive rats. Am J Physiol 269:H2017–2021, 1995.
4. Rothermund L, Luckert S, Kossmehl P, Paul M, Kreutz R. Renal endothelin ET(A)/ET(B) receptor imbalance differentiates salt-sensitive from salt-resistant spontaneous hypertension. Hypertension 37:275–280, 2001.
5. Savage P, Jeng AY. Upregulation of endothelin-1 binding in tissues of salt-loaded stroke-prone spontaneously hypertensive rats. Can J Physiol Pharmacol 80:470–474, 2002.
6. Ye DZ, Wang DH. Function and regulation of endothelin-1 and its receptors in salt sensitive hypertension induced by sensory nerve degeneration. Hypertension 39:673–678, 2002.
7. Doucet J, Gonzalez W, Michel JB. Endothelin antagonists in salt-dependent hypertension associated with renal insufficiency. J Cardiovasc Pharmacol 27:643–651, 1996.
8. Williams JM, Pollock JS, Pollock DM. Arterial pressure response to the antioxidant Tempol and ETB receptor blockade in rats on a high-salt diet. Hypertension 44:770–775, 2004.
9. Gariepy CE, Ohuchi T, Williams SC, Richardson JA, Yanagisawa M. Salt-sensitive hypertension in endothelin-B receptor-deficient rats. J Clin Invest 105:925–933, 2000.
10. Reinhart GA, Preusser LC, Burke SE, Wessale JL, Wegner CD, Opgenorth TJ, Cox BF. Hypertension induced by blockade of ET(B) receptors in conscious nonhuman primates: role of ET(A) receptors. Am J Physiol Heart Circ Physiol 283:H1555–1561, 2002.
11. Mortensen LH, Fink GD. Salt-dependency of endothelin-induced, chronic hypertension in conscious rats. Hypertension 19:549–554, 1992.
12. Pollock DM, Pollock JS. Evidence for endothelin involvement in the response to high salt. Am J Physiol Renal Physiol 281:F144–F150, 2001.
13. Ohkita M, Wang Y, Nguyen ND, Tsai YH, Williams SC, Wiseman RC, Killen PD, Li S, Yanagisawa M, Gariepy CE. Extrarenal ETB plays a significant role in controlling cardiovascular responses to high dietary sodium in rats. Hypertension 45:940–946, 2005.
14. Ahn D, Ge Y, Stricklett PK, Gill P, Taylor D, Hughes AK, Yanagisawa M, Miller L, Nelson RD, Kohan DE. Collecting duct-specific knockout of endothelin-1 causes hypertension and sodium retention. J Clin Invest 114:504–511, 2004.
15. Suzuki N, Matsumoto H, Miyauchi T, Goto K, Masaki T, Tsuda M, Fujino M. Endothelin-3 concentrations in human plasma: the increased concentrations in patients undergoing haemodialysis. Biochem Biophys Res Commun 169:809–815, 1990.
16. Matsumoto H, Suzuki N, Onda H, Fujino M. Abundance of endothelin-3 in rat intestine, pituitary gland and brain. Biochem Biophys Res Commun 164:74–80, 1989.
17. Alemayehu A, Breen L, Printz MP. A new inbred Wistar-Kyoto rat substrain exhibiting apparent salt sensitivity and borderline hypertension. Am J Physiol Heart Circ Physiol 283:H1181–1190, 2002.
18. Matrougui K, Loufrani L, Levy BI, Henrion D. High NaCl intake decreases both flow-induced dilation and pressure-induced myogenic tone in resistance arteries from normotensive rats: involvement of cyclooxygenase-2. Pharmacol Toxicol 89:183–187, 2001.
19. Nurkiewicz TR, Boegehold MA. High dietary salt alters arteriolar myogenic responsiveness in normotensive and hypertensive rats. Am J Physiol 275:H2095–2104, 1998.
20. Kohan DE, Padilla E. Osmolar regulation of endothelin-1 production by rat inner medullary collecting duct. J Clin Invest 91:1235–1240, 1993.
21. Herrera M, Garvin JL. A high-salt diet stimulates thick ascending limb eNOS expression by raising medullary osmolality and increasing release of endothelin-1. Am J Physiol Renal Physiol 288:F58–64, 2005.
22. Sasser JM, Pollock JS, Pollock DM. Renal endothelin in chronic angiotensin II hypertension. Am J Physiol Regul Integr Comp Physiol 283:R243–248, 2002.
23. Hoffman A, Grossman E, Goldstein DS, Gill JR Jr, Keiser HR. Urinary excretion rate of endothelin-1 in patients with essential hypertension and salt sensitivity. Kidney Int 45:556–560, 1994.
24. Jackson RW, Treiber FA, Harshfield GA, Waller JL, Pollock JS, Pollock DM. Urinary excretion of vasoactive factors are correlated to sodium excretion. Am J Hypertens 14:1003–1006, 2001.
25. Abdel-Sayed S, Brunner HR, Nussberger J. Volume expansion enhances plasma endothelin-1. Am J Hypertens 16:1057–1061, 2003.
26. Ohkita M, Gariepy CE. ETA blockage prevents polydipsia in the face of volume expansion and hypertension in salt-fed Wistar-Kyoto rats (abstract). In: Program of the 9th International Conference on Endothelin, Park City, UT, September 2005.