Hypertension Research (2012) 35, 849–854

www.nature.com/hr

ORIGINAL ARTICLE

Upregulation of endothelium-derived hyperpolarizing factor compensates for the loss of nitric oxide in mesenteric arteries of dahl salt-sensitive hypertensive rats

Kenichi Goto, Yasuo Kansui, Hideyuki Oniki, Toshio Ohtsubo, Kiyoshi Matsumura and Takanari Kitazono

This study was designed to determine whether a high-salt diet would alter endothelial function and, if so, the relative contributions of endothelium-derived hyperpolarizing factor (EDHF) and nitric oxide (NO) to any changes in vasomotor responses. Male Dahl salt-sensitive (DS) rats were given either a high-salt diet (8% NaCl, DS-H) or a low-salt diet (0.4% NaCl, DS-L) for 6 weeks. Membrane potentials and contractile responses were recorded from the isolated superior mesenteric arteries. After salt loading, DS-H developed hypertension, while DS-L remained normotensive. No difference was found in acetylcholine (ACh)-induced, endothelium-dependent relaxation between the groups. However, after treatment with indomethacin and NO synthase inhibitor, EDHF-like relaxation was significantly greater in DS-H than in DS-L. In contrast, NO-mediated relaxation was significantly smaller in DS-Hthan in DS-L. Iberiotoxin (IbTx), a specific blocker of large-conductance calcium-dependent potassium (BKCa) channels, attenuated EDHF-like relaxation in DS-H but not in DS-L. IbTx marginally inhibited EDHF-mediated hyperpolarization only in DS-H. Endothelium-independent relaxation in response to sodium nitroprusside or levcromakalim was similar in both groups. In conclusion, EDHF-like relaxation is upregulated through the activation of BKCa channels in the mesenteric arteries of DS-H. As the overall relaxation in response to ACh did not differ between the groups, the upregulation of EDHF appears to compensate for the loss of NO in the mesenteric arteries of DS-H.
Hypertension Research (2012) **35,** 849–854; doi:10.1038/hr.2012.36; published online 5 April 2012

Keywords: endothelium-derived hyperpolarizing factor; endothelial function; hypertension; large-conductance calcium-dependent potassium channel; salt

INTRODUCTION

In blood vessels, endothelium-dependent relaxation is determined by the balance of nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF) and endothelium-derived contracting factors.[1–6] In disease states, such as hypertension, this balance is disrupted that leads to endothelial dysfunction.[7,8] Although the mechanisms for endothelial dysfunction during hypertension may vary depending on the animal models studied, many previous studies have described diminished NO-mediated vasodilation as a principal factor for endothelial dysfunction during hypertension.[7,8]

In addition to reduced NO-mediated vasodilation, changes in EDHF-mediated responses have also been reported in several genetic and experimental animal models of hypertension.[9,10] We have previously shown that EDHF-mediated hyperpolarization and relaxation were decreased in the superior mesenteric arteries of 12-month-old spontaneously hypertensive rats (SHR) compared with age-matched normotensive Wistar-Kyoto rats and that the impairment of the EDHF pathway accounted, at least in part, for the endothelial dysfunction in this model.[11] Several mechanisms have been proposed to explain the reduced EDHF-mediated responses in mesenteric arteries of SHR: change in the expression profile of gap junctions,[12–14] increased activity of calcium-activated chloride channels[15] and reduced expression of small conductance calcium-dependent potassium channels.[16]

In contrast, Sofola *et al.*[17] have reported that EDHF-like relaxation is upregulated in the mesenteric arteries of Sprague–Dawley rats fed a high-salt diet to compensate for the loss of NO. Upregulation of EDHF during hypertension has also been reported in renal arteries of SHR.[18] In that study, EDHF-mediated responses were upregulated at the age of 2 months, whereas EDHF-mediated responses were virtually abolished at the age of 22 months compared with age-matched Wistar-Kyoto rats.[18] It is therefore plausible to speculate that although long-term exposure to hypertension would ultimately diminish EDHF-mediated responses, EDHF-mediated responses may be upregulated to maintain overall endothelial function in the early phase of hypertension. As impairment of EDHF would induce

Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
Correspondence: Dr K Goto, Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan.
E-mail: r09gotou2@fa.kyu-dent.ac.jp
Received 14 September 2011; revised 16 January 2012; accepted 17 February 2012; published online 5 April 2012

endothelial dysfunction, which may lead to the progress of atherosclerosis,[19] it is of clinical importance to reveal the underlying mechanisms of the upregulation of EDHF during hypertension.

In arterioles of Dahl salt-sensitive (DS) rats fed a high-salt diet for 6–7 weeks, acetylcholine (ACh)-induced, endothelium-dependent relaxations were preserved despite the impairment of the NO pathway.[20] These observations led us to speculate that EDHF-mediated responses would be upregulated to compensate for the loss of NO in this model. As increased activity and expression of large-conductance calcium-dependent potassium (BKCa) channels during hypertension have been well documented,[21,22] we also hypothesized that the upregulation of EDHF could be attributed to the enhanced input from BKCa channels. In this study, we have tested this possibility in the mesenteric arteries of DS rats fed a high-salt diet, an animal model of salt-sensitive hypertension.

MATERIALS AND METHODS

Handling of animals

This study was approved by the Committee on the Ethics of Animal Experimentation of Kyushu University. Male DS rats were given either a high-salt diet (8% NaCl, DS-H) or a low-salt diet (0.4% NaCl, DS-L) for 6 weeks, beginning at 6 weeks of age.

The systolic blood pressure and heart rate were measured in conscious rats with the tail-cuff method before and at the end of the treatment period. The rats were deeply anesthetized with ether and killed by decapitation. The main branch of the mesenteric artery was excised and bathed in cold Krebs solution having the following composition ($mmol\,l^{-1}$): Na^+, 137.4; K^+, 5.9; Mg^{2+}, 1.2; Ca^{2+}, 2.5; HCO_3^-, 15.5; $H_2PO_4^-$, 1.2; Cl^-, 134 and glucose, 11.5.

Isometric tension recording

Rings with intact endothelium were placed in 5-ml organ chambers filled with 36 °C Krebs solution that had been aerated with 93% O_2–7% CO_2 (pH 7.4). Isometric contractile responses were recorded as described previously.[11] The rings were contracted with norepinephrine (NE, $10^{-5}\,mol\,l^{-1}$), and then the relaxant effects of ACh were studied in the absence or presence of indomethacin ($10^{-5}\,mol\,l^{-1}$) and N^G-nitro-L-arginine (L-NNA, $10^{-4}\,mol\,l^{-1}$).

In some preparations, the rings were contracted with $77\,mmol\,l^{-1}$ KCl solution in the presence of indomethacin ($10^{-5}\,mol\,l^{-1}$), and the relaxation in response to ACh was observed. Relaxations in response to sodium nitroprusside and levcromakalim were studied in rings contracted with NE ($10^{-5}\,mol\,l^{-1}$).

Electrophysiological experiments

Transverse strips were placed in the experimental chamber (2 ml) with the endothelial layer up, and were then superfused with 36 °C Krebs solution aerated with 95% O_2 −5% CO_2 (pH 7.3 −7.4) at a rate of $3\,ml\,min^{-1}$. After equilibration for at least 60 min, the membrane potentials of vascular smooth muscle cells were recorded using a conventional microelectrode technique as described previously.[11] Thereafter, hyperpolarizations to ACh were recorded in the presence of indomethacin ($10^{-5}\,mol\,l^{-1}$) and L-NNA ($10^{-4}\,mol\,l^{-1}$).

Drugs and solutions

The following drugs were used: ACh chloride, NE hydrochloride, indomethacin, L-NNA, sodium nitroprusside, levcromakalim, catalase, iberiotoxin (Sigma, St Lois, MO, USA) and 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE; Cayman Chemical, Ann Arbor, MI, USA). Levcromakalim and 14,15-EEZE were dissolved in ethanol. Indomethacin was dissolved in $10\,mmol\,l^{-1}$ Na_2CO_3. Apocynin was dissolved in dimethyl sulfoxide. The other drugs used were dissolved in distilled water. All drugs were further diluted 1000-fold in Krebs solution to give the final chamber concentrations.

Statistics

Results are given as means ± s.e.m. The concentration–response curves of relaxation were analyzed by two-way analysis of variance followed by Scheffe's test for multiple comparisons. The concentrations of agonists causing half-maximal responses (EC50 value) were also calculated for relaxations using a nonlinear regression analysis. The EC50 values are expressed as the negative logarithm of the molar concentration (pD_2 values). Other variables were analyzed by one-way analysis of variance followed by Scheffe's test for multiple comparisons, or by the paired Student's *t*-test. A level of $P<0.05$ was considered statistically significant.

RESULTS

Systolic blood pressure, heart rate and body weight

The systolic blood pressure, heart rate and body weight of DS-H and DS-L groups are summarized in Table 1. After salt loading, DS-H developed hypertension, while DS-L remained normotensive. After salt loading, the systolic blood pressure was higher in DS-H than in DS-L, and the heart rate tended to be lower in DS-H than in DS-L, though the latter difference did not reach statistical significance. Body weight was lower in DS-H than in DS-L after salt loading.

Endothelium-dependent and endothelium-independent relaxation

In mesenteric arterial rings precontracted with NE ($10^{-5}\,mol\,l^{-1}$), there was no difference in the complete curves of ACh-induced, endothelium-dependent relaxation between DS-H and DS-L compared using two-way analysis of variance, although there was a significant reduction in maximal relaxation in DS-H (Figure 1a, Table 2). After pretreatment with indomethacin ($10^{-5}\,mol\,l^{-1}$) and L-NNA ($10^{-4}\,mol\,l^{-1}$) to inhibit prostanoid and NO production, respectively, ACh-induced, EDHF-like relaxation was significantly greater in DS-H than in DS-L (Figure 1b, Table 2).

When mesenteric arterial rings pretreated with indomethacin ($10^{-5}\,mol\,l^{-1}$) were contracted with $77\,mmol\,l^{-1}$ KCl, to inhibit prostanoid and EDHF production, respectively, ACh-induced, NO-mediated relaxation was significantly smaller in DS-H than in DS-L (Figure 2, Table 2). This relaxation was abolished by further incubation with L-NNA ($10^{-4}\,mol\,l^{-1}$) in both groups ($n=3$ each).

The relaxation in response to sodium nitroprusside, an NO donor, in mesenteric arterial rings precontracted with NE ($10^{-5}\,mol\,l^{-1}$) did not differ between DS-H and DS-L (Figure 3a, Table 2). The levcromakalim-induced relaxation in rings precontracted with NE ($10^{-5}\,mol\,l^{-1}$) was also similar between the two groups (Figure 3b, Table 2).

Effect of IbTx on EDHF-like relaxation

Additional incubation with iberiotoxin (IbTx, $10^{-7}mol\,l^{-1}$), a specific blocker of large-conductance calcium-dependent potassium (BKCa) channels, attenuated ACh-induced, EDHF-like relaxation in DS-H (pD_2 values: DS-H 6.8 ± 0.2 and DS-H + IbTx 6.6 ± 0.4, NS, $n=6$ each. Maximal relaxation: DS-H 65.3 ± 7.8 and DS-H + IbTx 46.2 ± 4.9%, $P<0.01$, $n=6$ each; Figure 4a). By contrast, IbTx did

Table 1 Systolic blood pressure, heart rate and body weight before and after 6 weeks of salt loading

	Blood pressure (mm Hg)		*Heart rate (b.p.m.)*		*Body weight (g)*	
	Before	*After*	*Before*	*After*	*Before*	*After*
DS-H	130 ± 3	225 ± 7*,†	519 ± 9	503 ± 12	187 ± 5	295 ± 11*,†
DS-L	129 ± 4	138 ± 3	527 ± 9	523 ± 7	196 ± 3	351 ± 4*

Abbreviations: DS, Dahl salt-sensitive; DS-H; DS rats fed a high-salt (8% NaCl) diet, DS-L; DS rats fed a low-salt (0.4% NaCl) diet.
Values are mean ± s.e.m. There were 11–13 rats in each group.
*$P<0.05$ *vs.* before.
†$P<0.05$ *vs.* DS-L.

not attenuate ACh-induced, EDHF-like relaxation in DS-L (pD_2 values: DS-L7.1 ± 0.1 and DS-L + IbTx 6.9 ± 0.1, not significant (NS), $n = 5$ each. Maximal relaxation: DS-L 57.0 ± 2.3 and DS-L + IbTx 57.8 ± 7.2%, NS, $n = 5$ each; Figure 4b).

The ACh-induced, EDHF-like relaxation in DS-H was not reduced by 14,15-EEZE (10^{-5} mol l^{-1}), a selective epoxyeicosatrienoic acid (EET) antagonist (pD_2 values: DS-H 7.2 ± 0.1 and DS-H + 14,15-EEZE 6.6 ± 0.3, NS, $n = 4$ each. Maximal relaxation: DS-H 63.3 ± 4.3 and DS-H + 14,15-EEZE 65.3 ± 3.5%, NS, $n = 4$ each). Catalase (2000 U ml^{-1}), a catalyst of H_2O_2 degradation, also failed to inhibit EDHF-like relaxation in DS-H (pD_2 values: DS-H 7.0 ± 0.2 and DS-H + catalase 7.0 ± 0.2, NS, $n = 6$ each. Maximal relaxation: DS-H 72.2 ± 2.3 and DS-H + catalase 76.3 ± 2.3%, NS, $n = 6$ each). Apocynin (10^{-5} mol l^{-1}), an inhibitor of NADPH oxidase, did not inhibit EDHF-like relaxation in DS-H (pD_2 values: DS-H 7.2 ± 0.1 and DS-H + apocynin 7.1 ± 0.1, NS, $n = 3$ each. Maximal relaxation: DS-H 59.3 ± 2.3 and DS-H + apocynin 58.0 ± 1.0%, NS, $n = 3$ each).

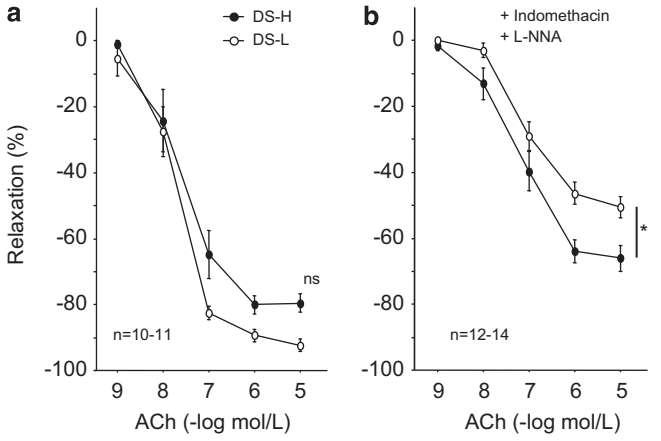

Figure 1 The concentration–response curves of relaxation in response to acetylcholine (ACh) in mesenteric arterial rings precontracted with norepinephrine (10^{-5} mol l^{-1}) in DS rats fed a high-salt diet (8% NaCl, DS-H) or a low-salt diet (0.4% NaCl, DS-L). (**a**) Without indomethacin (10^{-5} mol l^{-1}) and N^G-nitro-L-arginine (10^{-4} mol l^{-1} L-NNA). There was no significant difference in complete curves of relaxation between DS-H and DS-L when compared using two-way analysis of variance; (**b**) in the presence of indomethacin and L-NNA. Values are mean ± s.e.m. $^*P < 0.05$ *vs.* DS-L. Bar at the end of the data indicates statistical significance between the group data.

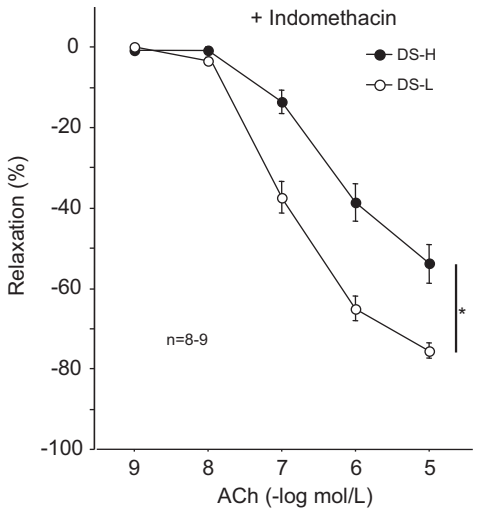

Figure 2 The concentration–response curves of relaxation in response to acetylcholine (ACh) in mesenteric arterial rings precontracted with 77 mmol l^{-1} KCl in Dahl salt-sensitive (DS) rats fed a high-salt diet (8% NaCl, DS-H) or a low-salt diet (0.4% NaCl, DS-L). Indomethacin (10^{-5} mol l^{-1}) was present throughout the experiments. Values are mean ± s.e.m. $^*P < 0.05$ *vs.* DS-L. Bar at the end of the data indicates statistical significance between the group data.

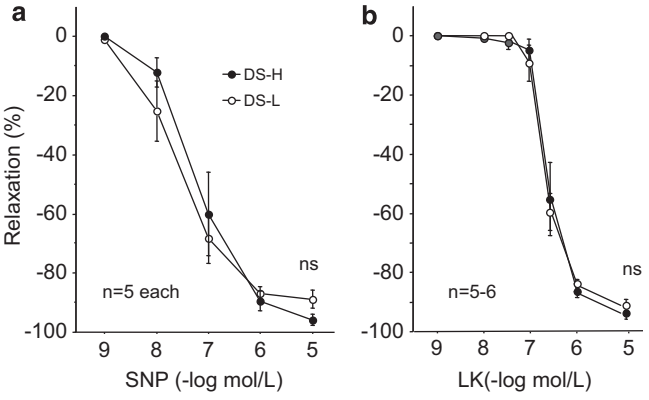

Figure 3 The concentration–response curves of relaxations in response to (**a**) sodium nitroprusside (SNP) and (**b**) levcromakalim (LK) in mesenteric arterial rings precontracted with norepinephrine (10^{-5} mol l^{-1}) in Dahl salt-sensitive (DS) rats fed a high-salt diet (8% NaCl, DS-H) or a low-salt diet (0.4% NaCl, DS-L). Values are mean ± s.e.m.

Table 2 Relaxation to ACh, sodium nitroprusside and levcromakalim in mesenteric arteries of DS rats

	Acetylcholine		*Acetylcholine (EDHF)* + *Indomethacin* + *L-NNA*		*Acetylcholine (NO)* + *Indomethacin*		*Sodium Nitroprusside*		*Levcromakalim*	
	pD_2	*Max, %*	pD_2	*Max, %*	pD_2	*Max, %*	pD_2	*Max, %*	pD_2	*Max, %*
DS-H	7.7 ± 0.3	80.5 ± 2.8*	7.3 ± 0.2	66.2 ± 4.0*	6.4 ± 0.1*	53.9 ± 4.8*	7.2 ± 0.2	97.6 ± 1.6	6.5 ± 0.1	93.4 ± 2.3
DS-L	7.8 ± 0.1	92.4 ± 2.0	7.0 ± 0.1	50.4 ± 4.0	7.0 ± 0.1	75.4 ± 1.8	7.5 ± 0.2	91.4 ± 3.1	6.6 ± 0.1	90.8 ± 1.9

Abbreviations: ACh, acetylcholine; DS, Dahl salt-sensitive; DS-H; DS rats fed a high-salt (8% NaCl) diet, DS-L; DS rats fed a low-salt (0.4% NaCl) diet; EDHF, endothelium-derived hyperpolarizing factor; L-NNA, N^G-nitro-L-arginine; NO, nitric oxide.
Values are mean ± s.e.m. Max indicates maximal relaxation to drugs. pD2 indicates negative logarithm of molar concentration of drug causing half-maximal relaxation. EDHF-like relaxation and NO-mediated relaxation were assessed in rings contracted with norepinephrine (10^{-5} mol l^{-1}) and 77 mmol l^{-1} KCl, respectively.
$^*P < 0.05$ *vs.* DS-L

Endothelium-dependent hyperpolarization in mesenteric arteries
ACh-induced, EDHF-mediated hyperpolarization was studied in the presence of indomethacin (10^{-5} mol l^{-1}) and L-NNA (10^{-4} mol l^{-1}) to abolish the influence of prostanoids and NO, respectively. After salt loading, the resting membrane potential of the mesenteric artery did not differ between DS-H and DS-L (DS-H 44.1 ± 1.1 and DS-L 45.8 ± 0.7 mV, NS, $n = 8$–10). There was no significant difference in ACh-induced, EDHF-mediated hyperpolarization in the resting state of the membrane between the two groups (Figure 5). Additional incubation with IbTx (10^{-7} mol l^{-1}) marginally inhibited ACh (10^{-5} mol l^{-1})-induced, EDHF-mediated hyperpolarization in DS-H but not in DS-L (Figure 5).

DISCUSSION

This study demonstrated that EDHF-like relaxation is upregulated in mesenteric arteries of DS rats fed a high-salt diet compared with those fed a low-salt diet. Inhibition of BKCa channels with IbTx eliminated the difference in EDHF-like relaxation between the two groups. These findings suggest that the activation of BKCa channels underpins the upregulation of EDHF-like relaxation in DS rats fed a high-salt diet. On the other hand, NO-mediated relaxation was reduced in DS rats fed a high-salt diet compared with those fed a low-salt diet. As the overall relaxation in response to ACh did not differ between the groups, upregulation of EDHF via the activation of BKCa channels appears to compensate for the loss of NO in mesenteric arteries of DS rats fed a high-salt diet.

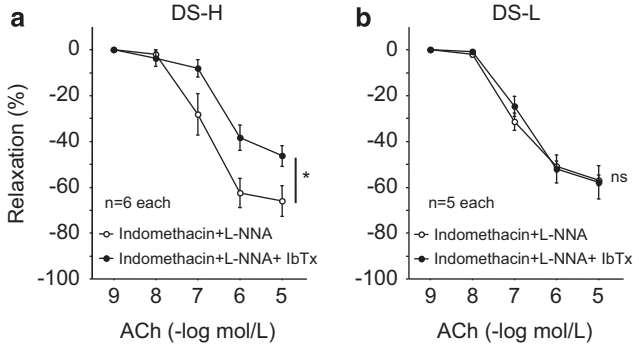

Figure 4 Effect of iberiotoxin (IbTx, 10^{-7} mol l^{-1}) on acetylcholine (ACh)-induced relaxations in mesenteric arterial rings precontracted with norepinephrine (10^{-5} mol l^{-1}) in (**a**) Dahl salt-sensitive (DS) rats fed a high-salt diet (8% NaCl, DS-H) and (**b**) DS rats fed a low-salt diet (0.4% NaCl, DS-L). Indomethacin (10^{-5} mol l^{-1}) and N^{G}-nitro-L-arginine (L-NNA, 10^{-4} mol l^{-1}) were present throughout the experiments. Values are mean ± s.e.m. $^{*}P<0.05$ *vs.* indomethacin + L-NNA. Bar at the end of the data indicates statistical significance between the group data.

In rat mesenteric arteries NO-mediated relaxation can be assessed by the relaxation in response to ACh in KCl-contracted rings in which EDHF-mediated hyperpolarization is absent.[11] In this study, NO-mediated relaxation was reduced in mesenteric arteries of DS rats fed a high-salt diet compared with those fed a low-salt diet. These findings are consistent with those of previous studies[20,23] and can be ascribed to increased generation of oxygen-derived free radicals in salt-sensitive hypertension.[20] Reduced endothelial NO synthase expression[24] and/or endothelial NO synthase uncoupling[25] might also relate to impaired NO-mediated relaxation in the mesenteric arteries of DS rats fed a high-salt diet.

Despite reduced NO-mediated vasodilation, ACh-induced, endothelium-dependent dilation was preserved in mesenteric arteries of DS rats fed a high-salt diet in this study. These results are in agreement with several previous studies demonstrating unaltered vasodilatory response to ACh in salt-induced hypertension despite the impairment of the NO pathway.[17,20] Although these results indicate that some compensatory mechanisms exist to maintain the vasodilatory response to ACh in salt-induced hypertension, few studies have shed light on the underlying mechanisms of this compensation.

ACh-induced relaxation resistant to the combined blockade of cyclooxygenase and NO synthase can be attributable to EDHF.[2–6] In contrast to the reduced NO-mediated relaxation, ACh-induced, EDHF-like relaxation was upregulated in mesenteric arteries of DS rats fed a high-salt diet in this study. As the overall endothelium-dependent vasodilation to ACh was preserved in the mesenteric arteries of DS rats fed a high-salt diet, it appears that upregulation of EDHF compensates for the loss of NO. This upregulation of EDHF

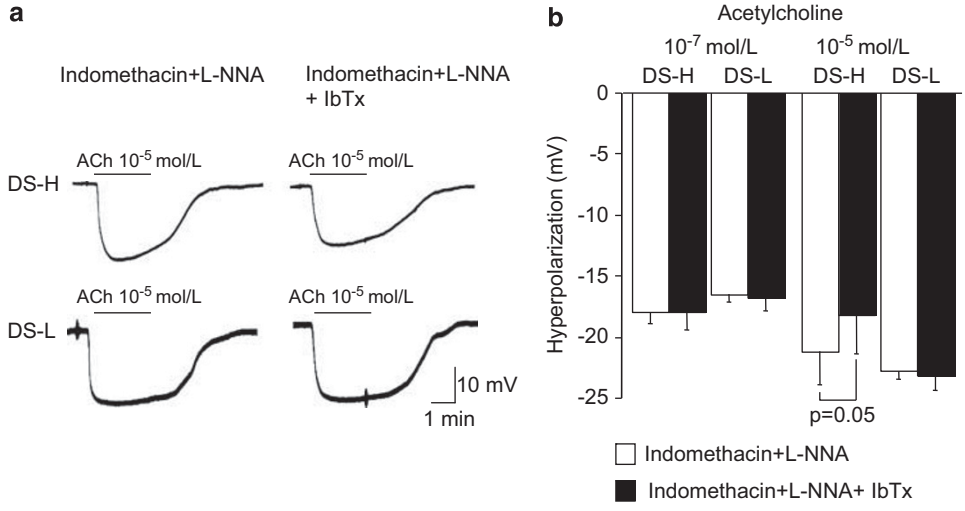

Figure 5 (**a**) Representative tracigs and (**b**) summarized data of the effect of iberiotoxin (IbTx, 10^{-7} mol l^{-1}) on ACh-induced hyperpolarization in mesenteric arteries of Dahl salt-sensitive (DS) rats fed a high-salt diet (8% NaCl, DS-H) and DS rats fed a low-salt diet (0.4% NaCl, DS-L). ACh was applied under resting conditions in the presence of indomethacin (10^{-5} mol l^{-1}) and L-NNA (10^{-4} mol l^{-1}). Values are mean ± s.e.m. There are four to five rats in each group.

was not because of a change in the responsiveness of smooth muscle cells as the relaxations induced by both sodium nitroprusside and levcromakalim were comparable between the two groups.

In the mesenteric arteries of the DS rats fed a high-salt diet in this study, IbTx had a substantial inhibitory effect on EDHF-like relaxation, suggesting the compensatory involvement of BKCa channels in EDHF-like relaxation in salt-induced hypertension. Although Sofola *et al.*[17] have previously reported that a high-salt diet augments EDHF-like relaxation, our present findings for the first time revealed the mechanism underlying the upregulation of EDHF in salt-induced hypertension. However, the mechanism by which a high-salt diet induces BKCa channel involvement in EDHF-mediated response could not be discerned from this results. Although it has been reported that NO hyperpolarizes and relaxes arterial smooth muscle cells through the opening of potassium channels in certain vascular beds,[26] the activation of BKCa by NO is unlikely in this study as we and others have previously shown that hyperpolarization produced by ACh is not mediated by endogenous NO in rat mesenteric arteries.[11,27] Likewise, the activation of BKCa by H_2O_2,[28] a candidate for EDHF,[29] or by other reactive oxygen species is unlikely as we found no significant effect of catalase or apocynin on EDHF-like relaxation in mesenteric arteries of DS rats fed a high-salt diet.

Recently, Gao *et al.*[30] demonstrated that a high-salt diet upregulates transient receptor potential vanilloid type 4 channel expression in mesenteric arteries of DS rats. It has also been reported that EETs, another candidate for EDHF,[31] stimulate the transient receptor potential vanilloid type 4 channels, which increases the calcium influx, and subsequently activates BKCa channels in rat smooth muscle cells.[32] Taken together, it is plausible to speculate that enhanced EDHF-like relaxation in the mesenteric arteries of DS rats fed a high-salt diet could be attributable to an enhanced EETs/transient receptor potential vanilloid type 4/BKCa signaling pathway. However, 14,15-EEZE, an inhibitor of the action of EETs, was without effect on EDHF-like relaxation in this study. Although the mediator activating BKCa channels in mesenteric arteries of DS rats fed a high-salt diet remains unclear, a mediator other than H_2O_2 or EETs might activate smooth muscle BKCa channels. Indeed, the contribution of H_2O_2- and EET-independent BKCa channels to EDHF-like relaxation has been reported in resistant mesenteric arteries of 11- to 12-month-old SHR.[14] Alternatively, the expression and/or function of BKCa channels might be increased in the mesenteric arteries of DS rats fed a high-salt diet. Further studies are needed to elucidate the mechanism by which a high-salt diet activates BKCa channels.

Despite the upregulation of EDHF-like relaxation in DS rats fed a high-salt diet, ACh-induced, EDHF-mediated hyperpolarization *per se* did not differ between the two groups, although IbTX marginally inhibited EDHF-mediated hyperpolarization in the high-salt diet group. Such a discrepancy between EDHF-like relaxation and EDHF-mediated hyperpolarization has also been reported in renal arteries of 2-month-old SHR.[18] The apparent mismatch in the data may result from differences in experimental methodologies. The vasorelaxant effects of ACh were studied in samples preconstricted with NE, whereas the membrane potentials were recorded in the absence of NE. As BKCa channel activity is facilitated when depolarizing voltages are applied and when the intracellular calcium concentration is increased,[33] the application of NE would favor the open probability of BKCa channels and thus would increase the BKCa channel contribution to EDHF-like relaxation in mesenteric arteries of DS rats fed a high-salt diet.

Our present electrophysiological data are in disagreement with our previous findings of impaired EDHF-mediated hyperpolarization in isolated mesenteric arteries of DS rats fed a high-salt diet.[34] In the previous study by Onaka *et al.*,[34] EDHF-mediated hyperpolarization was significantly reduced in mesenteric arteries of DS rats fed a high-salt diet compared with those fed a low-salt diet. One possible explanation for this discrepancy is the difference in the duration of salt loading. In our previous study, the rats were treated for 8 weeks,[34] whereas the duration of salt loading in this study was 6 weeks. The longer duration of salt loading might alter the properties of arterial ion channels and therefore reduce the EDHF-mediated hyperpolarization. Indeed, after 7 weeks of treatment, the membrane potential was significantly depolarized in mesenteric arteries of DS rats fed a high-salt diet compared with those fed a low-salt diet.[35] By contrast, no difference was found in the resting membrane potential between high- and low-salt diet groups in this study. Abel *et al.*[36] have also reported that a high-salt diet for 5 weeks did not alter membrane potentials in the caudal arteries of DS rats. Another possibility is that the development of heart failure could affect EDHF-mediated hyperpolarization in mesenteric arteries of DS rats fed a high-salt diet. Klotz *et al.*[37] reported that DS rats began to develop heart failure after 5 weeks of treatment with a high-salt diet (8% NaCl). Interestingly, it has been reported that ACh-induced, EDHF-like relaxation was reduced in mesenteric arteries of rats with chronic heart failure.[38] Thus, taken together, it is possible to speculate that the reduced EDHF-mediated hyperpolarization in DS rats fed a high-salt diet for 8 weeks is associated with the development of heart failure.

The EDHF system does exist in human arteries.[39–42] Although few studies have investigated the role of EDHF in human hypertension, Taddei *et al.*[41] have reported that EDHF partially compensates for the loss of NO to sustain endothelium-dependent vasodilation in patients with essential hypertension. With regard to salt-sensitive hypertensive patients, several studies have reported that impairment of the NO pathway is associated with endothelial dysfunction;[43,44] however, the role of EDHF in endothelial function in salt-sensitive hypertensive individuals remains unresolved and thus warrants further investigation.

In conclusion, EDHF-like relaxation is upregulated through the activation of BKCa in the mesenteric arteries of DS rats fed a high-salt diet. The upregulation of EDHF compensated for the loss of NO to maintain overall endothelial function in the early phase of salt-induced hypertension. The clinical relevance of our findings remains to be determined.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported in part by a Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (21890233), Tokyo, Japan.

1 Ignarro LJ. Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. *Circ Res* 1989; **65**: 1–21.
2 Feletou M, Vanhoutte PM. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br J Pharmacol* 1988; **93**: 515–524.
3 Chen G, Suzuki H, Weston AH. Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. *Br J Pharmacol* 1988; **95**: 1165–1174.
4 Hill CE, Phillips JK, Sandow SL. Heterogenous control of blood flow amongst different vascular beds. *Med Res Rev* 2001; **21**: 1–60.
5 Sandow SL. Factors, fiction and endothelium-derived hyperpolarizing factor. *Clin Exp Pharmacol Physiol* 2004; **31**: 563–570.
6 Dora KA. Coordination of vasomotor responses by the endothelium. *Circ J* 2010; **74**: 226–232.

7 Vanhoutte PM. Endothelial dysfunction in hypertension: the first step toward coronary arteriosclerosis. *Circ J* 2009; **73**: 595–601.
8 Boulanger CM. Secondary endothelial dysfunction: hypertension and heart failure. *J Mol Cell Cardiol* 1999; **31**: 39–49.
9 Goto K, Fujii K, Kansui Y, Iida M. Changes in endothelium-derived hyperpolarizing factor in hypertension and ageing: response to chronic treatment with renin–angiotensin system inhibitors. *Clin Exp Pharmacol Physiol* 2004; **31**: 650–655.
10 Feletou M, Vanhoutte PM. EDHF: new therapeutic targets? *Pharmacol Res* 2004; **49**: 565–580.
11 Fujii K, Tominaga M, Ohmori S, Kobayashi K, Koga T, Takata Y, Fujishima M. Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Circ Res* 1992; **70**: 660–669.
12 Kansui Y, Fujii K, Nakamura K, Goto K, Oniki H, Abe I, Shibata Y, Iida M. Angiotensin II receptor blockade corrects altered expression of gap junctions in vascular endothelial cells from hypertensive rats. *Am J Physiol Heart Circ Physiol* 2004; **287**: H216–H224.
13 Goto K, Rummery NM, Grayson TH, Hill CE. Attenuation of comducted vasodilation in rat mesenteric arteries during hypertension: role of inwardly rectifying potassium channels. *J Physiol* 2004; **561**: 215–231.
14 Ellis A, Goto K, Chaston DJ, Brackenbury TD, Meaney KR, Falck JR, Wojcikiewicz RJ, Hill CE. Enalapril treatment alters the contribution of epoxyeicosatrienoic acids but not gap junctions to endothelium-derived hyperpolarizing factor activity in mesenteric arteries of spontaneously hypertensive rats. *J Pharmacol Exp Ther* 2009; **330**: 413–422.
15 Goto K, Edwards FR, Hill CE. Depolarization evoked by acetylcholine in mesenteric arteries of hypertensive rats attenuates endothelium-dependent hyperpolarizing factor. *J Hypertens* 2007; **25**: 345–359.
16 Weston AH, Porter EL, Harno E, Edwards G. Impairment of endothelial SK(Ca) channels and of downstream hyperpolarizing pathways in mesenteric arteries from spontaneously hypertensive rats. *Br J Pharmacol* 2010; **160**: 836–843.
17 Sofola OA, Knill A, Hainsworth R, Drinkhill M. Change in endothelial function inmesenteric arteries of Sprague-Dawley rats fed a high salt diet. *J Physiol* 2002; **543**: 255–260.
18 Büssemaker E, Popp R, Fisslthaler B, Larson CM, Fleming I, Busse R, Brandes RP. Aged spontaneously hypertensive rats exhibit a selective loss of EDHF-like relaxation in the renal artery. *Hypertension* 2003; **42**: 562–568.
19 Vanhoutte PM. Endothelial dysfunction and atherosclerosis. *Eur Heart J* 1997; **18**: E19–E29.
20 Boegehold MA. Reduced influence of nitric oxide on arteriolar tone in hypertensive Dahl rats. *Hypertension* 1992; **19**: 290–295.
21 Rusch NJ, Liu Y, Pleyte KA. Mechanisms for regulation of arterial tone by Ca2+ dependent K+ channels in hypertension. *Clin Exp Pharmacol Physiol* 1996; **23**: 1077–1081.
22 Kamouchi M, Kitazono T, Nagao T, Fujishima M, Ibayashi S. Role of CA(2+)-activated K+ channels in the regulation of basilar arterial tone in spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol* 2002; **29**: 575–581.
23 Boegehold MA. Microvascular structure and function in salt-sensitive hypertension. *Microcirculation* 2002; **9**: 225–241.
24 Ni Z, Oveisi F, Vaziri ND. Nitric oxide synthase isotype expression in salt-sensitive and salt-resistant dahl rats. *Hypertension* 1999; **34**: 552–557.
25 Yamamoto E, Kataoka K, Shintaku H, Yamashita T, Tokutomi Y, Dong YF, Matsuba S, Ichijo H, Ogawa H, Kim-Mitsuyama S. Novel mechanism and role of angiotensin II-induced vascular endothelial injury in hypertensive diastolic heart failure. *Arterioscler Thromb Vasc Biol* 2007; **27**: 2569–2575.
26 Tare M, Parkington HC, Coleman HA, Neild TO, Dusting GJ. Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from the endothelium. *Nature* 1990; **346**: 69–71.
27 Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A. Sources of Ca2+ in relation to generation of acetylcholine-induced endothelium-dependent hyperpolarization in rat-mesenteric artery.. *Br J Pharmacol* 1997; **120**: 1328–1334.
28 Barlow RS, White RE. Hydrogen peroxide relaxes porcine coronary arteries by stimulating BKCa channel activity. *Am J Physiol Heart Circ Physiol* 1998; **275**: H1283–H1289.
29 Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J Clin Invest* 2000; **106**: 1521–1530.
30 Gao F, Sui D, Garavito RM, Worden RM, Wang DH. Salt intake augments hypotensive effects of transient receptor potential vanilloid 4. Functional significance and implication. *Hypertension* 2009; **53**: 228–235.
31 Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ Res* 1996; **78**: 415–423.
32 Earley S, Heppner TJ, Nelson MT, Brayden JE. TRPV4 forms a novel Ca2+ signaling complex with ryanodine receptors and BKCa channels. *Circ Res* 2005; **97**: 1270–1279.
33 Standen NB, Quayle JM. K^+ channel modulation in arterial smooth muscle. *Acta Physiol Scand* 1998; **164**: 549–557.
34 Onaka U, Fujii K, Abe I, Fujishima M. Antihypertensive therapy improves endothelium-dependent hyperpolarization. In: Vanhoutt PM (editor) *Endothelium-Dependent Hyperpolarization*. Harwood Academic Publishers, Amsterdam, 1999: 305–312.
35 Fujii K, Onaka U, Ohya U, Ohmori S, Tominaga M, Abe I, Takata Y, Fujishima M. Role of eicosanoids in alteration of membrane electrical properties in isolated mesenteric arteries of salt-loaded Dahl salt-sensitive rats. .*Br J Pharmacol* 1997; **120**: 1207–1214.
36 Abel PW, Trapani A, Matsuki N, Ingram MJ, Ingram FD, Hermsmeyer K. Unaltered membrane properties of arterial muscle in Dahl strain genetic hypertension. *Am J Physiol Heart Circ Physiol* 1981; **241**: H224–H227.
37 Klotz S, Hay I, Zhang G, Maurer M, Wang J, Burkhoff D. Development of heart failure in chronic hypertensive dahl rats: focus on heart failure with preserved ejection fraction. *Hypertension* 2006; **47**: 901–911.
38 Xu Y, Henning RH, Lipsic E, van Buiten A, van Gilst WH, Buikema H. Acetylcholine stimulated dilatation and stretch induced myogenic constriction in mesenteric artery of rats with chronic heart failure. *Eur J Heart Fail* 2007; **9**: 144–151.
39 Nakashima M, Mombouli JV, Taylor AA, Vanhoutte PM. Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries. *J Clin Invest* 1993; **92**: 2867–2871.
40 Urakami-Harasawa L, Shimokawa H, Nakashima M, Egashira K, Takeshita A. Importance of endothelium-derived hyperpolarizing factor in human arteries. *J Clin Invest* 1997; **100**: 2793–2799.
41 Taddei S, Versari D, Cipriano A, Ghiadoni L, Galetta F, Franzoni F, Magagna A, Virdis A, Salvetti A. Identification of a cytochrome P450 2C9-derived endothelium-derived hyperpolarizing factor in essential hypertensive patients. *J Am Coll Cardiol* 2006; **48**: 508–515.
42 Chadha PS, Liu L, Rikard-Bell M, Senadheera S, Howitt L, Bertrand RL, Grayson TH, Murphy TV, Sandow SL. Endothelium-dependent vasodilation in human mesenteric artery is primarily mediated by myoendothelial gap junctions intermediate conductance calcium-activated. K^+ channel and nitric oxide. *J Pharmacol Exp Ther* 2011; **336**: 701–708.
43 Higashi Y, Oshima T, Watanabe M, Matsuura H, Kajiyama G. Renal response to L-arginine in salt-sensitive patients with essential hypertension. *Hypertension* 1996; **27**: 643–648.
44 Bragulat E, De la Sierra A, Antonio MT, Coca A. Endothelial dysfunction in salt-sensitive essential hypertension. *Hypertension* 2001; **37**: 444–448.