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 F₂α, 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)

All blood vessels are lined by the endothelium, and the important role of an organic or functional abnormality of endothelial cells in the pathogenesis and pathophysiology of various diseases has attracted attention.

In 1980, Furchgott et al. found that acetylcholine induced endothelium-dependent relaxation in the rabbit aorta. Since then, various substances have been reported to induce endothelium-dependent relaxation in most of the blood vessels in mammals.²,³ Several pharmacological observations have strongly suggested that there is more than one endothelium-derived relaxing factor (EDRF)⁴,⁵ and that nitric oxide is one of these EDRFs.⁶,⁷

After the discovery of EDRF, studies by Vanhoutte and coworkers⁸–¹¹ have shown that endothelial cells produce not only EDRF but also endothelium-derived contracting factors (EDCF) after various stimulations. Several substances have been suggested as EDCFs. In isolated rabbit intrapulmonary arteries, acetylcholine-induced endothelium-dependent vasorelaxation is caused by thromboxane A₂ (TXA₂)–mediated contraction.¹² In canine basilar arteries, calcium ionophore A23187, arachidonic acid, and acetylcholine caused endothelium-dependent contractions. In the case of A23187 and arachidonic acid, TXA₂ contributes to the endothelium-dependent contractions.¹³ A recent publication has suggested that one of the EDCF may be the superoxide anion.¹⁴
Responses to Norepinephrine

Contraction induced by 10^{-7} M norepinephrine was
difference between these values, the rings from both
rats at concentrations of 10^{-8} to 10^{-5} M, and changes
in tension were evaluated. In the rings from SHR, the
contraction was 77.2±3.2% (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

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 concentra-
tions of 10^{-8} to 10^{-5} M to relax the rings. Fifteen minutes before the induction of contractions
by norepinephrine, indomethacin, which is a cyclo-
oxxygenase inhibitor (10^{-5} M), OKY-046, which is a
TXA_2 synthetase inhibitor (10^{-5} M), and ONO-3708, which is a TXA_2/prostaglandin H_2 (PGH_2)
receptor antagonist (10^{-6} M) were each added sepa-
rate to the bath solution, and each effect on relax-
ant 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 addition of acetylcholine were
evaluated. Immediately before the induction of contrac-
tions by norepinephrine, at the peak response to 10^{-7}
M norepinephrine and at the peak response to 10^{-3}
M acetylcholine after cumulative addition of 10^{-5} to
10^{-3} M acetylcholine, the organ bath solution (1 ml)
was taken, and the concentrations of PGE_2, 6-
keto-PGF_1alpha, and TXB_2 were each determined
by the RIA method of Jaffe et al.\textsuperscript{20} and Powell.\textsuperscript{21}

Responses to Exogenous Prostaglandins

PGE_2, PGF_2alpha, stable analogue of TXA_2 (STA_2),\textsuperscript{19} PGD_2 were added at cumulative concentra-
tions of 10^{-9} to 10^{-6} M and PGI_2 at 10^{-5} to 10^{-3} 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

Tranlycypromine, 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.
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**Drugs**

The following drugs from Sigma Chemical Co. (St. Louis, Missouri) were used: l-norepinephrine bitartrate, acetylcholine chloride, indomethacin, tranylcypromine, sodium nitroprusside, and PGI₂. Ono Pharmaceutical Company (Osaka, Japan) provided the PGF₂α, PGE₂, PGD₂, PGH₂, 9,11-epithio-11,12-methano-TXA₂ (STA₂), (E)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride monohydrate (OKY-046) (a TXA₂ synthetase inhibitor), and (9,11),(11,12)-dideoxa-9α,11α-dimethylmethano-11,12-methano-13,14-dihydro-13-aza-14-oxo-15-cyclopentyl-16,17,18,19,20-pentanor-15-epi-TXA₂ (ONO-3708) (a TXA₂ antagonist). Indomethacin was dissolved in distilled water containing 3 x 10⁻² M Na₂CO₃. PGI₂, PGF₂α, 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. Vehicle 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,

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**Figure 1.** Typical records of acetylcholine (ACh)-induced responses in spontaneously hypertensive rat aortic rings with (panel A) and without (panel B) endothelium. Rings were contracted with 10⁻⁷ M norepinephrine (NE), and ACh was cumulatively added at 10⁻⁸ to 10⁻⁵ M. Wo, wash out.

**Figure 2.** Line graph showing cumulative concentration-response curves for acetylcholine in spontaneously hypertensive rat (SHR) and Wistar-Kyoto (WKY) rat aortic rings with and without endothelium. Rate of relaxation was expressed as percentages to contraction induced by 10⁻⁷ M norepinephrine. Results are shown as mean±SEM. *p<0.05 between SHR and WKY rats.
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$_3$ and 6-keto-PGF$_{1a}$ increased about threefold to fourfold after acetylcholine stimulation. The concentration of PGE$_3$ 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$_{2\alpha}$ and TXB$_2$ did not change significantly (Figure 7).

Responses to Exogenous Prostaglandins

In the SHR aortic rings with endothelium, PGE$_{2\alpha}$, STA$_2$, PGH$_2$, PGE$_3$, and PGI$_1$ induced only contractions at the basal state. STA$_2$, PGE$_{2\alpha}$, and PGH$_2$ induced contractions at $10^{-7}$ M or more; on the other hand, PGE$_3$ and PGD$_2$ induced contractions only at a high concentration of $10^{-6}$ M and PGI$_1$ 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, PGE$_{2\alpha}$, STA$_2$, PGH$_2$, PGE$_3$, 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 tranilcypromine ($10^{-4}$ M) on relaxant responses to acetylcholine in rings from the SHR were evaluated. Because tranilcypromine reduced the norepinephrine-induced contractions, the rings were contracted with $10^{-6}$ M norepinephrine to obtain responses comparable with those in the control group. Tranilcypromine 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 $10^{-4}$ 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. 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.

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Figure 8. Line graph showing responses of spontaneously hypertensive rat aortic rings with endothelium to various prostaglandins (PG) and effects of ONO-3708 (10^{-6} M). Rate of contraction was expressed as percentages to contraction induced by 10^{-7} M norepinephrine. Results are shown as mean±SEM. **p<0.01 between presence and absence of ONO-3708. STA 2, a stable analogue of thromboxane A2, 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/PGH2 receptor antagonist. ONO-3708, which inhibits the actions of TXA2 and PGH2,19 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.16

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 al25 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 10^4 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. Tranlycypromine, 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 PG12 concentrations of 10^{-10} to 10^{-6} M. At 10^{-5} M, contractions were induced. Therefore, PG12 increased by acetylcholine stimulation is not likely to be involved in not only contractions but also relaxations in the rat aorta.


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References

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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

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