Endothelium-Derived Contracting Factor in Carotid Artery of Hypertensive Dahl Rats

Ming-Sheng Zhou, Yasuhiro Nishida, Qing-Hui Chen, Hiroaki Kosaka

Abstract—The present study is designed to investigate whether acetylcholine (ACh) elicits an endothelium-derived contracting factor (EDCF) and whether it contributes to decreased relaxant response induced by ACh in Dahl rats. Dahl salt-sensitive (DS) and -resistant (DR) rats were fed a 0.4% NaCl or an 8% NaCl diet for 4 weeks. High sodium intake significantly increased blood pressure in DS rats but not in DR rats. The carotid rings were suspended for isometric tension recording. ACh caused an endothelium-dependent contraction in carotid rings from hypertensive DS rats but not from normotensive Dahl rats. Atropine, indomethacin, SQ29548, or ONO-3708 (prostaglandin H₂ [PGH₂]/thromboxane A₂ [TXA₂] receptor antagonist) abolished ACh-induced contraction, and OKY-046 (inhibitor of TXA₂ synthetase) partially attenuated the contraction. High sodium intake significantly enhanced contraction evoked by U46619, a PGH₂/TXA₂ receptor agonist, in both DS and DR rats. In contrast, ACh-induced relaxation was significantly depressed in the rings from hypertensive DS rats, and ONO-3708 partially improved the depressed relaxation. Administration of ONO-8809 (an orally active PGH₂/TXA₂ receptor antagonist; 30 μg per body per day) for 4 weeks neither reduced blood pressure nor improved the depressed ACh-induced relaxation in hypertensive DS rats. These results suggest that ACh causes release of EDCF in carotid rings of hypertensive DS rats, which is likely to be PGH₂ and TXA₂. The EDCF contributed in part to the depressed ACh-induced relaxation. (Hypertension. 1999;34:39-43.)

Key Words: acetylcholine ■ arteries ■ rats, Dahl ■ endothelium-derived relaxing factor ■ hypertension, sodium dependent ■ receptors ■ prostaglandin ■ thromboxane

Endothelial cells modulate vascular smooth muscle tone through synthesis and release of endothelium-derived relaxing factor (EDRF) and endothelium-derived contracting factor (EDCF). The main component of EDRF has been identified to be nitric oxide. Increased production of EDCF has been reported in anoxia, hypertension, aging, and diabetes. Thromboxane A₂ (TXA₂) and prostaglandin H₂ (PGH₂) have been reported to be EDCF in spontaneously hypertensive rats (SHR). In the aorta of SHR, low concentrations of acetylcholine (ACh) induced an endothelium-dependent relaxation that was comparable to that observed in the aorta from normotensive control rats. However, relaxation to higher concentrations of ACh was attenuated in SHR because of concomitant release of an EDCF.

Salt-induced hypertension is associated with abnormality of vascular reactivity. Controversy exists regarding the release of EDCF in deoxycorticosterone acetate (DOCA) salt hypertension. Fortes et al showed that indomethacin corrected the decrease in relaxant response to ACh observed in the mesenteric arterioles of DOCA hypertensive rats. In contrast, Makynen et al reported that indomethacin did not affect relaxation induced by ACh in the mesenteric artery of DOCA hypertensive rats. Impaired ACh-induced relaxation has been reported in the aorta of hypertensive Dahl salt-sensitive rats (DS-HT). To our knowledge, there is no report that EDCF occurs in DS-HT.

The present study is thus designed to determine whether ACh elicits an EDCF and to clarify the mechanism underlying these decreased endothelium-dependent relaxations in the carotid rings of DS-HT. Next, we investigate whether oral administration of EDCF antagonist attenuates development of hypertension in DS-HT.

Methods

Tissue Bath Preparation

Male 6-week-old DS and Dahl salt-resistant (DR) rats were purchased from Yoshitomi Seyaku (Osaka, Japan). Both DS and DR rats were fed an 8% NaCl or a 0.4% NaCl diet for 4 weeks. Systolic arterial pressure (SAP) was measured in awake rats by the tail-cuff method (PS-100, Riken). SAP was measured in the morning in a quiet environment, and the average of 3 successive readings was recorded.

The carotid artery was removed and immediately placed into ice-cold modified Krebs-Ringer bicarbonate solution (composition in mmol/L: NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, glucose 11.1). The vessels were cleaned of adherent connective tissue, and the rings (3 mm) were cut. In some rings, the endothelium was removed mechanically by gentle rubbing of the intimate surface with a hair. A ring was mounted vertically between 2 stirrups in an organ bath with 10 mL of Krebs’ solution (37°C, with
95% O₂, 5% CO₂. One stirrup was fixed to the floor of organ bath, and the other was connected to an isometric force transducer (Randonoti). The rings were equilibrated under a resting tension of 1 g for at least 90 minutes and were exposed 2 or 3 times to 100 mmol/L KCl solution at 30-minute intervals.

Contraction and Relaxation Induced by ACh
Concentration-response curves to ACh were obtained in the quiescent rings with or without endothelium in the absence or presence of 100 μmol/L Nω-nitro-L-arginine methyl ester (L-NAME, a nitric oxide synthetase inhibitor). To identify EDCF, we studied the effects of various blocking agents on ACh-induced contraction in DS-HT. After contractile response to ACh was elicited in the presence of 100 μmol/L L-NAME, 1 μmol/L atropine (muscarinic receptor antagonist), 10 μmol/L indomethacin (inhibitor of cyclooxygenase), 10 μmol/L OKY-046 (thromboxane synthetase inhibitor), 1 μmol/L ONO-3708, or 1 μmol/L SQ29548 (PGH₂/ TXA₂ receptor antagonist) together with 100 μmol/L L-NAME was added to the organ bath 30 minutes before the second application of ACh.

In the endothelium-intact rings precontracted with 3 μmol/L serotonin, concentration-response curves to ACh were cumulatively determined in both DS and DR rats. The responses were also studied in the presence of 1 μmol/L ONO-3708.

ACh-induced contraction and relaxation were studied in the DS rats treated with an 8% NaCl diet and 30 μg per body per day ONO-8809 (an orally active TXA₂/PGR₁ receptor antagonist) for 4 weeks.

Contractions to Exogenous Prostaglandins
Contractions evoked by prostaglandin E₂ (PGE₂), PGF₂α, and U46619 (a stable analogue of TXA₂) were studied in both DS and DR rats. The responses were also studied in DS rats treated with 8% NaCl diet and ONO-8809.

Effects of Oral Administration of ONO-8809 on Blood Pressure
Ten DS rats were divided in random order into 2 groups: control group (rats fed an 8% NaCl diet for 4 weeks) and treated group (rats fed an 8% NaCl diet and 30 μg per body per day ONO-8809 for 4 weeks). SAP was weekly measured by the tail-cuff method in the awake rats. At the end of week 4, mean arterial pressure (MAP) was measured in the conscious rat as described previously. In brief, the left femoral artery and vein were cannulated. Three days after surgery, the arterial catheter was connected to a pressure transducer (DX-360, Nihon Koden). After an equilibration period of 30 minutes, MAP was noted, and then depressor response to bolus injections of ACh (1, 3, 10, and 30 μg/kg body wt) was elicited in each rat. The reduction in MAP was calculated from peak height.

Drugs
The following drugs from Sigma Chemical Co were used: 5-hydroxytryptamine creatinine sulfate, ACh hydrochloride, L-NAME, indomethacin, atropine sulfate, PGE₂, PGF₂α, U46619, and SQ29548. ONO Pharmaceutical Co provided OKY-046, ONO-3708, and ONO-8809. All concentrations of drugs used in vitro are expressed as final molar concentration.

Calculations and Statistical Analysis
The contraction for the in vitro experiment was expressed as a percentage of the contraction developed by 100 mmol/L KCl solution. In relaxant experiment, the rings were precontracted with 3 μmol/L serotonin, and the results are expressed as percent inhibition of the contraction. All data are expressed as mean±SEM, with P<0.05 considered significant. All statistical analyses were performed with a commercially available statistical package for the Macintosh personal computer (StatView-J, version 4.11, and Super-ANOVA, version 1.11, Abacus Concepts). Statistical analysis was performed with the use of ANOVA or Student’s t test.

Results
Blood Pressure
DS rats fed an 8% NaCl diet caused a significant increase in SAP (215±3 mm Hg; n=20) compared with DS rats fed a 0.4% NaCl diet (148±2 mm Hg; n=8). There was no significant difference in SAP between DR rats fed an 8% NaCl diet (136±6 mm Hg; n=6) and a 0.4% NaCl diet (135±2 mm Hg; n=6).

Endothelium-Dependent Contraction Induced by ACh
Ach 10⁻⁵ to 10⁻⁴ mol/L evoked a concentration-dependent contraction in endothelium-intact (Figures 1A and 2A) but not in endothelium-denuded rings from DS-HT (Figure 1B). The contraction was augmented in the presence of L-NAME (Figure 2B). ACh did not produce contraction response in the rings from normotensive DS or DR rats regardless of the presence of L-NAME (Figure 2).

The rings were pretreated with L-NAME to augment the contraction. Atropine (1 μmol/L), indomethacin (10 μmol/L), ONO-3708 (1 μmol/L), or SQ29548 (1 μmol/L) abolished ACh-induced contraction in the rings from DS-HT. OKY-046
(10 μmol/L) significantly attenuated the contraction. Oral administration of ONO-8809 slightly but significantly reduced ACh-induced contraction (Figure 3).

**Endothelium-Dependent Relaxation Induced by ACh**

In the rings precontracted with 3 μmol/L serotonin, ACh caused a relaxant response in all groups (Figures 1C to 1F and 4). The relaxation induced by $10^{-7}$ to $10^{-4}$ mol/L ACh was significantly depressed in the rings from DS-HT compared with those from normotensive DS rats. ONO-3708 1 μmol/L significantly increased but did not fully recover the relaxation induced by $10^{-7}$ to $10^{-4}$ mol/L ACh in the rings of DS-HT. ONO-3708 did not affect the relaxation in the rings from DR or from normotensive DS rats. Oral administration of ONO-8809 did not improve the depression of ACh-induced relaxation.

**Contractions Induced by Exogenous Prostaglandins**

U46619 evoked a dose-dependent contraction response in all groups (Figure 5A). High sodium intake significantly enhanced the response in both DS and DR rats. However, there was no significant difference in the contraction response between the 2 rat strains fed a diet of equivalent sodium. ONO-3708 significantly inhibited the contraction response evoked by U46619. Oral administration of ONO-8809 significantly attenuated enhanced contraction response evoked by U46619.

PGE$_2$ and PGF$_{2α}$ evoked a significant contraction response in both DS and DR rats fed a high sodium diet but not in the rats fed a normal sodium diet (Figures 5B and 5C). The contraction response was higher in DS rats fed a high sodium diet than in DR rats fed a high sodium diet. The maximal contractions evoked by $10^{-6}$ mol/L PGE$_2$ and $10^{-6}$ mol/L PGF$_{2α}$ in DS-HT were 35±6% and 42±7% of the contraction evoked by 100 mmol/L KCl, respectively.
Effect of ONO-8809 on Blood Pressure in Vivo

ONO-8809 did not significantly reduce SAP obtained by the tail-cuff method in DS-HT throughout weeks 1 to 4 (n = 5; data not shown). MAP measured directly through an arterial catheter in conscious rats was similar in the treated (139 ± 7 mm Hg; n = 5) and control groups (147 ± 5 mm Hg; n = 5).

A bolus injection of ACh (1, 3, 10, and 30 µg/kg body wt) caused a dose-dependent reduction in MAP in ONO-8809–treated and control groups. No significant difference of reduction in MAP was present between the 2 groups. A bolus injection of ACh (30 µg/kg body wt) reduced MAP by 64 ± 3 mm Hg in the ONO-8809–treated group (n = 5) and by 62 ± 6 mm Hg in the control group (n = 5).

Discussion

The present study demonstrated that ACh produced an endothelium-dependent contraction in the carotid rings of DS-HT but not in those of normotensive Dahl rats. To our knowledge, this is the first report that EDCF exists in DS-HT. Nitric oxide may inhibit the ACh-induced contraction, as L-NAME augmented the contraction. We also demonstrated that ACh-induced contraction is an endothelium-dependent reaction through muscarinic receptor in endothelial cells of DS-HT.

Several reports suggest that EDCF is a substance in the cyclooxygenase system produced and released in the state of hypertension. The present study showed that ACh induced an endothelium-dependent contraction in the carotid rings from DS-HT. The contraction was abolished by indomethacin, indicating that EDCF was an arachidonic acid metabolite product. Furthermore, PGH₂/TXA₂ receptor antagonist ONO-3708 or SQ29548 completely inhibited ACh-induced contraction. The TXA₂ synthetase inhibitor OKY-046 also attenuated ACh-induced contraction. However, OKY-046 was less effective than indomethacin, ONO-3708, or SQ29548. Thus, both PGH₂ and TXA₂ will operate as EDCF in the carotid artery of DS-HT.

Our results showed that a high sodium diet significantly enhanced the contraction evoked by PGE₂ or PGF₂α in both DS and DR rats, which was higher in DS rats than in DR rats. Tension developed by 10⁻⁶ mol/L PGE₂ or 10⁻⁶ mol/L PGF₂α was similar to that developed by 10⁻⁴ mol/L ACh in DS-HT.

Recently, some argued that prostaglandins other than PGH₂ may contribute to ACh-induced contraction in the aorta of SHR. ONO-3708 (1 µmol/L) has been reported to antagonize PGE₂ or PGF₂α, receptor activation. Thus, other prostaglandins such as PGE₂ or PGF₂α may be EDCF in DS-HT. However, this is less likely because 1 µmol/L SQ29548 abolished ACh-induced contraction. SQ29548 was regarded not to antagonize PGE₂ or PGF₂α receptor activity.

ACh-induced contraction can be explained either by increased production and/or release of EDCF from endothelial cells or by hypersensitivity to EDCF in the smooth muscle of DS-HT. Our results demonstrated that increased EDCF production and release from endothelial cells were mainly responsible for ACh-induced contraction in DS-HT. This conclusion is based on the appearance of ACh-induced contractions only in DS-HT but not in other Dahl rats and on the similarity of the response to U46619 in DS and DR rats fed a diet of equivalent salt. The present study also indicated that high sodium intake significantly increased sensitivity to EDCF in the smooth muscle of both DS and DR rats.

The present study revealed that the relaxant response induced by ACh was significantly weaker in the rings from DS-HT than in those from normotensive DS rats. ONO-3708 partially improved ACh-induced relaxation in the rings of DS-HT. The results suggest that ACh stimulates the carotid endothelial cells to release EDCF and EDRF in DS-HT. The EDCF released will weaken EDRF-induced relaxation. However, ONO-3708 did not fully recover ACh-induced relaxation in DS-HT, indicating that depression of ACh-induced relaxation was due to both simultaneous release of EDCF and reduced release of EDRF. The present study shows that mechanisms for decrease in endothelium-dependent relaxation induced by ACh differ between spontaneous and salt-induced hypertension. Impaired ACh-induced relaxation in SHR is not due to reduced EDRF but to release of an EDCF. Furthermore, the alteration of EDCF and EDRF released from endothelial cells may be heterogeneous in the state of hypertension. In the Dahl strain, EDCF was not released in DS-HT aorta and coronary artery.

The depression of ACh-induced relaxation is not improved by oral administration of ONO-8809 in DS-HT. The depression is due to both increased EDCF and reduced EDRF, which may have resulted from structural and functional deterioration of endothelial cells in hypertensive blood vessels. ONO-8809 could not prevent the deterioration of endothelial cells because ONO-8809 did not attenuate the development of hypertension in DS rats. The lack of antihypertensive effect is not explained by failure of pharmacological potency, because oral administration of ONO-8809 altered TXA₂/PGH₂ receptor activity (Figure 5A). It has been reported that blockage of TXA₂ synthetase induced a significant antihypertensive effect in SHR. The antihypertensive effect may be due to improvement of renal function and due to decrease in the pressor effects of TXA₂ and noradrenaline. In contrast to SHR, the depressor effect is not obtained in hypertensive Dahl rats, although blockage of TXA₂ synthetase ameliorates renal functional and structural lesions. Gomi et al postulated that the abnormality of renal thromboxane seems to play a minor role in the development of hypertension in DS rats in contrast to SHR.

What is the significance of endothelium-dependent contractions in hypertension? The occurrence of pronounced endothelium-dependent contraction may reflect fading of endothelial protective mechanisms and the premature aging of the hypertensive blood vessel wall. Because endothelium-dependent contractions only occur in DS-HT, a close relationship will exist between the endothelium-dependent contraction and hypertension.

In conclusion, ACh caused release of EDCF in carotid rings of DS-HT but not of normotensive Dahl rats. The EDCF is likely to be PGH₂ and TXA₂. Endothelium-dependent relaxation induced by ACh is significantly depressed in the rings from DS-HT compared with those from normotensive DS rats. The depression may be due to both simultaneous release of EDCF and reduced release of EDRF.
References
4. Rapport R, Williams SP. Role of prostaglandins in acetylcholine-induced contraction of aorta from spontaneously hypertensive and Wistar-Kyoto rats. Hypertension. 1996;28:64–75.
Endothelium-Derived Contracting Factor in Carotid Artery of Hypertensive Dahl Rats
Ming-Sheng Zhou, Yasuhiro Nishida, Qing-Hui Chen and Hiroaki Kosaka

Hypertension. 1999;34:39-43
doi: 10.1161/01.HYP.34.1.39

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/34/1/39

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/