Br. J. Pharmac. (1984), **81**, 301–307

Endothelium-dependent relaxation induced by angiotensin II and histamine in isolated arteries of dog

Noboru Toda

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

1 In helical strips of dog renal and mesenteric arteries pre-contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), endothelium-dependent relaxations were investigated. Removal of the endothelium was shown histologically by staining with silver nitrate and functionally by testing the inability of acetylcholine to induce arterial relaxations.

2 When the endothelium was removed, relaxation of renal arteries to angiotensin (Ang) II was markedly suppressed, whereas relaxations induced by PGI_2 or isoprenaline were attenuated only slightly. Removal of the endothelium attenuated the relaxant response of mesenteric arteries to histamine but did not significantly alter the response to PGI_2.

3 Treatment with indomethacin caused an additional attenuation of the relaxant response to histamine or a reversal of the Ang II-induced relaxation to a contraction in the arterial strips, from which the endothelium had been removed.

4 Relaxation of renal arteries induced by Ang II and of mesenteric arteries induced by histamine is postulated to result from PGI_2 released from the arterial wall. Therefore, it appears that the endothelium is a major site but not the only site responsible for drug-induced release of PGI_2.

Introduction

In earlier papers (Toda & Miyazaki, 1981; Toda, 1981), it has been postulated that relaxation induced by angiotensin (Ang II) of dog renal arterial strips is mediated by prostaglandin I_2 (PGI_2) released from the arterial wall via the stimulation of Ang II receptors, since the relaxation is reversed to a contraction by aspirin and indomethacin, cyclo-oxygenase inhibitors, abolished by Ang II antagonists, and suppressed by 15-hydroperoxyarachidonic acid and tranylcypromine, PGI_2 synthesis inhibitors, or by dexamethasone, an inhibitor of phospholipase A_2. A PGI_2-like substance is also detected in the superfusate of renal arteries in response to Ang II by the cascade method with isolated stomach of rat and dog coronary artery strips (Toda & Miyazaki, 1981). The PGI_2-releasing activity of Ang II has also been observed in the dog kidney and lung (Shebuski & Aiken, 1980; Dusting, 1981) and rat kidney and mesenteric artery (Shibouta *et al.*, 1979; Nolan *et al.*, 1981; Desjardins-Giasson *et al.*, 1982).

On the other hand, we have shown that the rapid phase of histamine-induced relaxation of isolated mesenteric arteries of the dog mediated via H_1 receptors is suppressed by cyclo-oxygenase inhibitors, suggesting the involvement of released PGI_2 (Toda *et al.*, 1982). The release of a PGI_2-like substance via H_1-receptors from dog mesenteric arteries and cultured human umbilical vein endothelial cells has been demonstrated (Baenziger *et al.*, 1980; Toda *et al.*, 1982).

PGI_2 has been shown to be synthesized mainly in endothelial cells or the intimal surface of blood vessels (Moncada *et al.*, 1977; Weksler *et al.*, 1978; MacIntyre *et al.*, 1978; Marcus *et al.*, 1978; Eldor *et al.*, 1981). In rabbit isolated aortae, from which endothelial cells are removed, relaxations induced by acetylcholine, Ca^{2+} ionophore A23187 and ATP are abolished or reversed to contractions (Furchgott & Zawadzki, 1980; Furchgott *et al.*, 1981). Since these relaxations are not influenced by aspirin and indomethacin, but suppressed by 5,8,11,14-eicosatetraynoic acid, an inhibitor of cyclo-oxygenase and lipoxygenase, or mepacrine, a phospholipase A_2 inhibitor, the involvement of lipoxygenase products but not cyclo-oxygenase products, released from the vascular wall in the relaxation is postulated.

The present study was carried out in order to determine the role of endothelial cells in the relaxation caused by Ang II in dog renal arteries and by histamine in dog mesenteric arteries, which is considered to result from the release of PGI_2.

Methods

Mongrel dogs of either sex, weighing 7 to 13 kg, were anaesthetized with intraperitoneal injections of sodium thiopentone (50 mg kg^{-1}) and killed by bleeding from the common carotid arteries. The kidney was rapidly removed. Intrarenal, interlobar branches of the renal artery (0.5 to 0.8 mm o.d.) were isolated. Distal portions of the superior mesenteric artery (0.6 to 0.8 mm) were also isolated. The arteries were cut into spiral strips, approximately 20 mm long. The strips were fixed vertically between hooks in a muscle bath containing the modified Ringer-Locke solution, which was aerated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37 ± 0.3°C. The hook fixing the upper end of the strips was connected to the lever of a force-displacement transducer (Nihonkohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 g sufficient to induce the maximum contraction (Toda *et al.*, 1978). Constituents of the solution were as follows (mM); Na^+ 144.8, K^+ 5.4, Ca^{2+} 2.2, Mg^{2+} 1.0, Cl^- 131.6, HCO_3^- 25.0, and glucose 5.6. The pH of the solution was 7.3 to 7.4. Before the start of experiments, the arterial strips were allowed to equilibrate for 60 to 90 min in the bathing media, during which time the bathing fluids were replaced every 10 to 15 min.

Isometric contractions and relaxations were recorded on an ink-writing oscillograph (Nihonkohden Kogyo Co.). The contractile response to 30 mM K^+ was first obtained. The arterial strips were washed three times with fresh media and equilibrated for 40 to 50 min. The strips were partially pre-contracted with $PGF_{2\alpha}$ (10^{-7} to 6×10^{-7} M); the contraction was in a range between 20 and 30% of the contraction induced by 30 mM K^+. Ang II in a concentration of 10^{-7} M or histamine in a concentration of 10^{-6} M was added, and after the tension had returned and stabilized, PGI_2 (10^{-8} M) and isoprenaline (10^{-7} M) were added. The concentration of Ang II used was sufficient to produce the maximum relaxation (Toda & Miyazaki, 1981), and the concentration of histamine was approximately the median effective concentration (Konishi *et al.*, 1981). At the end of each series of experiments, papaverine (10^{-4} M) was added to produce the maximum relaxation (Toda, 1974); relaxations induced by Ang II, histamine, PGI_2, isoprenaline or acetylcholine relative to those induced by papaverine are presented. The response to Ang II was obtained three times to confirm the reproducibility. The third response was taken as a control. After the third trial, tachyphylaxis did not develop (Toda & Miyazaki, 1981). Arterial strips had been treated for 30 min with aspirin or indomethacin, before Ang II or histamine was added.

One of two renal or mesenteric arterial strips obtained from the same dog was used as a control, and the intimal surface of the other strip was rubbed with filter paper as described by Furchgott & Zawadzki (1980). The endothelium was examined histologically by a silver staining procedure (Caplan *et al.*, 1974). The strips were immersed successively in the dark in the HEPES buffered solution (pH 7.4) containing 4.6% glucose for 150 s, 0.4% $AgNO_3$ in 4.2% glucose solution for 90 s, and 4.6% glucose solution for 60 s. Marked silver-stained demarcation of endothelial cells was seen only in the unrubbed strip in experiments with 4 pairs of mesenteric arteries and 3 pairs of renal arteries from different dogs. In rubbed and unrubbed strips pre-contracted with $PGF_{2\alpha}$, responses to 10^{-6} M acetylcholine were compared.

The results shown in the text and figures are expressed as mean values ± s.e.mean. Statistical analyses were made using Student's paired and unpaired *t* test. Drugs used were angiotensin II (Ang II, Protein Research Foundation, Osaka, Japan), prostaglandin I_2 sodium salt (PGI_2, Ono Pharmaceutical Co., Osaka), acetylsalicylic acid (aspirin), indomethacin, acetylcholine chloride, (±)-isoprenaline hydrochloride, prostaglandin $F_{2\alpha}$ and papaverine hydrochloride.

Results

Angiotensin II-induced relaxation in renal arteries

The addition of 10^{-7} M Ang II caused a slight, transient contraction followed by a moderate relaxation in renal arterial strips partially precontracted with $PGF_{2\alpha}$ (Figure 1). The Ang-induced relaxation was

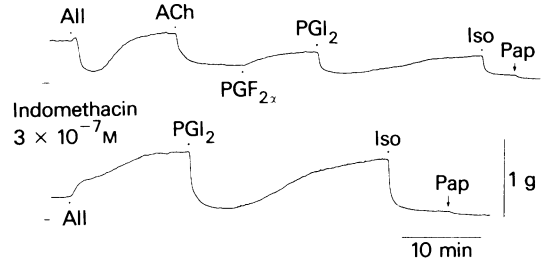

Figure 1 Responses of a renal arterial strip of the dog to angiotensin II (AII, 10^{-7} M), acetylcholine (ACh, 10^{-6} M), prostacyclin (PGI_2, 10^{-8} M) and isoprenaline (Iso, 10^{-7} M) before and after treatment with indomethacin. The strip was partially precontracted with $PGF_{2\alpha}$ (3×10^{-7} M for upper tracings and 2×10^{-7} M for the lower); horizontal lines just left of the tracings represent the level prior to the addition of $PGF_{2\alpha}$. In the upper tracing, $PGF_{2\alpha}$, 10^{-7} M was additionally applied to restore the active tone after acetylcholine. Pap = 10^{-4} M papaverine.

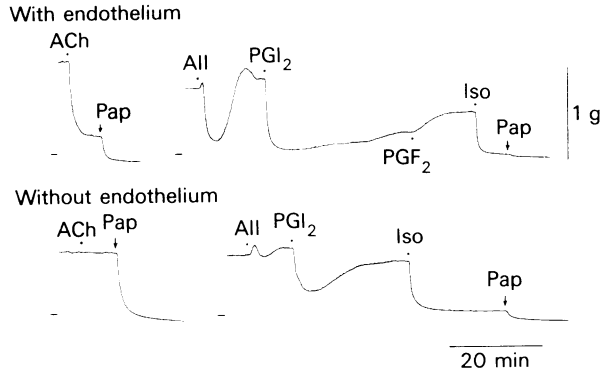

Figure 2 Responses to acetylcholine (ACh, 10^{-6} M), angiotensin II (A II, 10^{-7} M), prostacyclin (PGI_2, 10^{-8} M) and isoprenaline (Iso, 10^{-7} M) of dog renal arterial strips with (upper tracings) and without the endothelium (lower). Horizontal lines just left of the tracings represent the level prior to the addition of $PGF_{2\alpha}$ (4×10^{-7} M). After the addition of PGI_2 in the upper right tracing, $PGF_{2\alpha}$ (10^{-7} M) was added to restore the active tone. Pap = 10^{-4} M papaverine.

reversed to a contraction by treatment with indomethacin (3×10^{-7} M) or aspirin (5×10^{-5} M), although these drugs did not attenuate the relaxant response to PGI_2 and isoprenaline (Figure 1). This action of the octapeptide has been analyzed previously and the involvement of PGI_2, released from the arterial wall, in the relaxation has been suggested (Toda & Miyazaki, 1981; Toda, 1981).

Removal of the endothelium by rubbing reversed the acetylcholine-induced relaxation to a slight contraction in one of 23 renal arterial strips, abolished the relaxation as shown in Figure 2 in 9 strips, and markedly attenuated the relaxation in the remaining 13. Ang-induced relaxations were also reversed to slight contractions by rubbing in 5 out of 23 strips and markedly suppressed in the remaining 18 (Figure 2). The transient contraction induced by 10^{-7} M Ang II was not appreciably altered; mean values in control and rubbed strips were 192 ± 36 mg and 153 ± 34 mg ($n = 23$), respectively. Relaxant responses to PGI_2 and isoprenaline were attenuated only slightly. Quantitative data are summarized in Figure 3. Relax-

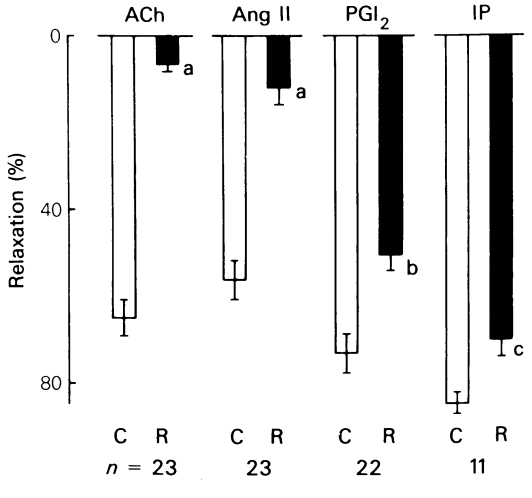

Figure 3 Relaxations induced by acetylcholine (ACh, 10^{-6} M), angiotensin II (Ang II, 10^{-7} M), prostacyclin (PGI_2, 10^{-8} M) and isoprenaline (Iso, 10^{-7} M) in control (open columns) and rubbed (solid columns) renal arterial strips. Relaxations induced by 10^{-4} M papaverine were taken as 100%; mean absolute values in control and rubbed strips were 718 ± 57 mg and 709 ± 66 mg ($n = 23$), respectively, with acetylcholine, 877 ± 74 mg and 797 ± 64 mg ($n = 23$), respectively, with Ang II, 1047 ± 77 mg and 847 ± 57 mg ($n = 22$), respectively, with PGI_2, and 588 ± 66 mg and 660 ± 57 mg ($n = 11$), respectively, with isoprenaline. n = number of preparations used. [a]Significantly different from control, $P < 0.001$;[b] $P < 0.01$;[c] $P < 0.02$.

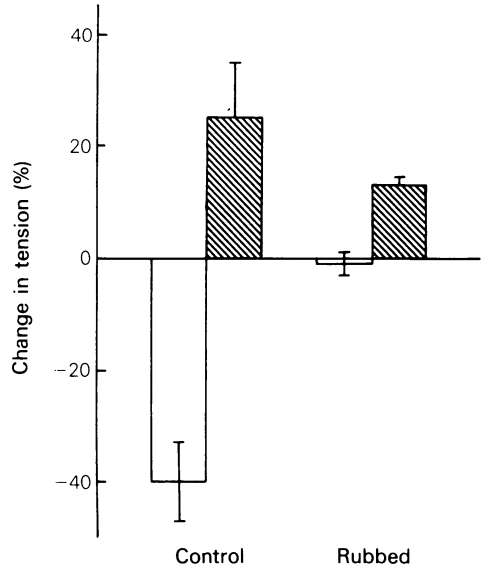

Figure 4 Modification by indomethacin (3×10^{-7} M) of the response to angiotensin II (Ang II) 10^{-7} M of control and rubbed renal arterial strips: open columns, non-treated; hatched columns, indomethacin-treated. Relaxations induced by 10^{-4} M papaverine were taken as 100%; mean absolute values in control and rubbed strips before treatment with indomethacin were 718 ± 130 mg and 623 ± 169 mg ($n = 5$), respectively. Contractions induced by 30 mM K^+ were taken as 100%; mean absolute values in control and rubbed strips treated with indomethacin were 3952 ± 489 mg and 3346 ± 405 mg ($n = 5$), respectively.

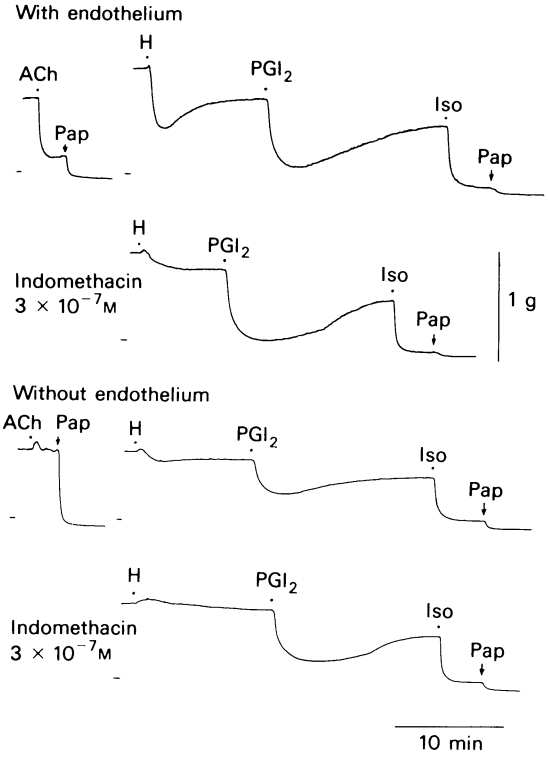

Figure 5 Responses of control (upper two tracings) and rubbed mesenteric arterial strips (lower two) to 10^{-6}M acetylcholine (ACh), 10^{-6}M histamine (H), 10^{-8}M prostacyclin (PGI_2) and 10^{-7}M isoprenaline (Iso) before and after treatment with indomethacin. Two strips obtained from the same dog were partially precontracted with $PGF_{2\alpha}$ (2×10^{-7}M); horizontal lines just left of the tracings represent the level prior to the addition of $PGF_{2\alpha}$. Pap, 10^{-4}M papaverine.

ations induced by acetylcholine (10^{-6}M) and Ang II (10^{-7}M) were markedly inhibited by removal of the endothelium, whereas those induced by PGI_2 (10^{-8}M) and isoprenaline (10^{-7}M) were attenuated to an appreciably smaller extent. Average inhibitions by rubbing of the relaxation induced by Ang II and PGI_2 were 78.1 and 31.5%, respectively. Such a difference does not appear to derive from a greater relaxation induced by PGI_2 than by Ang II (73.4 ± 4.6% vs. 56.2 ± 4.5% relative to relaxations induced by 10^{-4}M papaverine), since in preparations showing less relaxation induced by PGI_2 (mean value of 57.8%, $n = 10$), the inhibition of the response by rubbing was only 21.2%. Papaverine in a concentration of 10^{-4}M maximally relaxed the control and rubbed arterial strips; further increase in the concentration to 3×10^{-4}M did not produce an additional relaxation.

In 5 control strips, Ang-induced relaxations were reversed to contractions, by treatment with indomethacin as shown in Figure 4 (left two columns; significantly different, $P < 0.01$). Treatment of rubbed strips with indomethacin also reversed the slight relaxation to a contraction (right two columns), the difference again being statistically significant ($P < 0.01$).

Histamine-induced relaxation in mesenteric arteries

The addition of histamine in a concentration of 10^{-6}M produced a rapidly-developing relaxation in dog mesenteric arterial strips partially precontracted with $PGF_{2\alpha}$, the minimum level of tension being partially restored (Figure 5, top). The relaxant response was attenuated by removal of the endothelium. However, relaxations induced by PGI_2 and isoprenaline were not influenced. Figure 6 shows quantitative data obtained in control and rubbed strips. Relaxations induced by acetylcholine were reversed to contractions by removal of the endothelium in 8 out of 17 strips (Figure 5, lower left), abolished in 3 strips and markedly attenuated in the remaining 6. Histamine-induced relaxations were

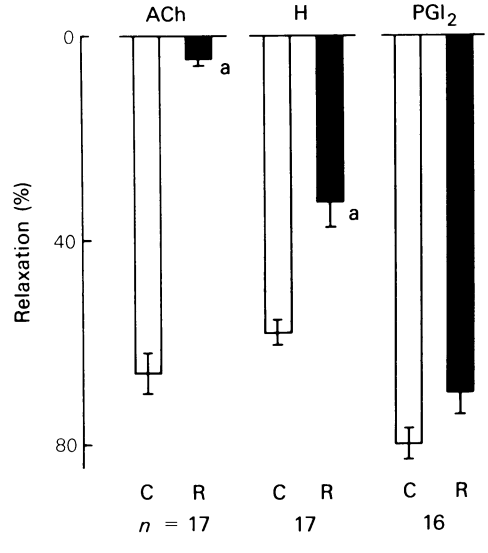

Figure 6 Relaxations induced by acetylcholine (ACh, 10^{-6}M), histamine (H, 10^{-6}M) and prostacyclin (PGI_2, 10^{-8}M) in control (open columns) and rubbed (closed columns) mesenteric arterial strips. Relaxations induced by 10^{-4}M papaverine were taken as 100%; mean absolute values in control and rubbed strips were 615 ± 50 mg and 536 ± 52 mg ($n = 17$), respectively, with acetylcholine, 696 ± 48 mg and 612 ± 66 mg ($n = 17$), respectively, with histamine, and 394 ± 49 mg and 441 ± 78 mg ($n = 16$), respectively, with PGI_2. [a]Significantly different from control, $P < 0.001$.

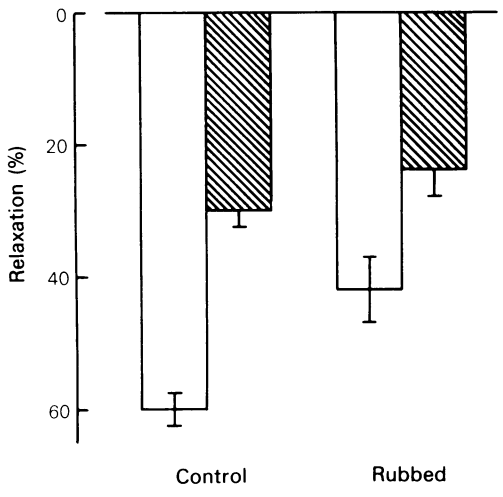

Figure 7 Modification by indomethacin (3×10^{-7} M) of the relaxant response to histamine of control and rubbed mesenteric arterial strips. Relaxations induced by 10^{-4} M papaverine were taken as 100%; mean absolute values in control and rubbed strips before treatment with indomethacin (open columns) were 690 ± 77 mg and 603 ± 132 mg ($n = 8$), respectively, and those in the strips treated with indomethacin (hatched columns) were 696 ± 118 mg and 561 ± 82 mg ($n = 8$), respectively.

moderately attenuated, while relaxations induced by PGI_2 tended to be decreased, the difference being statistically insignificant. Mean inhibitions of the relaxation induced by histamine and PGI_2 by rubbing were 44.2 and 11.8%, respectively. In 5 strips, in which only a moderate relaxation was induced by PGI_2 (62.6% vs. histamine-induced relaxation of 58.2%), the inhibition of the relaxation by rubbing was only 19.8%, which was appreciably less than the inhibition of the histamine-induced relaxation (44.2%).

Relaxant responses of 8 mesenteric arterial strips to histamine were significantly attenuated by treatment with indomethacin (3×10^{-7} M) ($P < 0.001$, Figures 5 and 7, left two columns). In the strips in which the histamine-induced relaxation was significantly attenuated by rubbing ($P < 0.05$, right open column as compared with left open column), indomethacin produced additional inhibition of the relaxation, the difference being statistically significant ($P < 0.05$, Figure 7, right two columns).

Discussion

Rubbing of the intimal surface of dog renal and mesenteric arterial strips abolished the relaxant response to acetylcholine almost completely, suggesting the removal of endothelial cells (Furchgott & Zawadzki, 1980). This removal was actually observed by histological examination of these arteries with silver staining. Such a removal of the endothelium markedly suppressed the relaxant response of renal arteries to Ang II but attenuated only slightly the response to exogenously applied PGI_2. The Ang-induced relaxations are associated possibly with the release of PGI_2 from the arterial wall (Toda & Miyazaki, 1981; Toda, 1981), since the relaxation is reversed to a contraction by cyclo-oxygenase inhibitors, and suppressed by 15-hydroperoxy-arachidonic acid, tranylcypromine or dexamethasone. Therefore, endothelial cells appear to be involved in the release of PGI_2 from the arterial wall. Three possibilities may be considered as the mechanism of Ang action; (1) arachidonic acid is released from endothelial cells and converted to PGI_2 at the same place; (2) arachidonic acid is released from endothelial cells and converted to PGI_2 in the other tissues, such as the smooth muscle, fibroblast and collagen; and (3) the substrate is released from these tissues and converted to PGI_2 in the endothelium. PGI_2 in a detectable amount is reported to be synthesized from arachidonic acid or PGG_2 exclusively in endothelial cells but not in medial smooth muscle cells and fibroblasts in culture (MacIntyre *et al.*, 1978). If this is the case in isolated renal arteries of dog, the second possibility could be excluded. The presence of Ang II receptors is indicated in cultured human vascular endothelial cells (Gimbrone & Alexander, 1975); stimulation of the receptors appears to activate phospholipase A_2 (Nolan *et al.*, 1981). Therefore, the first possibility is most likely. There is evidence supporting the idea that the intimal surface of arteries is the major site for generating PGI_2 (Moncada *et al.*, 1977; Eldor *et al.*, 1981).

Removal of the endothelium markedly suppressed the Ang-induced relaxation, whereas treatment with cyclo-oxygenase inhibitors reversed the relaxation to a contraction. The release of PGI_2 from renal arterial strips does not appear to be abolished by rubbing. Since even in the arteries showing complete abolition of relaxation induced by acetylcholine, the Ang-induced relaxation was not reversed to a contraction (Figure 2), the release of PGI_2 from arterial tissues other than the endothelium (Goldsmith, 1982) would be more probable rather than incomplete removal of endothelial cells.

It has been postulated that stimulation of H_1-receptors by histamine liberates PGI_2 from dog mesenteric and gastroepiploic arteries, resulting in the relaxation, since the rapid phase of relaxations is significantly attenuated by cyclo-oxygenase inhibitors and H_1-antagonists (Toda *et al.*, 1982). In

the arteries treated with H_1-antagonists, the response to histamine is not influenced by cyclo-oxygenase inhibitors. The histamine-induced relaxation was also attenuated by removal of the endothelium, while the relaxation induced by PGI_2 was not significantly influenced. Again, endothelial cells appear to play an important role in releasing PGI_2 from the arterial wall. Histamine has been demonstrated to stimulate PGI_2 synthesis via H_1-receptors in cultured human umbilical vein endothelial cells (Baenziger *et al.*, 1980). It appears that histamine receptors are present in endothelial cells; stimulation of the receptors activates phospholipase A_2, releases arachidonic acid and promotes the synthesis of PGI_2. Histamine reportedly releases other vasodilator substance(s), possibly lipoxygenase product(s), from the endothelium of rat isolated aortae, the release being also mediated by H_1-receptors (Van de Voorde & Leusen, 1983). This is not the case in dog mesenteric arteries, since histamine-induced relaxations of these arteries are suppressed by aspirin, indomethacin and tranylcypromine (Toda *et al.*, 1982). In the present study, indomethacin attenuated the relaxant response to histamine to a greater extent than removal of the endothelium, and caused a further attenuation of the response to histamine in preparations from which the endothelium was removed. Therefore, it is suggested that the endothelium is not the only site responsible for the release of PGI_2 from the arterial wall.

The author thanks Mr M. Nakajima and Miss I. Shimizu for excellent technical assistance. This work was supported in part by Scientific Research Fund from Takeda Medical Research Foundation.

References

BAENZIGER, N.L., FORCE, L.E. & BECHERER, P.R. (1980). Histamine stimulates prostacyclin synthesis in cultured human umbilical vein endothelial cells. *Biochem. biophys. Res. Commun.*, **92,** 1435–1440.

CAPLAN, B.A., GERRITY, R.G. & SCHWARTZ, C.J. (1974). Endothelial cell morphology in focal areas of in vivo Evans Blue uptake in the young pig aorta. I. Quantitative light microscopic findings. *Exp. mol. Path.*, **21,** 102–117.

DESJARDINS-GIASSON, S., GUTKOWSKA, J., GARCIA, R. & GENEST, J. (1982). Effect of angiotensin II and norepinephrine on release of prostaglandins E_2 and I_2 by the perfused rat mesenteric artery. *Prostaglandins*, **24,** 105–114.

DUSTING, G.J. (1981). Angiotensin-induced release of a prostacyclin-like substance from the lungs. *J. cardiovasc. Pharmac.*, **3,** 197–206.

ELDOR, A., FALCONE, D.J., HAJJAR, D.P., MINICK, D.R. & WEKSLER, B.B. (1981). Recovery of prostacyclin production by de-endothelialized rabbit aorta. Critical role of neointimal smooth muscle cells. *J. clin. Invest.*, **67,** 735–741.

FURCHGOTT, R.J. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288,** 373–376.

FURCHGOTT, R.F., ZAWADZKI, J.V. & CHERRY, P.D. (1981). Role of endothelium in the vasodilator response to acetylcholine. In *Vasodilation*, ed. Vanhoutte, P.M. & Leusen, I. pp. 49–66. New York: Raven Press.

GIMBRONE, M.A., Jr. & ALEXANDER, R.W. (1975). Angiotensin II stimulation of prostaglandin production in cultured human vascular endothelium. *Science*, **189,** 219–220.

GOLDSMITH, J.C. (1982). Contribution of the subendothelium to prostacyclin release after vascular injury. *J. Lab. clin. Med.*, **100,** 574–584.

KONISHI, M., TODA, N. & YAMAMOTO, M. (1981). Different mechanisms of action of histamine in isolated arteries of the dog. *Br. J. Pharmac.*, **74,** 111–118.

MACINTYRE, D.E., PEARSON, J.D. & GORDON, J.L. Localisation and stimulation of prostacyclin production in vascular cells. *Nature*, **271,** 549–551.

MARCUS, A.J., WEKSLER, B.B. & JAFFE, E.A. (1978). Enzymatic conversion of prostaglandin endoperoxide H_2 and arachidonic acid to prostacyclin by cultured human endothelial cells. *J. biol. Chem.*, **253,** 7138–7141.

MONCADA, S., HERMAN, A.G., HIGGS, E.A. & VANE, J.R. (1977). Differential formation of prostacyclin (PGX or PGI_2) by layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. *Thromb. Res.*, **11,** 323–344.

NOLAN, R.D., DUSTING, G.J. & MARTIN, T.J. (1981). Phospholipase inhibition and the mechanism of angiotensin-induced prostacyclin release from rat mesenteric vasculature. *Biochem. Pharmac.*, **30,** 2121–2125.

SHEBUSKI, R.J. & AIKEN, J.W. (1980). Angiotensin II stimulation of renal prostaglandin synthesis elevates circulating prostacyclin in the dog. *J. cardiovasc. Pharmac.*, **2,** 667–677.

SHIBOUTA, Y., INADA, Y., TERASHITA, Z., NISHIKAWA, K., KIKUCHI, S. & SHIMAMOTO, K. (1979). Angiotensin II-stimulated release of thromboxane A_2 and prostacyclin (PGI_2) in isolated, perfused kidneys of spontaneously hypertensive rats. *Biochem. Pharmac.*, **28,** 3601–3609.

TODA, N. (1974). The action of vasodilating drugs on isolated basilar, coronary and mesenteric arteries of the dog. *J. Pharmac. exp. Ther.*, **191,** 139–146.

TODA, N. (1981). Mechanism of vascular smooth muscle relaxation induced by angiotensins. In *Vasodilatation.* ed. Vanhoutte, P.M. & Leusen, I. pp. 151–157. New York: Raven Press.

TODA, N., HATANO, Y. & HAYASHI, S. (1978). Modification by stretches of the mechanical response of isolated cerebral and extracerebral arteries to vasoactive agents. *Pflügers Arch.*, **374,** 73–77.

TODA, N., KONISHI, M. & MIYAZAKI, M. (1982). Involvement of endogenous prostaglandin I_2 in the vascular action of histamine in dogs. *J. Pharmac. exp. Ther.*, **223,** 257–262.

TODA, N. & MIYAZAKI, M. (1981). Angiotensin-induced relaxation in isolated dog renal and cerebral arteries. *Am. J. Physiol.*, **240,** H247–H254.

VAN DE VOORDE, J. & LEUSEN, I. (1983). Role of the endothelium in the vasodilator response of rat thoracic aorta to histamine. *Eur. J. Pharmac.*, **87,** 113–120.

WEKSLER, B.B., LEVY, C.W. & JAFFE, E.C. (1978). Stimulation of endothelial cell prostacyclin production by thrombine, trypsin, and the ionophore A 23187. *J. clin. Invest.*, **62,** 923–930.

(Received July 6, 1983.)