Prostaglandin H₂ May Be the Endothelium-Derived Contracting Factor Released by Acetylcholine in the Aorta of the Rat

Toshio Kato, Yoshio Iwama, Kenji Okumura, Hidekazu Hashimoto, Takayuki Ito, and Tatsuo Satake

The present experiment was performed to identify endothelium-derived contracting factor produced by acetylcholine stimulation in the aorta of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. The rings of the thoracic aorta were obtained from age-matched SHR and WKY rats, and changes in isometric tension were recorded. The relaxant responses to acetylcholine in the aortic rings from SHR were significantly weaker than those from WKY rats. The relaxant responses to acetylcholine were significantly enhanced by pretreatment with a cyclooxygenase inhibitor (indomethacin) or thromboxane A₂/prostaglandin H₂ receptor antagonist (ONO-3708) in aortic rings from both SHR and WKY rats. A thromboxane A₂ synthetase inhibitor (OKY-046) did not affect the acetylcholine-induced relaxation in the aortic rings from SHR or WKY rats. In the organ bath solution, after acetylcholine stimulation, prostaglandin E₂ and 6-keto-prostaglandin F₁α concentrations increased but not prostaglandin F₂α and thromboxane B₂ concentrations. Exogenous prostaglandin H₂, a stable analogue of thromboxane A₂, and prostaglandin F₂α induced contractions of the SHR rings at a lower concentration than prostaglandin E₂, prostaglandin D₂, and prostaglandin I₂. These contractile responses to various prostaglandins were markedly inhibited by pretreatment with ONO-3708. A prostacyclin synthetase inhibitor did not affect the relaxant responses to acetylcholine in the SHR rings. These results show that endothelium-derived contracting factor is produced and released by acetylcholine stimulation not only in the aorta of SHR but also in those of WKY rats and suggest that prostaglandin H₂, a precursor of the released prostaglandins, is a strong candidate for endothelium-derived contracting factor produced by acetylcholine stimulation. (Hypertension 1990;15:475–481)
Recently, endothelin was identified as an EDCF. This substance is the most potent mammalian vasconstrictor peptide and has characteristic constricting effects. The endothelin-induced contraction is resistant to cyclooxygenase inhibitors, lipoxygenase inhibitors, and a-adrenergic, histaminergic, and serotoninergic antagonists.15

In the aorta of spontaneously hypertensive rats (SHR), acetylcholine causes the simultaneous release of EDRF and an EDCF that differs from endothelin, which is a peptide, and is considered to be a cyclooxygenase product.16 However, the details remain obscure. We performed the present study to identify the EDCF, which is produced by acetylcholine stimulation in the SHR aorta.

Methods

The thoracic aorta from male SHR and normotensive Wistar-Kyoto (WKY) rats matched for age (30–32 weeks) and body weight (330–400 g) was used. Blood pressure measured by the tail-cuff method in the unanesthetized state was 191.9±3.4 mm Hg in SHR (n=8) and 140.6±1.5 mm Hg in WKY rats (n=8).

The rats were decapitated, and the thorax was opened. The thoracic aorta was immediately removed and placed in cold Krebs-Henseleit solution with the following composition (mM): NaCl 118, KCl 4.7, CaCl2 2.55, MgSO4 1.18, KH2PO4 1.18, NaHCO3 24.88, glucose 11.1, and Ca-2Na EDTA 0.026. The fat and connective tissues on the vascular surface were removed, and the vessel was cut into rings (5 mm in length) with scissors. After two stainless steel wires were inserted into the vascular lumen, the rings were replaced at 30-minute intervals. The resting tension to apply unnecessary tension to the aortic rings or damage their luminal surface. The rings were allowed to equilibrate for 90 minutes, during which buffer was replaced at 20-minute intervals. The rate of tension was adjusted to 1.0 g. The intimal surface was removed by gentle rubbing with a swab inserted into the vascular lumen. Removal of the intima was confirmed by the absence of responses to 10⁻⁷ M acetylcholine.

Responses to Norepinephrine

Norepinephrine was cumulatively added to the bath solution for the aortic rings from SHR or WKY rats at concentrations of 10⁻⁸ to 10⁻³ M, and changes in tension were evaluated. In the rings from SHR, the contraction induced by 10⁻⁷ M norepinephrine was 77.2±3.2% (n=6) of the maximum contraction (10⁻⁶ M norepinephrine). In the rings from WKY rats, the contraction induced by 10⁻⁷ M norepinephrine was 75.7±2.0% (n=6) of the maximum contraction (10⁻⁶ M norepinephrine). Because there was no significant difference between these values, the rings from both SHR and WKY rats were contracted with 10⁻⁷ M norepinephrine.

Responses to Acetylcholine and Effects of Inhibitors and an Antagonist

The aortic rings with and without endothelium from SHR and WKY rats were contracted with 10⁻⁷ M norepinephrine. After the contraction reached a plateau, acetylcholine was added at cumulative concentrations of 10⁻⁸ to 10⁻⁵ M to relax the rings. Fifteen minutes before the induction of contractions by norepinephrine, indomethacin,17 which is a cyclooxygenase inhibitor (10⁻⁵ M), OKY-046,18 which is a TXA2 synthetase inhibitor (10⁻⁵ M), and ONO-3708,19 which is a TXA2/prostaglandin H2 (PGH2) receptor antagonist (10⁻⁶ M) were each added separately to the bath solution, and each effect on relaxant responses to acetylcholine was evaluated. The rates of relaxation were expressed as percentages to the contraction induced by 10⁻⁷ M norepinephrine.

All the following experiments were done in the aortic rings from SHR alone.

Concentrations of Prostaglandins in the Bath Solution

In the SHR rings, changes in the concentrations of various prostaglandins (PGs) in the bath solution before and after the addition of acetylcholine were evaluated. Immediately before the induction of contractions by norepinephrine, at the peak response to 10⁻⁷ M norepinephrine and at the peak response to 10⁻⁵ M acetylcholine after cumulative addition of 10⁻⁸ to 10⁻⁵ M acetylcholine, the organ bath solution (1 ml) was obtained, and the concentrations of PGE2, 6-keto-PGF1α, and TXB2 using a radioimmunoassay (RIA) kit prepared by New England Nuclear (Boston, Massachusetts) and PGE2α using an RIA kit prepared by Clinical Assays (Cambridge, Massachusetts) were determined by the RIA method of Jaffe et al.20 and Powell.21

Responses to Exogenous Prostaglandins

PGE2α, a stable analogue of TXA2 (STA2),19 PGE2α and PGD2 were added at cumulative concentrations of 10⁻⁹ to 10⁻⁶ M and PGI2 at 10⁻⁷ to 10⁻⁵ M to the bath solution for the SHR aortic rings with and without endothelium at the basal state and at the precontracted (by norepinephrine 10⁻⁷ M) state, and vascular responses and the effects of ONO-3708 (10⁻⁶ M) on these responses were evaluated. The rate of contraction was expressed as percentages to the contraction induced by 10⁻⁷ M norepinephrine. The tests were performed at 40-minute intervals with replacement of the buffer every 20 minutes.

Effects of a Prostacyclin Synthetase Inhibitor

Tranylcypromine,22 a prostacyclin synthetase inhibitor, was added to the bath solution at a concentration of 10⁻⁴ M 15 minutes before the induction of contraction by norepinephrine, and its effects on relaxant responses to acetylcholine in the rings from the SHR were evaluated.
Kato et al. Endothelium-Derived Contracting Factor

DRUGS

The following drugs from Sigma Chemical Co. (St. Louis, Missouri) were used: l-norepinephrine bitartrate, acetylcholine chloride, indomethacin, tranilcipromine, sodium nitroprusside, and PGI2. Ono Pharmaceutical Company (Osaka, Japan) provided the PGF2α, PGE2, PGD2, PGH2, and STAc (ONO-3708) (a TXA2/PGH2 antagonist). Indomethacin was dissolved in distilled water containing 3 x 10^-2 M Na2CO3. PGI2, PGF2a, PGE2, PGD2, PGH2, and STA2 were dissolved in ethanol. The final ethanol concentration in the bath solution was 0.1% or less. The other drugs were dissolved in distilled water.

Vehicles did not affect the acetylcholine-induced relaxations or the resting tension. Results were expressed as mean±SEM. For statistical analysis, Student’s t test for paired or unpaired observations and the Wilcoxon test were used. Values of p<0.05 were considered to be significant.

RESULTS

RESPONSES TO ACETYLCHOLINE

In the aortic rings from both SHR and WKY rats, the maximum relaxation was observed with 10^{-7} M acetylcholine. At higher concentrations, acetylcholine induced contractions (Figure 1A, Figure 2). In the rings without endothelium from SHR and WKY rats, responses to acetylcholine were negligible (Figure 1B, Figure 2). The acetylcholine-induced relaxation in rings from the SHR was significantly weaker than that in rings from the WKY rats at acetylcholine concentrations of 10^{-7} to 10^{-5} M, and contractile responses observed at high acetylcholine concentrations disappeared (Figure 3). Similarly, in rings from the WKY rats relaxant responses were significantly enhanced at acetylcholine concentrations of 10^{-6} to 10^{-5} M, resulting in similar responses in rings from both SHR and WKY rats (Figure 3).

OKY-046 (10^{-5} M) did not affect the relaxant responses to acetylcholine in rings from the SHR or WKY rats (Figure 4).

To determine whether TXA2 is involved, the effects of ONO-3708 (10^{-6} M) were evaluated. In aortic rings from the SHR, relaxant responses were enhanced by pretreatment with ONO-3708 at acetylcholine concentrations of 10^{-7} to 10^{-5} M, and contractile responses observed at high acetylcholine concentrations disappeared (Figures 5 and 6). Similarly,
Indomethacin, OKY-046, and ONO-3708 did not significantly affect the contractions induced by $10^{-7}$ M norepinephrine in the rings from SHR and WKY rats. ONO-3708 ($10^{-6}$ M) did not significantly affect the relaxant responses to sodium nitroprusside in the rings from SHR and WKY rats (data not shown).

Concentrations of Prostaglandins in the Bath Solution

In rings from the SHR, the concentrations of PGE$_2$ and 6-keto-PGF$_{1a}$ increased about threefold to fourfold after acetylcholine stimulation. The concentration of PGE$_2$ in the bath solution after acetylcholine stimulation was about $10^{-10}$ M, and that of 6-keto-PGF$_{1a}$ was about $10^{-9}$ M. The concentrations of PGF$_{2a}$ and TXB$_2$ did not change significantly (Figure 7).

Responses to Exogenous Prostaglandins

In the SHR aortic rings with endothelium, PGF$_{2a}$, STA$_2$, PGH$_3$, PGE$_2$, PGD$_2$, and PGI$_2$ induced only contractions at the basal state. STA$_2$, PGF$_{2a}$, and PGH$_3$ induced contractions at $10^{-7}$ M or more; on the other hand, PGE$_2$ and PGD$_2$ induced contractions only at a high concentration of $10^{-6}$ M and PGI$_2$ at $10^{-5}$ M (Figure 8). ONO-3708 at a concentration of $10^{-6}$ M inhibited all contractile responses to the various PGs (Figure 8). In the SHR aortic rings without endothelium at the basal state, results similar to those in the SHR aortic rings with endothelium were obtained (data not shown). In the SHR aortic rings with and without endothelium, PGF$_{2a}$, STA$_2$, PGH$_3$, PGE$_2$, and PGD$_2$ also induced only contractions at the precontracted state. Results similar to those at the basal state were obtained, although the percentage of contraction was reduced (data not shown).

Effects of a Prostacyclin Synthetase Inhibitor

The contractions in the rings from SHR induced by PGI$_2$ at high concentrations presented the possibility that PGI$_2$ is EDCF. Therefore, the effects of tranylcypromine ($10^{-4}$ M) on relaxant responses to acetylcholine in rings from the SHR were evaluated. Because tranylcypromine reduced the norepinephrine-induced contractions, the rings were contracted with $10^{-6}$ M norepinephrine to obtain responses comparable with those in the control group. Tranylcypromine had no significant effect on acetylcholine-induced relaxation in rings from the SHR (Figure 9). PGI$_2$ is known to have potent vasodilating effects. In the SHR aortic rings with and without endothelium at the basal state, contractions were induced with $10^{-5}$ M PGI$_2$, but there were no significant changes at $10^{-9}$ to $10^{-6}$ M. At the precontracted state, negligible or only slight relaxations were observed at PGI$_2$ concentrations of $10^{-9}$ to $10^{-6}$ M. Contractions were induced at $10^{-7}$ M (data not shown).

Discussion

Acetylcholine-induced endothelium-dependent relaxations have been reported to be weaker in the
aorta of SHR than in those of WKY rats. These weaker relaxations in the SHR aorta have been suggested to be due to EDCF produced and released by acetylcholine stimulation in the SHR aorta. The present study confirmed significantly weaker relaxant responses to acetylcholine at concentrations of $10^{-7}$ to $10^{-5}$ M in the SHR aortic rings than in the WKY rat aortic rings. Indomethacin and ONO-3708 enhanced the relaxations at acetylcholine concentrations of $10^{-7}$ to $10^{-5}$ M in the SHR rings and at $10^{-6}$ to $10^{-5}$ M in the WKY rat aortic rings, resulting in similar responses in the SHR and WKY rat aortic rings. ONO-3708 did not affect the relaxations to sodium nitroprusside and the contractions induced by norepinephrine. Therefore, the relaxant responses to acetylcholine would not be enhanced by the direct effect of ONO-3708 on the vascular smooth muscle. These results suggest that a substance that is inhibited by indomethacin and ONO-3708 is produced and released by acetylcholine stimulation in the endothelium. This substance (EDCF) produced and released simultaneously with EDRF seems to weaken acetylcholine-induced relaxations. EDCF has been considered to be present only in SHR. However, our results suggest that EDCF is also produced and released by acetylcholine stimulation in the WKY rat aorta. Endothelium-dependent relaxations have been reduced in vascular smooth muscle of the rat with increasing age. The existence of EDCF in WKY rats suggests that EDCF may participate in the reduced endothelium-dependent relaxations with increasing age. Therefore, age and blood pressure may promote endothelium-dependent contractions in the aorta of the rat. Whether the difference in response between the SHR and WKY rat aortic rings is caused by a difference in the amount of EDCF produced by acetylcholine stimulation or that in the sensitivity of the smooth muscle to EDCF remains to be clarified.

Reports have suggested a substance in the cyclooxygenase system to be the EDCF produced and released by acetylcholine stimulation in the endothelium. This substance (EDCF) produced and released simultaneously with EDRF seems to weaken acetylcholine-induced relaxations. EDCF has been considered to be present only in SHR. However, our results suggest that EDCF is also produced and released by acetylcholine stimulation in the WKY rat aorta. Endothelium-dependent relaxations have been reduced in vascular smooth muscle of the rat with increasing age. The existence of EDCF in WKY rats suggests that EDCF may participate in the reduced endothelium-dependent relaxations with increasing age. Therefore, age and blood pressure may promote endothelium-dependent contractions in the aorta of the rat. Whether the difference in response between the SHR and WKY rat aortic rings is caused by a difference in the amount of EDCF produced by acetylcholine stimulation or that in the sensitivity of the smooth muscle to EDCF remains to be clarified.

Reports have suggested a substance in the cyclooxygenase system to be the EDCF produced and released by acetylcholine stimulation in the endothelium. This substance (EDCF) produced and released simultaneously with EDRF seems to weaken acetylcholine-induced relaxations. EDCF has been considered to be present only in SHR. However, our results suggest that EDCF is also produced and released by acetylcholine stimulation in the WKY rat aorta. Endothelium-dependent relaxations have been reduced in vascular smooth muscle of the rat with increasing age. The existence of EDCF in WKY rats suggests that EDCF may participate in the reduced endothelium-dependent relaxations with increasing age. Therefore, age and blood pressure may promote endothelium-dependent contractions in the aorta of the rat. Whether the difference in response between the SHR and WKY rat aortic rings is caused by a difference in the amount of EDCF produced by acetylcholine stimulation or that in the sensitivity of the smooth muscle to EDCF remains to be clarified.

Reports have suggested a substance in the cyclooxygenase system to be the EDCF produced and released by acetylcholine stimulation in the endothelium. This substance (EDCF) produced and released simultaneously with EDRF seems to weaken acetylcholine-induced relaxations. EDCF has been considered to be present only in SHR. However, our results suggest that EDCF is also produced and released by acetylcholine stimulation in the WKY rat aorta. Endothelium-dependent relaxations have been reduced in vascular smooth muscle of the rat with increasing age. The existence of EDCF in WKY rats suggests that EDCF may participate in the reduced endothelium-dependent relaxations with increasing age. Therefore, age and blood pressure may promote endothelium-dependent contractions in the aorta of the rat. Whether the difference in response between the SHR and WKY rat aortic rings is caused by a difference in the amount of EDCF produced by acetylcholine stimulation or that in the sensitivity of the smooth muscle to EDCF remains to be clarified.
released from the rat aorta. This was confirmed by the present study. To identify EDCF, the possibility of TXA₂ involvement was evaluated at first. This EDCF was not inhibited by OKY-046, a TXA₂ synthetase inhibitor, but was inhibited by ONO-3708, a TXA₂/PGF₂α receptor antagonist. ONO-3708, which inhibits the actions of TXA₂ and PGH₂, also inhibited contractions induced by PGF₂α, PGE₂, PGD₂, and PGI₂. This result shows that ONO-3708 is not a selective antagonist to TXA₂ and PGH₂. Consequently, EDCF seems to be a cyclooxygenase product or products other than TXA₂ as reported previously.⁴⁶

Next, the concentrations of various PGs in the organ bath solution were determined. After acetylcholine stimulation, the concentrations of PGE₂ and 6-keto-PGF₁α increased about threefold to fourfold. The concentration of PGE₂ in the bath solution after acetylcholine stimulation was about 10⁻¹⁰ M and that of 6-keto-PGF₁α (i.e., PGI₂) was about 10⁻⁹ M. Lüscher et al.⁵⁵ have obtained similar results. The effects of various exogenous PGs on the blood vessel were then evaluated. PGF₂α, STA₂, PGH₂, PGE₂, PGD₂, and PGI₂ caused contractions. PGE₂ and PGD₂ induced contractions only at a high concentration of 10⁻⁶ M and PGI₂ at 10⁻⁵ M. The concentrations of PGE₂ and PGI₂ measured in the bath solution were very low, and the concentrations that induced contractions were about 10⁷ times higher. Although the degree of transfer of a substance produced in the tissue to the solution is not known, the local concentrations of the PGs released from the endothelium within the blood vessel wall would be much higher than those measured in the organ chamber. Tranylcypromine, a prostacyclin synthetase inhibitor of acetylcholine-induced relaxations, had no effect, which is consistent with the result of the previous report,¹⁶ which suggests that PGI₂ is not increased in the tissue to the degree that induces vascular contractions. Therefore, there is only a slight possibility that PGI₂ or PGE₂ is EDCF. In addition, that there was no increase in the concentrations of PGF₂α and TXB₂ in the solution excludes the possibility that PGF₂α or TXA₂ is EDCF. These results suggest a cyclooxygenase product or products other than TXA₂, PGE₂, PGI₂, or PGF₂α as the EDCF produced and released by acetylcholine stimulation in the SHR and WKY rat aorta.

PGI₂ is produced in endothelial cells and has potent vasodilating effects. When administered exogenously, it is known to induce biphasic responses in some types of blood vessels: relaxation is observed at low concentrations and contraction at high concentrations.²⁶⁻³⁰ After acetylcholine stimulation, the concentration of PGI₂ in the solution increased. The degree of the involvement of this increased PGI₂ in relaxations in the rat aorta was evaluated. The aortic rings at the basal state showed no changes in tension at PGI₂ concentrations of 10⁻⁹ to 10⁻⁶ M. Contractions were induced at 10⁻⁵ M. The rings contracted with 10⁻⁷ M norepinephrine showed neg-
ligible change or only slight relaxation at PGI₂ concentrations of $10^{-9}$ to $10^{-6}$ M. At $10^{-5}$ M, contractions were induced. Therefore, PGI₂ increased by acetylcholine stimulation is not likely to be involved in not only contractions but also relaxations in the rat aorta.

In the present experiment, we could not identify EDCF produced by acetylcholine stimulation in the rat aorta but found that: 1) There is a very slight possibility that the final product in the cyclooxygenase system is an EDCF. 2) Exogenous PGH₂ induced contractions at $10^{-5}$ M or more. 3) The contractions induced by PGH₂ were inhibited by ONO-3708. 4) The concentrations of PGI₂ and PGE₂ increased after acetylcholine stimulation in the organ bath solution. The concentration of PGI₂ in the bath solution after acetylcholine stimulation was about $10^{-9}$ M and that of PGE₂ was about $10^{-10}$ M. 5) The volume of the aortic ring was about 1/10³ to 1/10⁴ of that of the organ bath solution. Taking the concentrations of PGs measured in the organ bath solution and a volume ratio of the vascular tissue to the organ bath solution into consideration, the concentration of PGH₂, a precursor of the released PGs, would be at least $10^{-5}$ M or more in the vascular tissue. Therefore, it seems that the concentration of PGH₂ produced in the tissue is sufficient to induce vascular contractions. These observations suggest that PGH₂ is a strong candidate for EDCF produced by acetylcholine stimulation in the rat aorta.

Acknowledgment

We thank Miss Keiko Sakai for secretarial assistance.

References

30. Van Dam J, Maddox YT, Ramwell PW, Kot PA: Role of the vascular endothelium in the contractile response to prostacyclin in the isolated rat aorta. J Pharmacol Exp Ther 1986;239:390–394

Key Words: endothelium • acetylcholine • prostaglandin synthase • aorta • spontaneously hypertensive rats • Wistar-Kyoto rats
Prostaglandin H2 may be the endothelium-derived contracting factor released by acetylcholine in the aorta of the rat.

T Kato, Y Iwama, K Okumura, H Hashimoto, T Ito and T Satake

*Hypertension*. 1990;15:475-481
doi: 10.1161/01.HYP.15.5.475

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 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/15/5/475

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/