Role of Endothelium in the Response to Prostaglandin H₂ in Isolated Dog Arteries

Tomio OKAMURA, Seiji INOUE, Yoshiyuki MINAMI, Hideki OKUNISHI and Noboru TODA*

Department of Pharmacology, Shiga University of Medical Sciences, Seta, Ohtsu 520-21, Japan

Accepted January 5, 1989

Abstract—Prostaglandin (PG) H₂ produced a transient contraction followed by a relaxation in helical strips of dog coronary, mesenteric and renal arteries contracted with PGF₂α. The contraction was in the order of mesenteric>renal>coronary artery. Removal of endothelium abolished the contraction in these arteries and significantly potentiated the relaxation only in mesenteric arteries. The relaxation was greater in mesenteric arteries than in renal and coronary arteries, denuded of endothelium. PG₁₂-induced relaxations were not influenced by endothelium denudation. In the arteries contracted with K⁺, PGH₂-induced relaxations were attenuated, compared to those contracted with PGF₂α. Treatment with ONO3708, an antagonist of vasoconstrictor PGs, abolished the PGH₂-induced contraction and potentiated the relaxation in the K⁺-contracted arteries. The relaxant response was suppressed by diphloretin phosphate, a PG receptor antagonist, as was the response to PG₁₂. PGH₂-induced contractions in dog coronary, mesenteric and renal arteries would be due to vasoconstrictor PGs produced preferentially in the endothelium. However, production of PG₁₂ from PGH₂ in endothelial and subendothelial tissues do not appear to differ.

Endothelium importantly regulates vascular tone by producing vasodilator and constrictor substances such as prostaglandins (PGs), endothelium-derived relaxing factor (EDRF) and angiotensin II. In addition to studies on the interesting topic EDRF (1), major attention has also been directed to the release of cyclooxygenase products from vascular endothelial and subendothelial tissues and their action on vascular smooth muscle. Enzymatic production of PGH₂ from ¹⁴C-arachidonic acid (cyclooxygenase activity) is evidently higher in the endothelium than in the smooth muscle of the bovine aorta, whereas the production of PG₁₂ from ¹⁴C-PGH₂ does not differ in these tissues (2). The uneven distribution of PG synthetases is reflected by different responsiveness to arachidonic acid and PGH₂ in isolated dog cerebral arteries with intact and damaged endothelium (3). Responses to arachidonic acid metabolites differ in the smooth muscles of a variety of arteries (4–7), and the ability to produce PGs from arachidonic acid or PGH₂ is expected to differ in various arteries with and without endothelium.

The present study was therefore undertaken to compare responses to PGH₂ and PG₁₂ of coronary, mesenteric and renal arteries isolated from dogs and determine their dependence on endothelium.

Materials and Methods

Mongrel dogs of either sex, weighing 8 to 14 kg, were anesthetized with intraperitoneal injections of thiopental sodium (50 mg/kg) and sacrificed by bleeding from the common carotid arteries. Anterior descending and circumflex branches of the coronary arteries were isolated from the heart, and interlobar branches of the renal artery were isolated from the kidneys. Distal portions of the mesenteric artery were removed. Human coronary arteries were obtained during autopsy within
4 hours after death (8), and human gastroduodenal arteries were isolated from the stomach resected during operations for stomach ulcer. The arteries were helically cut into strips of approximately 20 mm long. The specimen was vertically fixed between hooks in a muscle bath containing the modified Ringer-Locke solution, which was maintained at 37±0.3°C and aerated with a mixture of 95% O_2 and 5% CO_2. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer (Nihon Kohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 g, which is optimal for inducing the maximal contraction (9). Constituents of the solution were as follows: 120 mM NaCl, 5.4 mM KCl, 2.2 mM CaCl_2, 1.0 mM MgCl_2, 25.0 mM NaHCO_3 and 5.6 mM dextrose. Before the start of experiments, all strips were allowed to equilibrate for 90 to 120 min in the bathing media, during which time the medium was replaced every 10 to 15 min.

Isometric contractions and relaxations were displayed on an ink-writing oscillograph (Nihon Kohden Kogyo Co.). The contractile response to 30 mM K^+ was first obtained, and the preparations were repeatedly washed and equilibrated. The arterial response was obtained by adding agents directly to the bathing media. To test the relaxant effect of PG H_2, PG_12 or acetylcholine, the arterial strips were partially contracted with PGF_2a (10^{-8} to 10^{-7} M) or small amounts of K^+ (11 to 15 mM); the contraction was in a range between 20 and 35% of that induced by 30 mM K^+. At the end, papaverine (10^{-4} M) was added to attain the maximal relaxation, and the agonist-induced relaxation relative to that caused by papaverine was calculated. Preparations had been treated for 20 to 30 min with blocking agents such as ONO3708 (10^{-7} M) or diphloretin phosphate (DPP, 10^{-5} M), before the concentration-response curve for agonists was obtained.

In some preparations, the endothelium was removed by gently rubbing the intimal surface with a cotton pellet, and the response of endothelium-denuded strips was compared with that of strips with intact endothelium obtained from the same dogs. Removal of endothelium was determined by the abolition of relaxation induced by 10^{-6} M acetylcholine or 10^{-2} M substance P and histologically verified by the method reported by Caplan et al. (10).

Results shown in the text and figures are expressed as mean values±S.E.M. Statistical analyses were made using Student’s paired and unpaired t-tests.

Drugs used were prostaglandins (PGs) F_2α, H_2 and I_2 sodium salt, ONO3708 [(9,11), (11,12) - dideoxa - 9α,11α-dimethylmethano-11,12 - methano-13,14 - dihydro - 13 - azo-14- oxo-15 - cyclopentyl-16,17,18,19,20-pentanor -15-epi-thromboxane A2], OKY-046 {(E)-3- [4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride monohydrate} (Ono Pharmaceutical Co., Osaka, Japan); diphloretin phosphate (Leo Co., Helsingborg, Sweden); tranylcyromine hydrochloride (Nakarai Chemicals, Ltd., Kyoto, Japan); acetylcholine chloride and papaverine hydrochloride (Dainippon Pharmaceutical Co., Osaka). PGH_2 was prepared following the procedure described by Yoshimoto et al. (11) and dissolved in acetone to make a concentrated solution of 40 μM. Ampoules containing a small quantity of the PGH_2 solution were stored at -74°C. The stock solution of 50 μl was directly added to 20 ml bathing media. ONO3708 was dissolved in ethanol to make a stock solution, which was diluted by 50 mM sodium carbonate-bicarbonate buffer solution (pH 9.2) before use.

**Results**

**Coronary artery:** Helical strips of dog coronary arteries partially contracted with PGF_2α responded to 10^{-7} M PGH_2 with a transient contraction followed by a relaxation. Removal of endothelium abolished the contraction, but did not significantly alter the relaxation (Fig. 1). K^+ (30 mM)-induced contractions did not differ in control and deendothelialized arteries (2247±313 mg vs. 1900±306 mg, N=9). Relaxations caused by PG_12 (10^{-8} and 10^{-7} M) were not influenced by endothelium-denudation. The equipotent concentration of PG_12 to produce the same magnitude of relaxation to that caused by PGH_2 (10^{-7} M) in the endothelium-denuded arteries was calculated to be 5.4×10^{-8} M.
Fig. 1. Responses to PGH2 and PGI2 of dog coronary artery strips with and without endothelium (E), partially contracted with PGF2α. Relaxations induced by 10^{-4} M papaverine were taken as 100% relaxation; mean absolute values in the intact and endothelium-denuded arteries with PGH2 were 558±57 mg and 556±86 mg (N=9), respectively, and those with PGI2 were 461±61 mg and 453±63 mg (N=9), respectively. Contractions induced by 30 mM K+ were taken as 100% contraction; the mean absolute value in the intact arteries with endothelium was 2247±313 mg (N=9). a, Significantly different from the value in intact arteries, P<0.01. Vertical bars represent S.E.M.

Similar results with PGH2 and PGI2 were also obtained in 2 out of 2 human coronary artery strips with intact and damaged endothelium. Typical recordings are shown in Fig. 2.

Contractions induced by PGH2 were potentiated and the relaxations to PGH2 were attenuated in dog arteries contracted with K⁺ (13 to 15 mM), compared to those obtained in the PGF2α-contracted arteries (cf. Figs. 1 and 3). PGI2-induced relaxations were also decreased in the arteries contracted with K⁺. Treatment with 10^{-7} M ONO3708 abolished the contraction and potentiated the relaxation (Fig. 3). Relaxant responses to 10^{-8} and 10^{-7} M PGI2 were not influenced by ONO3708.

Mesenteric artery: The addition of PGH2 (10^{-7} M) produced a transient contraction followed by a relaxation in mesenteric artery strips partially contracted with PGF2α (Fig. 4). The contraction was abolished by removal of endothelium, whereas the relaxation was significantly potentiated. Endothelium-denudation did not alter the response to PGI2. The concentration of exogenous PGI2 equipotent to 10^{-7} M PGH2 in the arteries denuded of endothelium was calculated to be 1.0×10^{-7} M.

In 3 out of 3 human gastroepiploic artery strips with endothelium that were contracted with K⁺, PGH2 (10^{-7} M) produced phasic and tonic contractions, while PGI2 elicited a relaxation (Fig. 5). The contractile responses were reversed to relaxations by endothelium denudation. The PGH2-induced contraction in the arteries with endothelium was suppressed by the treatment with ONO3708. Dog mesenteric arteries contracted with K⁺ also responded to PGH2 with a phasic contraction followed by a sustained contraction, both of which were abolished or reversed to a...
Fig. 2. Responses to PGH₂ (10⁻⁷ M) and PGI₂ (10⁻⁸ to 10⁻⁶ M) of human coronary artery strips with and without endothelium, partially contracted with PGF₂α (3×10⁻⁸ M). The strips were obtained from the anterior descending artery of the same heart. PA=10⁻⁴ M papaverine.

Fig. 3. Modification by ONO3708 (10⁻⁷ M) of responses to PGH₂ and PGI₂ of dog coronary artery strips with endothelium, partially contracted with K⁺. Relaxations induced by 10⁻⁴ M papaverine were taken as 100% relaxation; mean absolute values in control (C) and ONO3708-treated (ONO) arteries with PGH₂ were 423±50 mg and 438±47 mg (N=4), respectively, and those with PGI₂ were 493±10 mg and 448±64 mg (N=4), respectively. Contractions induced by 30 mM K⁺ were taken as 100% contraction; the mean absolute value in the control arteries was 2220±517 mg (N=4). a, Significantly different from the value in control arteries, P<0.05. Vertical bars represent S.E.M.
Fig. 4. Responses to PGH\(_2\) and PGI\(_2\) of dog mesenteric artery strips with and without endothelium (E), partially contracted with PGF\(_2\alpha\). Relaxations induced by 10\(^{-4}\) M papaverine were taken as 100% relaxation; mean absolute values in the intact and endothelium-denuded arteries with PGH\(_2\) were 620±62 mg and 618±36 mg (N=7), respectively, and those with PGI\(_2\) were 196±38 mg and 194±40 mg (N=6), respectively. Contractions induced by 30 mM K\(^+\) were taken as 100% contraction; the mean absolute value in the arteries with endothelium was 2533±481 mg (N=7). a, Significantly different from the value in intact arteries, p<0.01; b, P<0.05.

The PGH\(_2\)-induced contraction was potentiated and the relaxation was reversed to a sustained contraction in the arteries contracted with K\(^+\) (cf. Figs. 8 and 9). The contractions were abolished or reversed to relaxations by treatment with 10\(^{-7}\) M ONO3708 (Fig. 9) but were unaffected by 10\(^{-5}\) M OKY-046 (N=2), a thromboxane A\(_2\) synthesis inhibitor (12). The relaxation induced by PGH\(_2\) in the arteries denuded of endothelium was potentiated by treatment with ONO3708 (Fig. 9) and suppressed by 10\(^{-4}\) M tranylcypromine, a PGI\(_2\) synthesis inhibitor (13); mean values of the relaxations before and after tranylcypromine were 48.1±6.2% and 18.3±3.4%, respectively (N=3, P<0.02).
Fig. 5. Responses to PGH₂ (10⁻⁷ M) and PGI₂ (10⁻⁸ and 10⁻⁷ M) of human gastroepiploic artery strips with and without endothelium, partially contracted with K⁺ (11 to 13 mM). Concentration of ONO3708 =10⁻⁷ M, PA=10⁻⁴ M papaverine.

Fig. 6. Modification by ONO3708 (10⁻⁷ M) of responses to PGH₂ and PGI₂ of dog mesenteric artery strips with endothelium, partially contracted with K⁺. Relaxations induced by 10⁻⁴ M papaverine were taken as 100% relaxation; mean absolute values in control (C) and ONO3708-treated (ONO) arteries with PGH₂ were 420±48 mg and 505±82 mg (N=6), respectively, and those with PGI₂ were 412±50 mg and 295±32 mg (N=5), respectively. Contractions induced by 30 mM K⁺ were taken as 100% contraction; the mean absolute value in the control arteries was 2354±676 mg (N=6). a, Significantly different from the value in control arteries, P<0.01; b, P<0.02.
Discussion

Helical strips of dog coronary, mesenteric and renal arteries responded to PGH₂ with a transient contraction, which was abolished by removal of endothelium. In an earlier report, PGH₂ and arachidonic acid were also demonstrated to produce endothelium-dependent contractions in isolated dog cerebral arteries (3). The contraction was reversed to a relaxation in the arteries treated with ONO3708 in a concentration (10⁻⁷ M) sufficient to selectively suppress the contractile response to PGF₂α, PGE₂, and a TXA₂ analog (14). The antagonist failed to inhibit the PG₁₂-induced relaxation (present study, 14). Dependence of the PGH₂-induced contraction on endothelium and susceptibility to ONO3708 were also observed in human coronary and gastroepioloic arteries. Therefore, as far as the arteries used in the present study are concerned, PGH₂ produces contractions, possibly mediated by conversion to vasoconstrictor PGs, including PGF₂α and PGE₂ (4), which are preferentially synthesized in the endothelium. TXA₂ may not be involved in the contraction, since the PGH₂-induced contraction in renal (present study) as well as cerebral arteries (3) is not influenced by treatment with a TXA₂ synthesis inhibitor, OKY-046. In renal arteries with damaged endothelium, PGH₂-induced relaxations were significantly potentiated by ONO3708 (Fig. 9, right), suggesting that the vasoconstrictor PGs were also produced in subendothelial tissues.

PGH₂ produced moderate or marked relaxations in coronary, mesenteric and renal arteries as well as in isolated dog cerebral (14, 15) and bovine coronary arteries (16). The PGH₂-induced relaxation in mesenteric arteries was suppressed by DPP, a PG receptor antagonist (5, 17), as was the relaxation by PG₁₂ (present study, 18, 19). Treatment with tranylcypromine or 15-hydroperoxy arachidonic acid, PGI₂ synthesis inhibitors (13), markedly inhibited the PGH₂-induced relaxation in dog renal (present
Fig. 8. Responses to PGH₂ and PGI₂ of dog renal artery strips with and without endothelium (E), partially contracted with PGF₂α. Relaxations induced by 10⁻⁴ M papaverine were taken as 100% relaxation; mean absolute values in the intact and endothelium-denuded arteries with PGH₂ were 574±72 mg and 754±73 mg (N=16), respectively, and those with PGI₂ were 573±51 mg and 778±75 mg (N=16), respectively. Contractions induced by 30 mM K+ were taken as 100% contraction; the mean absolute value in the intact arteries was 2921±281 mg (N=16). a, Significantly different from the value in intact arteries, P<0.001.

The PGH₂-induced relaxation was markedly suppressed in the arteries contracted with K⁺, compared to that in the PGF₂α-contracted arteries. PGH₂ relaxed the PGF₂α-contracted arteries with damaged endothelium to a greater extent than the endothelium-intact arteries contracted with K⁺ and treated with ONO3708 (52 vs. 38% in coronary arteries, Figs. 1 and 3; 88 vs. 68% in mesenteric arteries, Figs. 4 and 6; 58 vs. 52% in renal arteries, Figs. 8 and 9). PGI₂-induced relaxations were greater in the arteries contracted with PGF₂α than in the K⁺-contracted arteries. However, the lack of relaxation caused by PGH₂ in the K⁺-contracted arteries may not be explained solely by a reduced sensitivity to PGI₂ but also by an increased production of PGs responsible for arterial contractions.

Responses to PGH₂ in the coronary, mesenteric, renal (present study) and cerebral arteries (3, 15) with intact and damaged endothelium are quantitatively compared in Fig. 10. The magnitude of relaxation after endo-
Fig. 9. Modification by ONO3708 (10^{-7} M) of responses to PGH_{2} of dog renal artery strips with and without endothelium (E), partially contracted with K^+. Relaxations induced by 10^{-4} M papaverine were taken as 100% relaxation; mean absolute values in control (C) and ONO3708-treated (ONO) arteries with endothelium were 604±92 mg and 592±44 mg (N=5), respectively, and those in the arteries without endothelium were 484±77 mg and 438±47 mg (N=5), respectively. Contractions induced by 30 mM K^+ were taken as 100% contraction; mean absolute values in the control arteries with and without endothelium were 2415±503 mg and 2832±575 mg (N=5), respectively. a, Significantly different from the value in control arteries, p<0.001; b, P<0.02; c, P<0.05.

E, Partially contracted with K^+; E (+), Treated with ONO3708; E (−), Control; PGH_{2}, Prostaglandin H_{2}; C, Control; ONO, ONO3708.

Potency of PGH_{2} for arterial relaxation may be related mainly to the action of PGI_{2} synthesized in the arterial wall and also to the production of PGI_{2} (equipotent concentrations: 10, 5.4, 4.0 and 2.8×10^{-8} M in mesenteric, coronary, renal and cerebral arteries denuded of endothelium, data with cerebral artery from Toda et al. (3)). On the basis of contraction in the arteries with intact endothelium and potentiation by endothelium denudation of the relaxation, the endothelium-dependent contraction by PGH_{2} metabolites might be greater in cerebral and mesenteric arteries than in coronary and renal arteries.

References
1 Furchgott, R.F.: Role of endothelium in response of vascular smooth muscle. Circ. Res. 53, 557–573 (1983)
2 DeWitt, D.L., Day, J.S., Sonnenburg, W.K. and

References
1 Furchgott, R.F.: Role of endothelium in response of vascular smooth muscle. Circ. Res. 53, 557–573 (1983)
2 DeWitt, D.L., Day, J.S., Sonnenburg, W.K. and

Smith, W.L.: Concentrations of prostaglandin endoperoxide synthase and prostaglandin I_{2} synthase in the endothelium and smooth muscle of bovine aorta. J. Clin. Invest. 72, 1882–1888 (1983)
3 Toda, N., Inoue, S., Bian, K. and Okamura, T.: Endothelium-dependent and independent responses to prostaglandin H_{2} and arachidonic acid in isolated dog cerebral arteries. J. Pharmacol. Exp. Ther. 244, 297–302 (1988)
4 Toda, N. and Miyazaki, M.: Responses of isolated dog cerebral and peripheral arteries to prostaglandins after application of aspirin and polyphloretin phosphate. Stroke 9, 490–498 (1978)
5 Toda, N.: Mechanism of action of carbocyclic thromboxane A_{2} and its interaction with prostaglandin I_{2} and verapamil in isolated arteries. Circ. Res. 51, 675–682 (1982)
6 Toda, N.: Different responsiveness of a variety of isolated dog arteries to prostaglandin D_{2}. Prostaglandins 23, 99–112 (1982)
7 Hayashi, S., Park, M.K. and Kuehl, T.J.: Effects of prostaglandins and arachidonic acid on baboon cerebral and mesenteric arteries. Prostaglandins
Fig. 10. Comparison of the response to PGH₂ of dog cerebral (Cer.), coronary (C), renal (R) and mesenteric (M) artery strips with and without endothelium, partially contracted with PGF₂a. Relaxations induced by 10⁻⁴ M papaverine were taken as 100% relaxation, and contractions induced by 30 mM K⁺ were taken as 100% contraction. Data with cerebral arteries were quoted from an earlier report (3).
boxanes. Science 195, 409–412 (1977)

17 Eakins, K.E., Fex, H., Fredholm, B., Hogberg, B. and Veige, S.: On the prostaglandin inhibitory action of polyphloretin phosphate. Adv. Biosci. 9, 135–138 (1973)

18 Toda, N.: Responses of human, monkey and dog coronary arteries in vitro to carbocyclic thromboxane A₂ and vasodilators. Br. J. Pharmacol. 83, 399–408 (1984)

19 Toda, N., Inoue, S., Okamura, T. and Okunishi, H.: Mechanism underlying relaxations caused by prostaglandins and thromboxane A₂ analog in isolated dog arteries. J. Cardiovasc. Pharmacol. 11, 354–362 (1988)

20 Dusting, G.J., Moncada, S. and Vane, J.R.: Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. Prostaglandins 13, 3–15 (1977)