Original Article

Sodium Chloride Loading Does Not Alter Endothelium-Dependent Vasodilation of Forearm Vasculature in Either Salt-Sensitive or Salt-Resistant Patients with Essential Hypertension

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The purpose of this study was to determine whether a high NaCl intake impairs endothelium-dependent and -independent vasodilation of forearm circulation in salt sensitive (SS) patients with essential hypertension. We evaluated the effects of intra-arterial acetylcholine (ACh) and isosorbide dinitrate (ISDN) on forearm hemodynamics in 29 patients with essential hypertension, while consuming a low NaCl (50 mmol/d) or high NaCl (340 mmol/d) diet for 1 week. The forearm blood flow (FBF) was measured by strain-gauge plethysmography. Patients were classified as SS ($n=12$) or salt resistant (SR; $n=17$) based on salt-induced changes in blood pressures. The FBF responses of ACh and ISDN were similar in the SS and SR patients while on either NaCl diet, and was not altered by salt loading (ACh, SS: low NaCl 22.8 ± 4.3 vs. high NaCl 21.1 ± 3.6 ml/min per 100 ml, SR: low NaCl 22.5 ± 4.0 vs. high NaCl 23.3 ± 4.1 ml/min per 100 ml; ISDN, SS: low NaCl 13.9 ± 2.1 vs. high NaCl 14.1 ± 2.2 ml/min per 100 ml, SR: low NaCl 13.8 ± 2.3 vs. high NaCl 14.0 ± 2.2 ml/min per 100 ml). There were no significant differences in the vascular responses to ACh and ISDN in the presence of N^G -monomethyl-L-arginine, a nitric oxide synthase inhibitor, in either group for either NaCl diet. These findings suggest that forearm resistance artery endothelial function may not be influenced by salt loading in either SS patients which finding may play a role in determining salt sensitivity in patients with essential hypertension or SR patients. (*Hypertens Res* 2001; 24: 711–716)

Key Words: acetylcholine, sodium, nitric oxide, hypertension, essential hypertension

Introduction

Essential hypertension is associated with endothelial dysfunction. Several lines of evidence have demonstrated im-

pairment of endothelium-dependent vasodilation in the brachial (1–4), coronary (5), renal (6), and small arteries (7) of patients with essential hypertension. Furthermore, the alteration of endothelial function plays an important role in the pathogenesis, maintenance, and development of hyperten-

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sion (8).

It is well known that NaCl loading increases blood pressure in a subgroup of hypertensive patients (9–11). It is well known that NaCl loading increases blood pressure in the subgroup of hypertensive patients known as salt sensitive (SS) hypertensives (9–11). The kidney plays a critical role in the determination of salt sensitivity because of its direct role in modulating sodium and water balance. Several studies in experimental hypertensive animal models suggest that changes in salt intake may alter endothelial function through nitric oxide (NO) production (12–14). In previous studies, we have found that renal endothelial dysfunction is worsened by a high NaCl diet in SS patients (15). However, it is controversial whether a high NaCl intake impairs endothelium-dependent and -independent vasodilation of forearm circulation in SS patients (16–18).

Therefore, to evaluate the effects of NaCl on forearm endothelial function in patients with essential hypertension, we measured forearm vascular responses to acetylcholine (ACh), an endothelium-dependent vasodilator, and isosorbide dinitrate (ISDN), an endothelium-independent vasodilator. Studies were performed both in the absence and presence of *N*^G-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthase, during the consumption of low and high NaCl diets.

Methods

Patients

We studied 29 Japanese patients with essential hypertension (17 men and 12 women; mean age: 52 ± 3 years). Hypertension was defined as a systolic blood pressure >140 mmHg and/or a diastolic blood pressure >90 mmHg, recorded in a sitting position on at least three different occasions. Measurements were obtained in the outpatient clinic of Hiroshima University Faculty of Medicine. Patients with secondary forms of hypertension were excluded on the basis of a complete history, physical examination, radiologic and ultrasound examinations, urinalysis, plasma renin activity (PRA) measurements, plasma aldosterone and norepinephrine concentrations, serum creatinine, potassium, calcium, and free thyroxine concentrations, and measurements of the 24-h urinary excretion of 17-hydroxycorticosteroids, 17-ketogenic steroids, and vanillylmandelic acid. Patients with a history of cardiovascular or cerebrovascular disease, hypercholesterolemia, diabetes mellitus, liver disease, and renal disease were excluded from the study. The study protocol was approved by the ethics committee of the Hiroshima University Faculty of Medicine. Informed consent was obtained from all patients.

NaCl Dietary Manipulation

No patient had received antihypertensive medications prior

to the study. One week before the study, patients were given a regular diet containing 170 mmol of NaCl daily to allow the systemic sodium balance and blood pressure to stabilize. Patients were then placed on a low NaCl diet (50 mmol/day) for 1 week, followed by a high NaCl diet (340 mmol/day) for 1 week. The NaCl content of the high NaCl diet was increased by the addition of Slow-Sodium tablets (10 mmol NaCl per tablet; Mission Pharmaceutical Co., San Antonio, USA) to the diet. It is well known that dietary factors including minerals other than sodium affect blood pressure (19). The dietary contents of potassium (100 mmol/day) and calcium (40 mmol/day) were kept constant throughout the study. The caloric intake was maintained at 40 cal/kg daily. Meals were prepared in the Hiroshima University Hospital kitchen. Compliance with the diet was confirmed by measuring the 24-h urinary excretion of sodium, chloride, and potassium throughout the study.

Endothelial Function in Forearm Vasculature

The effects of ACh (Daiichi Pure Chemicals, Tokyo, Japan) and ISDN (Eisai Co., Ltd., Tokyo, Japan) on forearm hemodynamics during each NaCl dietary intake period were evaluated in 29 patients with essential hypertension. On the seventh morning of each NaCl dietary intake period, after an overnight fast, the patients were placed in a supine position in a quiet, dark, air-conditioned room maintained at a constant temperature (22°C to 25°C). A 23-gauge polyethylene catheter (Hakkow Co., Okayama, Japan) was inserted under local anesthesia (1% lidocaine) into the left brachial artery for the infusion of ACh and ISDN and for the recording of arterial pressure using an AP-641G pressure transducer (Nihon Kohden Kogyo, Tokyo, Japan). Another catheter was inserted into the left deep antecubital vein to obtain blood samples. After 30 min in the supine position, basal forearm blood flow (FBF) was measured and baseline fasting serum concentrations of total cholesterol, creatinine, glucose, electrolytes, angiotensin converting enzyme (ACE) activity, plasma concentration of angiotensin II (Ang II), PRA, and nitrate/nitrite (NO_x) were also obtained. ACh (7.5, 15, and 30 μ g/min) and ISDN (0.75, 1.5, and 3.0 μ g/min) were infused intra-arterially for 5 min at each dose using a constant rate infusion pump (Terfusion STG-523, Termo Co., Tokyo, Japan). FBF was measured during the last 2 min of the infusion. These studies were carried out in a randomized fashion and each study was performed after FBF had returned to baseline. In preliminary studies, after the infusion of a maximal dose of ACh (30 μ g/min) or ISDN (3.0 μ g/min), the FBF returned to baseline values within 30 min. Therefore, a 30-min recovery period followed the ACh and ISDN infusions.

After a 30-min rest period, L-NMMA (CLINALFA Co., Laufelfingen, Switzerland) was infused intra-arterially at a dose of 8 μ mol/min for 5 min while the basal FBF and arterial blood pressure were recorded. We then administered ACh

Table 1. Baseline Clinical Characteristics of SS and SR Patients during Low and High NaCl Diets

	SS patients (n=12)		SR patients (n=17)	
	Low NaCl	High NaCl	Low NaCl	High NaCl
Body weight (kg)	65.8±2.0	66.5±2.2*	66.7±2.4	67.4±2.5*
Mean blood pressure (mmHg)	102.6±2.9	114.3±3.3* [‡]	103.6±2.6	107.1±2.8*
Heart rate (bpm)	63.3±2.2	60.2±2.4	62.8±2.1	61.7±2.3
Serum creatinine (μmol/l)	86.8±5.4	88.3±5.1	85.9±5.6	84.8±5.0
Total cholesterol (mmol/l)	4.5±0.3	4.4±0.2	4.4±0.3	4.4±0.3
Renin-angiotensin system				
PRA (ng/ml/h)	1.93±0.35	0.21±0.04	2.27±0.46	0.23±0.03*
Angiotensin II (pg/ml)	26.9±8.2	16.3±6.4*	24.7±5.9	15.8±3.8*
Serum ACE activity (IU/l)	12.8±0.8	12.6±0.9	12.4±0.8	12.6±0.8
Plasma NOx (μmol/l)	33.8±4.6	36.4±6.9	42.8±6.5	41.3±7.2
Urinary NOx excretion (μmol/mmol creatinine)	126.3±18.6	93.4±14.9*	122.8±17.5	139.7±20.2
Urinary sodium excretion (mmol/day)	44±3	320±16*	41±2	332±20*
Forearm hemodynamics				
FBF (ml/min/100 ml tissue)	4.5±0.3	4.4±0.3	4.5±0.3	4.6±0.3
FVR (mmHg/ml/min/100 ml tissue)	22.8±1.2	25.9±1.5* [‡]	23.0±1.3	23.3±1.4*
Smoker (No. per group)	3		5	

SS, salt-sensitive; SR, salt-resistant; NOx, nitrite/nitrate; ACE, angiotensin converting enzyme; PRA, plasma renin activity; FBF, forearm blood flow; FVR, forearm vascular resistance. All results are the means ± SEM. * $p < 0.05$ vs. low NaCl diet. [‡] $p < 0.05$ vs. SR patients.

(7.5, 15, and 30 μg/min) and ISDN (0.75, 1.5, and 3.0 μg/min) for 5 min at each dose after the infusion of L-NM MA (8 μmol/min). The FBF was measured during the last 2 min of the infusion for each dose.

Measurement of FBF

FBF was measured by using a mercury-filled Silastic strain-gauge plethysmograph (EC-5R; DE Hokanson, Inc., Bellevue, USA) as previously described (2, 4). Briefly, a strain-gauge was attached to the upper part of the left arm and connected to a plethysmography device, and was supported above the right atrium. A wrist cuff was inflated to a pressure of 50 mmHg above the systolic blood pressure 1 min before each measurement and throughout the measurement of FBF to exclude the hand circulation from the measurements. The upper arm congesting cuff was inflated to 40 mmHg for 7 s during each 15-s cycle using a rapid cuff inflator (EC-20; DE Hokanson, Inc.) to occlude venous outflow from the arm. The FBF output signal was transmitted to a recorder (U-228; Advance Co., Nagoya, Japan). FBF is expressed as ml/min per 100 ml of forearm tissue volume. The mean of 4 plethysmographic measurements was used for the analysis of FBF at baseline and during the administration of drugs. Forearm vascular resistance (FVR) was calculated as the mean arterial pressure divided by FBF.

Analytical Methods

Patients in whom NaCl loading induced a 10% change in

mean blood pressure were classified as SS and patients in whom NaCl loading induced a <10% change were classified as salt-resistant (SR).

Routine chemical methods were used to determine serum concentrations of total cholesterol, HDL cholesterol, triglycerides, creatinine, glucose, insulin, and electrolytes. PRA and plasma Ang II were assayed by radioimmunoassay. Serum ACE activity was assayed by a colorimetric assay. The urine concentration of NOx was determined by the Griess method.

Statistical Analysis

Results are presented as the means ± SEM. Values of $p < 0.05$ were considered to indicate statistical significance. The Mann-Whitney *U* test was used to evaluate differences between SS and SR patients with respect to baseline parameters. Comparisons between the two groups with respect to changes in parameters were performed with adjusted means by ANCOVA using the baseline data as covariates. Comparisons of time-response and dose-response curves of parameters were analyzed by two-way ANOVA for repeated measures with Bonferroni correction. The data were analyzed using the software package StatView IV (SAS Institute Inc., Cary, USA) and Super ANOVA (Abacus Concepts, Berkeley, USA).

Fig. 1. Effects of acetylcholine on forearm blood flow (FBF) and forearm vascular resistance (FVR) during low and high NaCl diets in salt sensitive (SS) and salt resistant (SR) patients.

Results

Salt-Sensitivity

We identified 12 SS patients (7 men and 5 women; mean age: 54 ± 4 years) and 17 SR patients (10 men and 7 women; mean age: 52 ± 3 years).

Clinical Characteristics

The baseline clinical characteristics of the SS and SR patients obtained while the patients were on low and high NaCl diets are summarized in Table 1. A high NaCl intake significantly increased body weight ($p < 0.01$) and the urinary excretion of sodium ($p < 0.001$) and significantly decreased PRA ($p < 0.001$) and Ang II ($p < 0.001$) in all of the patients. The mean arterial pressure ($p < 0.01$) and FVR ($p < 0.01$) increased significantly during the high NaCl diet in both groups. Furthermore, the increase in the FVR ($p < 0.05$) was significantly greater in the SS patients than in the SR patients. A high NaCl diet significantly decreased the excretion of NO_x in the SS patients ($p < 0.05$) but not in the SR patients. The other parameters were similar between the 2 groups while on either NaCl diet.

Endothelial Function in Forearm Circulation

The vasodilatory effects of ACh and ISDN were similar in the 12 SS and 17 SR patients while on either NaCl diet, and were not altered by salt loading (Figs. 1 and 2).

The infusion of L-NMMA significantly decreased basal FBF from 4.5 ± 0.3 to 2.4 ± 0.1 ml/min per 100 ml in the SS

Fig. 2. Effects of isosorbide dinitrate on forearm blood flow (FBF) and forearm vascular resistance (FVR) during low and high NaCl diets in salt sensitive (SS) and salt resistant (SR) patients.

patients and from 4.5 ± 0.3 to 2.5 ± 0.1 ml/min per 100 ml in the SR patients on a low NaCl diet ($p < 0.01$, respectively) and from 4.4 ± 0.3 to 2.4 ± 0.1 ml/min per 100 ml in the SS patients and from 4.6 ± 0.3 to 2.5 ± 0.1 ml/min per 100 ml in the SR patients on a high NaCl diet ($p < 0.01$, respectively) and significantly increased FVR from 22.8 ± 1.2 to 42.7 ± 2.9 mmHg/ml/min per 100 ml in the SS patients and from 23.0 ± 1.3 to 41.4 ± 3.1 mmHg/ml/min per 100 ml in the SR patients on a low NaCl diet ($p < 0.01$, respectively) and from 25.9 ± 1.5 to 47.6 ± 3.3 mmHg/ml/min per 100 ml in the SS patients and from 23.3 ± 1.4 to 42.8 ± 2.9 mmHg/ml/min per 100 ml in the SR patients on a high NaCl diet ($p < 0.01$, respectively). L-NMMA significantly reduced the FBF and FVR responses to ACh and ISDN in the SS and SR groups for both NaCl diets. There were no significant differences in the vascular responses to ACh or ISDN in the presence of L-NMMA in either group for either NaCl diet (Figs. 3 and 4). No significant changes were observed in the arterial blood pressure or heart rate in the 2 groups during intra-arterial infusion of ACh or ISDN either in the absence or presence of L-NMMA.

Discussion

The present study demonstrated that ACh-induced or ISDN-induced forearm vasodilation is not affected by changes in dietary NaCl intake in patients with essential hypertension. Although patients were divided into SS and SR groups, changes in NaCl intake did not alter endothelial function in either SS or SR patients.

In the present study, neither endothelium-dependent nor -independent vasodilation was affected by changes in dietary salt intake in patients with essential hypertension. There

Fig. 3. Effects of acetylcholine after N^G -monomethyl-L-arginine (L-NMMA) infusion on forearm blood flow (FBF) and forearm vascular resistance (FVR) during low and high NaCl diets in salt sensitive (SS) and salt resistant (SR) patients.

have been only three clinical studies concerning the effects of NaCl intake on forearm endothelial function in humans. Stein *et al.* (16) showed that endothelium-independent vasodilation induced by sodium nitroprusside is enhanced while endothelium-dependent vasodilation induced by methacholine is not changed in the forearms of healthy individuals on a high-salt diet. Normotensive individuals may have different vascular responses to methacholine and sodium nitroprusside after salt loading. Miyoshi *et al.* (17) reported that the impairment of endothelium-dependent vasodilation, but not endothelium-independent vasodilation, is independent of NaCl intake in SS patients but not in SR patients, and that forearm vascular responses are not affected by changes in NaCl intake in either SS or SR patients. These findings are consistent with our result that a high salt diet did not alter endothelium-dependent vasodilation in the forearm circulation. In contrast, Bragulat *et al.* (18) have recently shown that endothelium-dependent vasodilation, but not endothelium-independent vasodilation, is blunted in SS patients compared with that in SR patients. However, because they measured the FBF responses to ACh and sodium nitroprusside before dietary manipulation, their results did not directly demonstrate the effect of dietary salt intake on endothelial function in the SS or SR patients. Although in the present study we measured the FBF responses to ACh and ISDN during a regular salt diet (170 mmol/day) before salt dietary manipulation, we did not find different vascular responses between SS and SR patients (data not shown). These discrepancies among the present and previous studies may be due to the severity of hypertension in selected patients and to differences in dietary salt intake.

It is well established that the kidney plays an important role in determining salt sensitivity through the regulation of

Fig. 4. Effects of isosorbide dinitrate after N^G -monomethyl-L-arginine (L-NMMA) infusion on forearm blood flow (FBF) and forearm vascular resistance (FVR) during low and high NaCl diets in salt sensitive (SS) and salt resistant (SR) patients.

the urinary excretion of sodium. Salazar *et al.* (20) reported that the regulation of renal sodium excretion and blood pressure during salt loading are NO-dependent. Transplantation of kidneys from normotensive Dahl SR rats into hypertensive Dahl SS rats can normalize blood pressure, while transplantation of Dahl SS kidneys into Dahl SR rats can cause salt-induced hypertension, suggesting that abnormal kidney function in Dahl SS rats is responsible for the salt-induced hypertension (21). In addition, many studies have reported that abnormal renal function in salt-induced hypertension in Dahl SS rats, including a reduced responsiveness to endothelium-dependent vasoactive agents and NO production, may play an important role in initiating salt-induced hypertension (12–14). In our previous study, we have shown that NaCl loading impairs L-arginine-induced renovascular relaxation in SS patients through the decreased renal production of NO (15). These observations suggest that the kidney is involved in the pathogenesis, maintenance, and development of SS hypertension.

Several lines of evidence from experimental studies indicate that the renal blood flow is lower in Dahl SS rats than in Dahl SR rats on a high-salt diet, but there are no differences between Dahl SS and SR rats with respect to blood flow in the brain, heart, lungs, liver, spleen, skeletal muscle, or skin (22, 23). In addition, oral L-arginine treatment significantly increases renal blood flow without changing blood flow in other organs of Dahl SS rats fed a high-salt diet (22). These findings suggest that abnormal NO production might be more prominent in the kidney than in other organs.

In conclusion, changes in dietary NaCl intake did not affect forearm resistance artery endothelial function in patients with essential hypertension in this study. Future studies on the effects of changes in dietary NaCl intake on endothelial

function in other organs such as the brain, heart, and retina, which are target organs for the detrimental effects of high blood pressure, are necessary.

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