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Mechanisms Underlying Relaxations Caused by Angiotensin II and Its Analogs in Isolated Rabbit Mesenteric Artery

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In the present study, we characterized the angiotensin II (AII)-induced relaxations in the phenylephrine-precontracted rabbit mesenteric arteries with endothelium. 1) AII-induced relaxation was consistently observed in the rabbit mesenteric arteries with and without endothelium, but not in the aortic segment with endothelium. 2) AII-induced endothelium-dependent relaxation was markedly inhibited by N^{W}-nitro-L-arginine (L-NNA, 100 μM), methylene blue (10 μM) and LY83583 (10 μM), respectively. 3) Inhibition of cyclooxygenase with indomethacin (10 μM) strongly decreased the vasorelaxant response to AII irrespective of the presence of endothelium. 4) 7-Ethoxyresorufin (1 μM) and clotrimazole (1 μM), inhibitors of cytochrome P-450-dependent arachidonic acid metabolism, greatly attenuated the vasodilator response to AII. 5) Carbacyclin, arachidonic acid and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) caused concentration-dependent relaxations in the mesenteric artery with endothelium, which were inhibited by L-NNA and methylene blue. 6) AII and $PGF_{2\alpha}$ significantly stimulated cyclic GMP formation in the mesenteric arteries with endothelium, which was inhibited by L-NNA and methylene blue, respectively. 7) AII enhanced synthesis of $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ from the arterial segments with endothelium, which was inhibitable by indomethacin, but not by L-NNA.

In conclusion, the vasorelaxant responses to AII of the rabbit mesenteric artery with endothelium are subserved by arachidonic acid and its metabolites produced via activation of cyclooxygenase and cytochrome P-450 enzyme as well as by nitric oxide.

Key Words: Angiotensin II, Rabbit mesenteric artery, Vasorelaxation

INTRODUCTION

Angiotensin II (AII) is the most prominent member of the renin-angiotensin system due to its potent vasoconstrictor activity which is mediated by stimulation of the AT_1 receptors on the vascular smooth muscle (Timmermans et al, 1991) and systemic administration of AII causes an elevation of arterial blood pressure due to increased systemic vascular resistance (Jackson & Garrison, 1996; Timmermans et al, 1991).

Otherwise, AII acts as a vasodilator in certain vascular beds (Haberl et al, 1990; Hasegawa et al, 1993). Toda and Miyazaki (1981) have demonstrated the angiotensin-induced relaxation in isolated dog renal and cerebral arteries. Yamaguchi and Nishimura (1988) have also reported the [Sar^1-Val^5]AII-induced relaxation of fowl aorta. It is known that the presence of endothelium and release of endothelium-derived relaxing factor by a number of agents balance the vasomotor tone (Furchgott & Zawadzki, 1980; Moncada

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et al, 1991). Recently, we observed that AII induced a concentration-dependent relaxation at lower concentration ranges of AII (0.1 ~ 100 nM) in the mesenteric arteries irrespective of the presence of endothelium, but it was not the same case in the aorta. It is not clarified what signal transduction is related with the AII-induced relaxation.

Thus, this study was designed to characterize the AII-induced vasorelaxation in the rabbit mesenteric arteries with endothelium. Particular attention was directed to gathering informations about the mechanism(s) underlying AII-induced endothelium-dependent relaxations in relation with activation of nitric oxide synthase, cyclooxygenase and cytochrome P-450. Further, we measured the AII-induced synthesis of prostanoids (prostacyclin and prostaglandin $F_{2\alpha}$, $PGF_{2\alpha}$) and the AII- and $PGF_{2\alpha}$-stimulated guanosine 3',5'-monophosphate (cyclic GMP) production in the rabbit mesenteric arteries with endothelium.

METHODS

Measurement of force development

Adult New Zealand White rabbits that weighed 2.5 to 3 kg (either sex) were sacrificed by a blow on the skull and exsanguinated. Thoracic aorta, abdominal aorta and mesenteric arteries with the second order branches were immediately removed to the oxygenated physiological salt solution (PSS) at room temperature. After they were cleaned up free from fat and connective tissues, they were dissected into rings approximately 2 mm in length. The mean diameter of the second order branches of the mesenteric arteries was 0.64 ± 0.37 mm.

When the denudation of endothelium was necessary, the inside of the rings was rubbed with a wooden stick and, thereafter, the ring segments were immersed and shaken in PSS containing CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate, 0.3%) for 10 sec and rinsed in fresh PSS. The PSS had the following composition (mM): NaCl 130, KCl 4.7, NaH_2PO_4 1.18, $MgSO_4$ 1.17, $CaCl_2$ 1.6, $NaHCO_3$ 14.9 and dextrose 5.5. The isolated rings were suspended between stainless steel hooks and mounted in 4-ml organ baths filled with warmed (37°C) and oxygenated (95% O_2, 5% CO_2) PSS. Isometric force was measured with force displacement transducers (FT 0.3, Grass Instrument Co., Cambridge, MA). A tension of 2 g was applied and the rings were equilibrated for 90 min by changing the PSS and adjusting the preload to 2 g.

After a stable baseline was obtained, the rings were contracted with phenylephrine at concentration of 1 ~ 3 μM which produced 70 ~ 80% of KCl (100 mM)-induced contraction. Thereafter, acetylcholine (1 μM) was added to the baths to examine the functional integrity of the endothelium. Only tissues which relaxed by more than 50% of the phenylephrine-induced tone following addition of acetylcholine were considered to have undamaged endothelium. After replacing the contents of the baths with fresh buffer every 15 min, the rings were contracted with phenylephrine (1 ~ 3 μM) and the responses of the tissues induced by AII (0.1 nM ~ 1 μM) were recorded. The same experiment was repeated 1 hour later with the same tissue in the presence of several inhibitors. AII inhibitors were added to the baths 20 min before addition of AII. Relaxation was expressed as a percentage of the steady state contraction height induced by phenylephrine (1 ~ 3 μM). If the steady height of the established contraction was not maintained, the strip was discarded.

Cyclic nucleotide determination

Rabbits were sacrificed by a blow on the skull and exsanguinated from carotid arteries. The mesenteric artery and its branches were rapidly isolated. Cyclic GMP was measured with a radioimmunoassay kit purchased from Amersham Life Science Products, and the cyclic GMP products were expressed as fmol/mg protein.

Measurement of prostanoids

The isolated mesenteric arterial segments were washed with PSS and then preincubated in PSS for 30 min at 37°C. Thereafter, the segments were further incubated for 20 min (37°C) in the presence of AII with and without antagonists (i.e., indomethacin and L-NNA). After determination of weight, the tissue was chopped with scissors and tris buffer (50 mM, pH 8.0 at 25°C) was added (3 ml/g tissue) and then sonicated for 90 min at below 10°C. The amounts of $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ (a stable metabolite of PGI_2) released into the buffer medium were determined by

radioimmunoassay (according to 6-keto $PGF_{1\alpha}$ and $PGF_{2\alpha}$ assay system, code TRK790, Amersham).

Drugs

Angiotensin I, Angiotensin II (human Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), angiotensin III, phenylephrine HCl, indomethacin, acetylcholine chloride, aminoguanidine, dexamethasone, methylene blue, L-NNA, 7-ethoxyresorufin, clotrimazole, arachidonic acid, carbacyclin, PGE_2 and $PGF_{2\alpha}$ were purchased from Sigma Chemical Co (St. Louis, MO). L-NNA were dissolved in 0.05 N of HCl as a stock (10 mg/ml) and sonicated. LY 83583 (6-anilino-5,8-quinolinedione, 10 mM) was supplied by Eli Lilly (Indianapolis, IN) and dissolved in 10 mM ethanol.

Statistics

The results are expressed as means±S.E.M. Statistical differences between groups were determined by analysis of variance or Student's *t*-test. Significance was set at $p<0.05$.

RESULTS

In the present study, to characterize the AII-induced relaxation in the ring segments of the second order branches of rabbit mesenteric arteries, we used phenylephrine (1~3 μM) to evoke contraction. The mean contraction heights evoked by 1~3 μM phenylephrine were 3.7±0.4 g (n=54) and 3.9±0.4 g (n=14) in the presence and the absence of endothelium, between which there was no significant difference (Fig. 1).

Comparison of angiotensin II action with acetylcholine

In the ring segments of mesenteric arteries with and without endothelium, AII caused concentration-dependent contractions under basal tension as shown in Fig. 1. Otherwise, in mesenteric arterial segments precontracted with phenylephrine (1~3 μM), AII exerted concentration-dependent relaxations. AII-induced relaxation was significantly larger in the segments with endothelium than in the segments without endothelium (Fig. 2). The concentration of AII which exerts 50% of the maximum relaxation -log EC_{50s} was 9.37±0.28 M in the segments with endothelium and 8.15±0.41 M in the segments without endothelium. In the preliminary study, when the experiment for AII-induced relaxation was repeated with one-hour interval in the absence of inhibition, a significant reduction in AII-induced relaxation was not observed

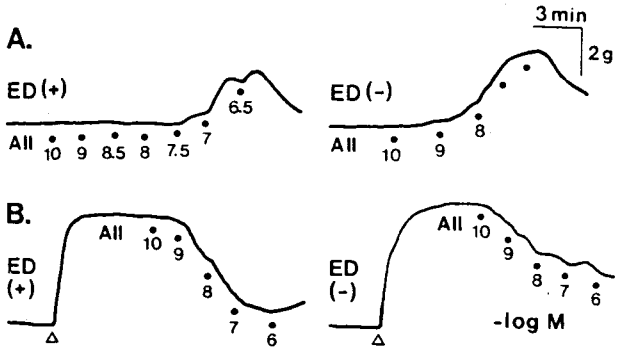

Fig. 1. Tracing graphs showing typical representation of angiotensin II (AII)-induced dual actions of the second order branches of rabbit mesenteric artery with (+) and without (−) endothelium (ED): AII-induced contraction in the arterial segment with (left) and without endothelium (right) (A). AII caused potent concentration-dependent relaxation in the precontracted ring segment with endothelium (ED, left), but less in the segment without endothelium (right). △, 3 μM Phenylephrine (B).

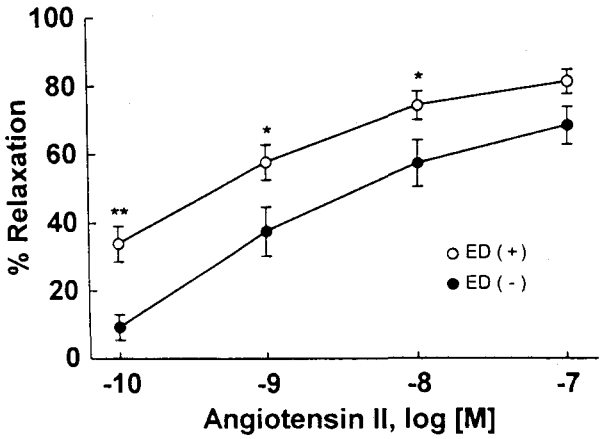

Fig. 2. Angiotensin II-induced endothelium (ED)-dependent (n=26) and -independent (n=13) relaxations in the rabbit isolated mesenteric arterial segments. *, $p<0.05$; **, $p<0.01$: significantly different from the corresponding values.

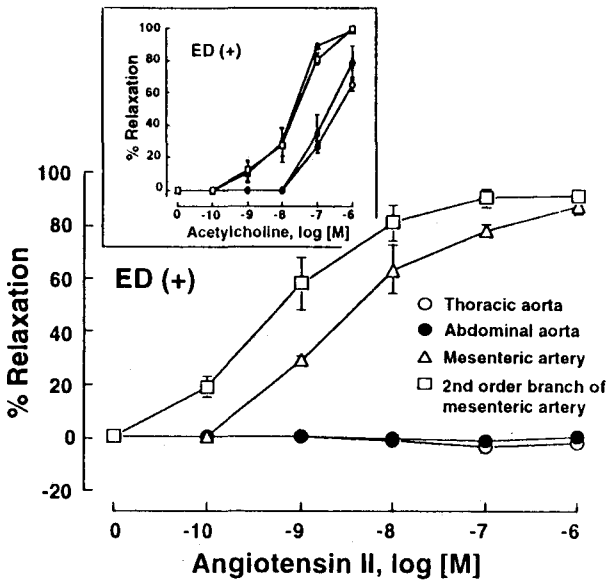

Fig. 3. Line graphs showing regional differences in the angiotensin II (AII)-induced, endothelium (ED)-dependent relaxation in the thoracic, abdominal aorta, main mesenteric artery and second order branches of mesenteric artery in comparison with acetylcholine (Inset). The results represent means ± S.E.M. from 4 ~ 5 experiments. AII-induced relaxation was not observed in the thoracic and abdominal aorta in contrast to acetylcholine-induced one.

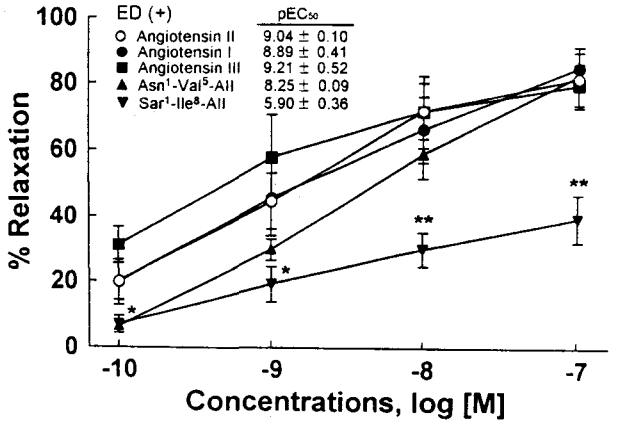

Fig. 4. Angiotensin II analog-induced endothelium-dependent relaxation in the rabbit mesenteric arterial ring segments. The results represent means ± S.E.M. from 4 ~ 5 experiments. *, $p<0.05$; **, $p<0.01$: significantly different from the corresponding values of angiotensin II.

(data not shown).

Difference between mesenteric artery and aorta

In the phenylephrine-induced precontraction of mesenteric artery with endothelium, AII consistently elicited a relaxation but it was not the same case in the aorta even in the presence of endothelium, while acetylcholine caused a relaxation in both preparations with endothelium (Fig. 3). The second order branches of the mesenteric arteries showed relatively larger relaxation than the main arteries.

Relaxant effects of angiotensin II analogs

As shown in Fig. 4, AII and its analogs, angiotensin I, angiotensin III, Asn^1-Val^5-AII and Sar^1-Ile^8-AII caused concentration-dependent relaxations in the second order branches of mesenteric arteries with this order of potency with -log EC_{50s} of 9.04 ± 0.10, 8.89 ± 0.41, 9.21 ± 0.52, 8.25 ± 0.09 and 5.90 ± 0.36 M, respectively.

Effects of L-arginine analog and guanylate cyclase inhibitors

When the mesenteric arteries with endothelium were pretreated with L-NNA (100 μM), the contraction heights induced by phenylephrine (1 ~ 3 μM) were significantly larger than those observed in the absence of L-NNA ($p<0.05$) (Table 1). In the precontracted mesenteric artery, AII-induced relaxation was mark-

Table 1. Comparison of phenylephrine (1-3 μM)-induced contractions in the mesenteric artery with endothelium in the absence and the presence of some inhibitors

Inhibitors	n	Contractions, g	p value
Vehicle	54	3.7 ± 0.4	
L-NNA (300 μM)	6	4.4 ± 0.6	0.05
Indomethacin (10 μM)	8	4.4 ± 0.6	0.05
Clotrimazole (1 μM)	5	3.3 ± 0.6	NS
7-Ethoxyresorufin (1 μM)	5	5.9 ± 0.3	0.05
Dexamethasone (10 μM)	6	4.2 ± 0.6	NS
Aminoguanidine (100 μM)	4	4.0 ± 0.8	NS

Each value represents mean ± S.E.M.
n, number of experiments; L-NNA, N_w-nitro-L-arginine

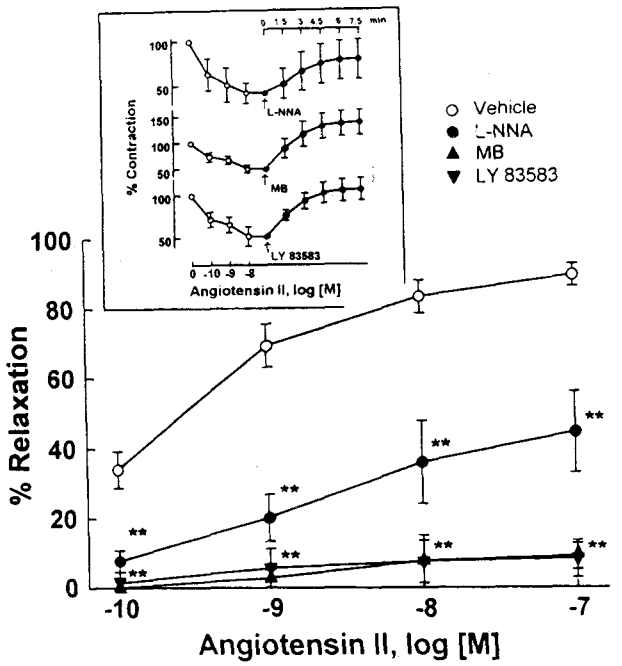

Fig. 5. Effects of pretreatment with N_w-nitro-L-arginine (L-NNA, 100 μM), methylene blue (MB, 10 μM), and LY 83583 (10 μM) on the angiotensin II-induced endothelium-dependent relaxation of the rabbit mesenteric arteries, respectively. Between vehicle and inhibitor-treated groups, the difference observed was significant at $p < 0.05$ in every concentration. Inset: reverse of AII-induced relaxation to contraction by postapplication (↑) of L-NNA (100 μM), MB (10 μM) and LY 83583 (10 μM), respectively. Each symbol represents mean ± S.E.M. from 4 ~ 6 experiments.

edly inhibited by L-NNA (100 μM), methylene blue (10 μM) and LY83583 (10 μM) as demonstrated in Fig. 5. Alternatively, the AII-induced relaxation turned to contraction by postapplication of L-NNA (100 μM), methylene blue (10 μM) and LY83583 (10 μM), respectively (Fig. 5, Inset).

Effect of cyclooxygenase inhibitor

In the mesenteric arteries pretreated with indomethacin (10 μM), phenylephrine-induced contraction heights were significantly larger than those without indomethacin (Table 1). The AII-induced relaxation was markedly inhibited by indomethacin, a cyclooxygenase inhibitor, in the mesenteric arteries with and without endothelium (Fig. 6).

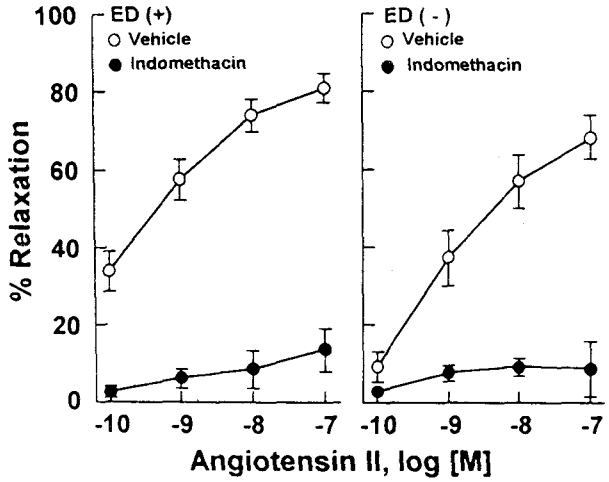

Fig. 6. Effect of indomethacin (10 μM) on the angiotensin II-induced, endothelium-dependent and -independent relaxations of the rabbit mesenteric resistance arteries with (+) and without (−) endothelium (ED). Each symbol represents mean ± S.E.M. from 4 ~ 8 experiments. The difference observed was significant at $p < 0.05$ between vehicle and indomethacin-treated groups.

Effect of cytochrome P-450-dependent arachidonic acid metabolism inhibitors

The phenylephrine-induced contraction in the mesenteric artery without endothelium was significantly larger than that with endothelium by pretreatment with 7-ethoxyresorufin (1 μM) but it was not the case for clotrimazole (1 μM) (Table 1). Nontheless, pretreatment with either 7-ethoxyresorufin (1 μM) or clotrimazole (1 μM) caused a substantial reduction in AII (0.1 ~ 100 nM)-induced relaxations (Table 1, Fig. 7).

Effects of dexamethasone and aminoguanidine

To identify whether phospholipase A_2 activation is involved in response to AII, we examined effect of dexamethasone, an inhibitor of phospholipase A_2 and inducible nitric oxide synthase in comparison with aminoguanidine, an inhibitor of inducible nitric oxide synthase. AII-induced relaxation was significantly suppressed by dexamethasone (10 μM) but not by aminoguanidine (100 μM) (Fig. 8). We further assessed the effect of time-dependence of dexamethasone pretreatment (20, 40 and 60 min). However, the incubation time longer than 20 min showed little

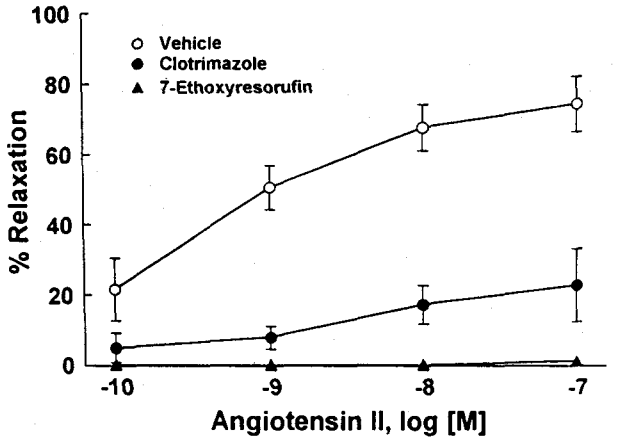

Fig. 7. Effect of 7-ethoxyresorufin (1 μM) and clotrimazole (1 μM), inhibitors of cytochrome P-450-dependent arachidonic acid metabolism, on the angiotensin II-induced relaxation in the rabbit mesenteric arteries with endothelium. Each symbol represents mean ± S.E.M. from 5 ~ 11 experiments. Between vehicle and inhibitor-treated groups the difference observed was significant at $p < 0.05$.

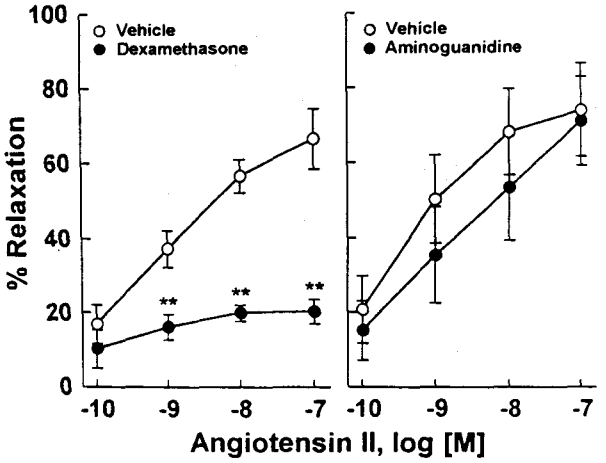

Fig. 8. Effect of dexamethasone (10 μM) and aminoguanidine (100 μM) on the angiotensin II-induced relaxation in the rabbit mesenteric arteries with endothelium. Each symbol represents means ± S.E.M. from 3 ~ 6 experiments. **, $p < 0.01$: significantly different between vehicle and dexamethasone-treated groups.

difference from 20 min incubation (data not shown).

Eicosanoid-induced relaxation

We identified the relaxant effects of $PGF_{2\alpha}$, PGE_2,

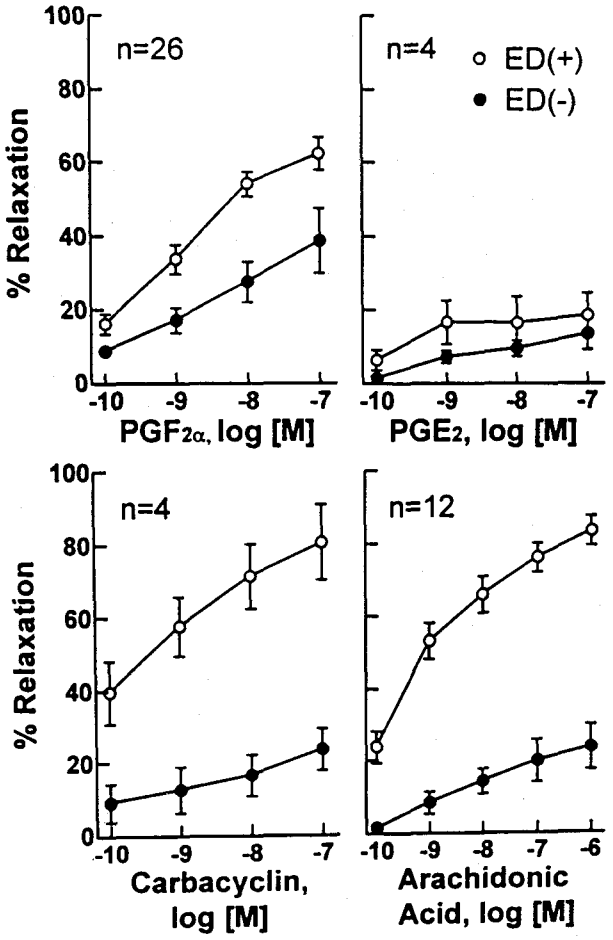

Fig. 9. Line graphs showing $PGF_{2\alpha}$, PGE_2, carbacyclin and arachidonic acid-induced relaxation of phenylephrine-precontracted rabbit mesenteric arteries with [ED(+)] and without endothelium [ED(−)]. The results represent means ± S.E.M. n, numbers of experiments.

carbacyclin (a stable analog of prostacyclin) and arachidonic acid in the rabbit mesenteric artery with and without endothelium as shown in Fig. 9. Both carbacyclin and arachidonic acid caused concentration-dependent relaxations. The relaxation was significantly enhanced in the mesenteric artery with endothelium by carbacyclin and arachidonic acid with -log EC_{50s} of 9.0 ± 0.9 M and 8.7 ± 0.1 M, respectively. However, the relaxant effect of $PGF_{2\alpha}$ (EC_{50}, 8.0 ± 0.8 M) was larger but less prominent than carbacyclin and arachidonic acid. PGE_2 caused little relaxation.

Cyclic nucleotide formation

In another series of experiments, we determined the AII- and $PGF_{2\alpha}$-induced stimulation of guanylate cyclase in the rabbit mesenteric arteries without and with endothelium (Table 2). In the preparations without endothelium, cyclic nucleotide levels were influenced by neither AII (10 nM) nor $PGF_{2\alpha}$ (10 nM). In the mesenteric arteries with intact endothelium, a significant stimulation of cyclic GMP production was observed by 10 nM of AII (basal cyclic GMP level, 7.9±2.0 to 31.7±3.2 fmol/mg protein, $p<0.05$) and 10 nM of $PGF_{2\alpha}$ (to 29.7±3.4 fmol/mg protein, $p<0.05$). Increased cyclic GMP production by AII and $PGF_{2\alpha}$ was significantly decreased by treatment with L-NNA (100 μM) and methylene blue (10 μM), respectively.

Table 2. Cyclic GMP formation in isolated rabbit mesenteric arterial segments without (−) and with (+) endothelium (ED) upon treatment with angiotensin II (10 nM) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10 nM) alone and in combination with N_w-nitro-L-arginine (L-NNA) and methylene blue

Inhibitors	Cyclic GMP, fmol/mg protein		
	Vehicle	Angiotensin II	$PGF_{2\alpha}$
ED (−)	7.4±1.9	9.0±1.2	6.9±0.5
ED (+)	7.9±2.0	31.7±3.2[a,b]	29.7±3.4[a,b]
+ L-NNA (100 μM)	7.0±0.7	13.3±2.3[c]	19.6±1.6[c]
+ Methylene blue (10 μM)	8.3±0.9	11.2±2.8[c]	18.3±1.5[c]

Each value represents mean±S.E.M. from 4 experiments which were conducted as duplicate. a, $p<0.05$ vs. vehicle; b, $p<0.05$ vs. values obtained in the mesenteric arteries without endothelium; c, $p<0.05$ vs. values determined in the absence of inhibitors.

Synthesis of prostanoids

The production of $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ from rabbit mesenteric arteries was not significantly different between arterial segments with and without endothelium in the absence of AII. In the arterial segments with endothelium, AII (10 nM) significantly enhanced the production of both $PGF_{2\alpha}$ (basal $PGF_{2\alpha}$, 39.8±2.8 to 104.1±12.7 ng/mg protein, $p<0.05$) and 6-keto $PGF_{1\alpha}$ (basal 6-keto $PGF_{1\alpha}$, 4.782±0.60 to 8.24±0.97 ng/mg protein, $p<0.05$). The increased production by AII of both $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ was significantly inhibited by pretreatment with indomethacin (10 μM) but not by L-NNA (100 μM) (Table 3).

Table 3. Synthesis of prostaglandin $F_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ in isolated rabbit mesenteric arterial segments without (−) and with (+) endothelium (ED) upon treatment with angiotensin II alone and in combination with indomethacin or N_w-nitro-L-arginine (L-NNA)

Treatment	ED	Synthesis of Prostanoids (ng/mg protein)	
		$PGF_{2\alpha}$	6-keto $PGF_{1\alpha}$
Vehicle	−	41.4±6.3	2.58±0.43
	+	39.8±2.8	4.78±0.60
Angiotensin II (10 nM)	+	104.1±12.7[a,b]	8.24±0.97[a,b]
Angiotensin II (10 nM) + indomethacin (10 μM)	+	40.9±6.1[c]	2.94±0.71[c]
Angiotensin II (10 nM) + L-NNA (100 μM)	+	80.4±9.8[a]	8.59±0.58[a]

Each value represents mean±S.E.M. from 4 experiments which were conducted as duplicate. a, $p<0.05$ vs vehicle; b, $p<0.05$ vs. values obtained in the mesenteric arteries without endothelium; c, $p<0.05$ vs. values of angiotensin II alone.

DISCUSSION

In the present study, AII caused a vasorelaxation in the precontracted mesenteric arterial segments as demonstrated in the fowl aorta (Yamaguchi & Nishimura, 1988) and a vasoconstriction under basal tension. The relaxant response could be displayed at low concentration ranges of AII (0.1～10 nM). AII analogs including angiotensin I, angiotensin III, Asn^1-Val^5-AII and Sar^1-Ile^8-AII also caused relaxations with this order of potency, indicating that the vasorelaxation is not specific to AII.

In our results, AII induced a relaxation independently of the presence of endothelium, which showed some difference from the report of Hasegawa et al. (1993). They observed AII-induced endothelium-dependent relaxation in the aortic smooth muscle of domestic fowl. In the mesenteric artery with intact endothelium, the magnititude of relaxation to AII was significantly larger than that observed in the endothelium-denuded segments, indicating release of some relaxing factor from endothelium.

In the present study, we employed three groups of inhibitors to characterize the relaxing factors. Firstly, AII-induced relaxation of the mesenteric arteries with endothelium was significantly inhibited by pretreatment with L-NNA (L-arginine analog, Palmer et al, 1988), methylene blue (Gruetter et al, 1981; Martin et al, 1985) and LY83583 (guanylate cyclase inhibitors, Schmidt et al, 1985). These results were consistent with the previous report of Vesely (1983), in that AII stimulated guanylate cyclase activity in homogenates of the aorta. Based on these findings, one possible mechanism by which AII causes vasodilation is the release of endothelium-derived relaxing factor (nitric oxide) (Furchgott & Zawadzki, 1980; Hasegawa et al, 1993; Ito et al, 1991). This postulation was further confirmed by the increased formation of cyclic GMP by AII in the rabbit mesenteric artery with endothelium.

Secondly, treatment with indomethacin, a cyclooxygenase inhibitor, strongly suppressed the relaxation in response to AII irrespective of the presence of endothelium and inhibited the AII-induced synthesis of prostanoids ($PGF_{2\alpha}$ and prostacyclin) in the mesenteric artery. These results provide two considerations: one is that nitric oxide is not the sole mediator of AII-induced relaxation and the prostanoids synthesized in the smooth muscle as well as endothelial cells are involved. The endothelial production of vasodilator prostaglandins in response to AII was reported by Gimbrone & Alexander (1975) and by Toda and Miyazaki (1981). In our results, an enhanced production of both $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ in response to AII was inhibited by indomethacin. In line with these results, arachidonic acid, carbacyclin, a stable analog of prostacyclin and $PGF_{2\alpha}$ caused endothelium-dependent and -independent relaxations in the low concentrations (0.1～100 nM). Thus, as Jaiswal et al (1992; 1993) have indicated, endogenous prostaglandins including arachidonic acid which are released from the endothelium and smooth muscle are suggested to be related with AII-induced relaxation.

Thirdly, the fact that $PGF_{2\alpha}$-induced increases in both cyclic GMP formation and vasorelaxation are inhibited by L-NNA and methylene blue indicates that endogenously released $PGF_{2\alpha}$ evoke a relaxation by mediation of endothelium-derived nitric oxide (Kawai & Ohhashi, 1991). Recently, Davidge et al (1995) and Salvemini et al. (1993) have demonstrated increased production of prostaglandins by endothelium-derived nitric oxide through activation of the constitutive and inducible forms of cyclooxygenase. However, it remains unclear in the present study. Moreover, under pretreatment with indomethacin in the presence or the absence of endothelium, the phenylephrine-induced contraction was significantly enlarged, suggesting that the vasodilator effect of prostaglandins manifests predominantly in association with release of EDRF (NO) or vasodilator prostaglandin (e.g., prostacyclin) at the resting state.

Instead of estimating the mobilization of arachidonic acid, we used dexamethasone, an inhibitor of phospholipase A_2 (Flower & Blackwell, 1979). Under treatment with dexamethasone for 20 min, the AII-induced relaxation was markedly inhibited. These results lead us to speculate that dexamethasone may interfere with the release of arachidonic acid by inhibiting the phospholipase A_2. In this case, the inhibitory effect of dexamethasone on the inducible nitric oxide synthase could be ruled out, because aminoguanidine, an inhibitor of inducible nitric oxide synthase, was without effect (Laszlo et al, 1995).

On the other hand, the evidence for the notion that the nitric oxide/prostanoid-independent vasodilatory response to AII may be mediated by a cytochrome P-450-derived arachidonic acid metabolite (Oyekan et al, 1990; Quilley et al, 1991) comes from the finding

that AII-induced relaxation was markedly inhibited by pretreatment with the cytochrome P-450 inhibitors, 7-ethoxyresorufin and clotrimazole (Hecker et al, 1994). However, it is difficult to reconcile the release of vasorelaxant mediators from the smooth muscle in terms of an unstable epoxide inasmuch as we have not demonstrated the release of cytochrome P-450 metabolites. Although we do not have a direct evidence for implication of cytochrome P-450-dependent metabolism of arachidonic acid in the AII-induced vasorelaxation, the speculation is based on a physiological function with the inhibitory effect of 7-ethoxyresorufin and clotrimazole.

In summary, the results of this study demonstrate that AII-induced relaxation of the isolated rabbit mesenteric arteries with endothelium comprises three components: the production of arachidonic acid and its metabolites via cyclooxygenase and cytochrome P-450 enzyme systems as well as the synthesis of nitric oxide. However, it is unidentified whether nitric oxide released in response to AII directly activates cyclooxygenase enzyme in the rabbit mesenteric artery.

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