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The role of endothelial and non-endothelial prostaglandins in the relaxation of isolated blood vessels of the rabbit induced by acetylcholine and bradykinin

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1 Strips of rabbit extrapulmonary, coeliac and mesenteric arteries were mounted in organ baths for isotonic recording of changes in tissue length. The formation by the strips of the vasodilator prostaglandins PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) and PGE_2 was determined by specific radioimmunoassays.

2 Removal of vascular endothelium initially increased and then permanently decreased the basal prostaglandin release of the tissues.

3 Acetylcholine (ACh) relaxed strips of all three arteries if the endothelium was intact. ACh also stimulated the formation of PGI_2 and PGE_2 from all three tissues; about 60% of these prostaglandins originated from endothelial cells. Indomethacin caused complete inhibition of prostaglandin formation and a slight inhibition of the ACh-relaxation (not statistically significant). Complete inhibition of the ACh relaxation was achieved with nordihydroguaiaretic acid (NDGA). NDGA also partially inhibited prostaglandin formation. These data suggest that in blood vessels that are also prostaglandin-sensitive, the ACh relaxation is predominantly mediated by a non-prostaglandin endothelium-derived relaxing factor.

4 Bradykinin was more potent that ACh in releasing prostaglandins from the same arteries. This release was activated in subendothelial components of the vascular wall. Neither this prostaglandin release nor the bradykinin-induced relaxations were significantly reduced in endothelium-denuded arteries. Indomethacin completely blocked the bradykinin-induced prostaglandin release and the bradykinin relaxation. NDGA caused a moderate inhibition of the bradykinin-induced prostaglandin release and slightly attenuated the bradykinin relaxation (neither effect of NDGA was statistically significant).

5 Under all experimental conditions (control, indomethacin, NDGA) and with all three arteries there was a good correlation between the bradykinin-induced prostaglandin release and the respective mechanical response. No such correlation could be found for ACh.

6 Prostaglandin-dependent relaxations of the coeliac and mesenteric artery are probably mediated by endogenous PGI_2. The extrapulmonary artery is rather insensitive to PGI_2 and is probably relaxed mainly by endogenous PGE_2.

Introduction

Several vasodilator agents, like acetylcholine (ACh; Furchgott & Zawadski, 1980; Chand & Altura, 1981a; Furchgott, 1981; 1983), calcium ionophore A 23187 (Furchgott, 1981; 1983; Singer & Peach, 1983), ADP and ATP (De Mey & Vanhoutte, 1981; Furchgott, 1981), substance P (Zawadzki *et al.*, 1981) histamine (H_1-receptor mediated, Van de Voorde & Leusen, 1983) and melittin (Förstermann & Neufang, 1985b) relax isolated arteries by acting on endothelial cells. Endothelium-dependent relaxations are believed to be mediated by some unknown humoral factor(s), referred to as endothelium-derived relaxing factor (EDRF), which is released by endothelial cells and diffuses to the smooth muscle cells (Furchgott & Zawadzki, 1980; Furchgott, 1983; 1984; Griffith *et al.*,

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1984; Förstermann *et al.*, 1984b). However, endothelial cells are also the major source of vasodilator prostaglandins, especially prostaglandin I_2 (PGI_2, Moncada *et al.*, 1977; Herman *et al.*, 1977; Ingerman-Wojenski *et al.*, 1981) and several of the above substances stimulate prostaglandin-release. Therefore the present paper deals with the release and significance of dilator prostaglandins in the relaxation of prostaglandin-sensitive arteries by two different stimuli: ACh and bradykinin.

The ACh-induced vasodilatation is considered as EDRF-mediated (Furchgott & Zawadski, 1980; Chand & Altura, 1981b,c; Furchgott, 1983). However, ACh also releases vasodilator prostaglandins from several arteries (Beetens *et al.*, 1983; Busse *et al.*, 1984; Förstermann & Neufang, 1984). This is irrelevant for the relaxation of rabbit aorta, since it is insensitive to dilator prostaglandins (Bunting *et al.*, 1976; Förstermann *et al.*, 1984a). However, in prostaglandin-sensitive blood vessels, an indomethacin-sensitive component of the ACh relaxation could be expected. This may be responsible for the higher sensitivity of such arteries towards ACh reported by Furchgott (1984).

In the case of bradykinin-induced vasodilatation, important species differences seem to exist. Bradykinin-induced relaxations of isolated arteries of the dog and man are endothelium-dependent and resistant to cyclo-oxygenase inhibition (Altura & Chand, 1981; Cherry *et al.*, 1981; 1982). In contrast, in isolated arteries of cat and rabbit the vasodilator effect of bradykinin could still be elicited if the endothelium had been removed, and the relaxation was reversed with non-steroidal anti-inflammatory drugs (Cherry *et al.*, 1981; 1982). These results are surprising since they suggest a mediation of the bradykinin effect by a vasodilator cyclo-oxygenase product of non-endothelial origin although endothelial cells are generally thought to be the major source of PGI_2 (Moncada *et al.*, 1977; Herman *et al.*, 1977; Ingerman-Wojenski *et al.*, 1981).

Since all this evidence comes from inhibitor experiments and thus is indirect, the present experiments were designed to measure directly the amounts of PGI_2 and PGE_2 released by three different arteries during their exposure to ACh and bradykinin. The site of origin of these prostaglandins (endothelium or subendothelial structures) was determined. The effect of the cyclo-oxygenase inhibitor indomethacin and of the lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA) on this prostaglandin release was tested. The sensitivities of the three tissues towards dilator prostaglandins were investigated and the mechanical effects observed were correlated with the amounts of prostaglandins actually released.

Portions of this work were presented at the 2nd International Symposium on Prostaglandins, May 1984, in Nürnberg-Fürth, FRG (Förstermann & Neufang, 1985a).

Methods

Preparation of vascular strips

Rabbits of either sex (2.5–3.5 kg bodyweight) were killed by a blow on the head and exsanguination. Then a portion of the pulmonary artery about 1 cm distal from the heart (henceforth referred to as the extrapulmonary artery), the coeliac artery and the superior mesenteric artery were rapidly dissected out and rinsed with ice-cold Krebs-bicarbonate solution (composition see below). The coeliac and mesenteric arteries were cut into helical strips of about 1×10 mm and in the case of the pulmonary artery cut-open rings (transverse strips) of the same size were used. The set-up used for the subsequent experiments has been described previously (Förstermann *et al.*, 1984a). Briefly, the strips were mounted in 3.5 ml organ baths containing Krebs-bicarbonate solution of the following composition (mM): NaCl 120.0, KCl 4.75, $NaHCO_3$ 25.0, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.7 and glucose 6.4. The incubation medium was kept at 37°C and continuously bubbled with a mixture of O_2 (95%) and CO_2 (5%). During the whole procedure damage to the intimal surface of the tissues was carefully avoided in order to obtain strips with an intact endothelium. In other strips the endothelium was deliberately removed by careful abrasion with a razor blade (rubbing procedure). This treatment did not reduce the contractility of the tissues. Inspection of the tissues by light microscopy after silver staining showed that virtually all endothelial cells were removed by this procedure.

Recording of changes in tissue length

Changes in length of the strips were recorded by an isotonic lever system with loads respectively of 1.5 g on the extrapulmonary artery and 0.5 g on both the coeliac and mesenteric arteries. The bath fluid was changed every 12 min throughout the experiments. After an equilibration period of about 1 h, a stable baseline length was reached. Then the tissues were exposed to noradrenaline (NA; 1×10^{-7}M, extrapulmonary artery, or 3×10^{-7}M, other two arteries) at hourly intervals (after 5 wash-out periods) in order to produce submaximal contractions (Förstermann *et al.*, 1984a). NA was dissolved and diluted in HCl 10^{-3}M containing ascorbic acid 1 mg ml^{-1} and added to the organ bath in a volume of 50 µl. When two NA precontractions had given reproducible responses (usually 2 or 3 h after the beginning of the experiment) ACh (10^{-8}M–10^{-6}M) was added to the bath 7 min after the contractile agonist. At that time a stable contraction plateau had been reached (cf. Figures 1a–3a). ACh was used as a functional test for endothelial integrity (strips with intact endothelium relaxed whereas strips without endothelium contracted to ACh, Furchgott & Zawadzki, 1980). Strips

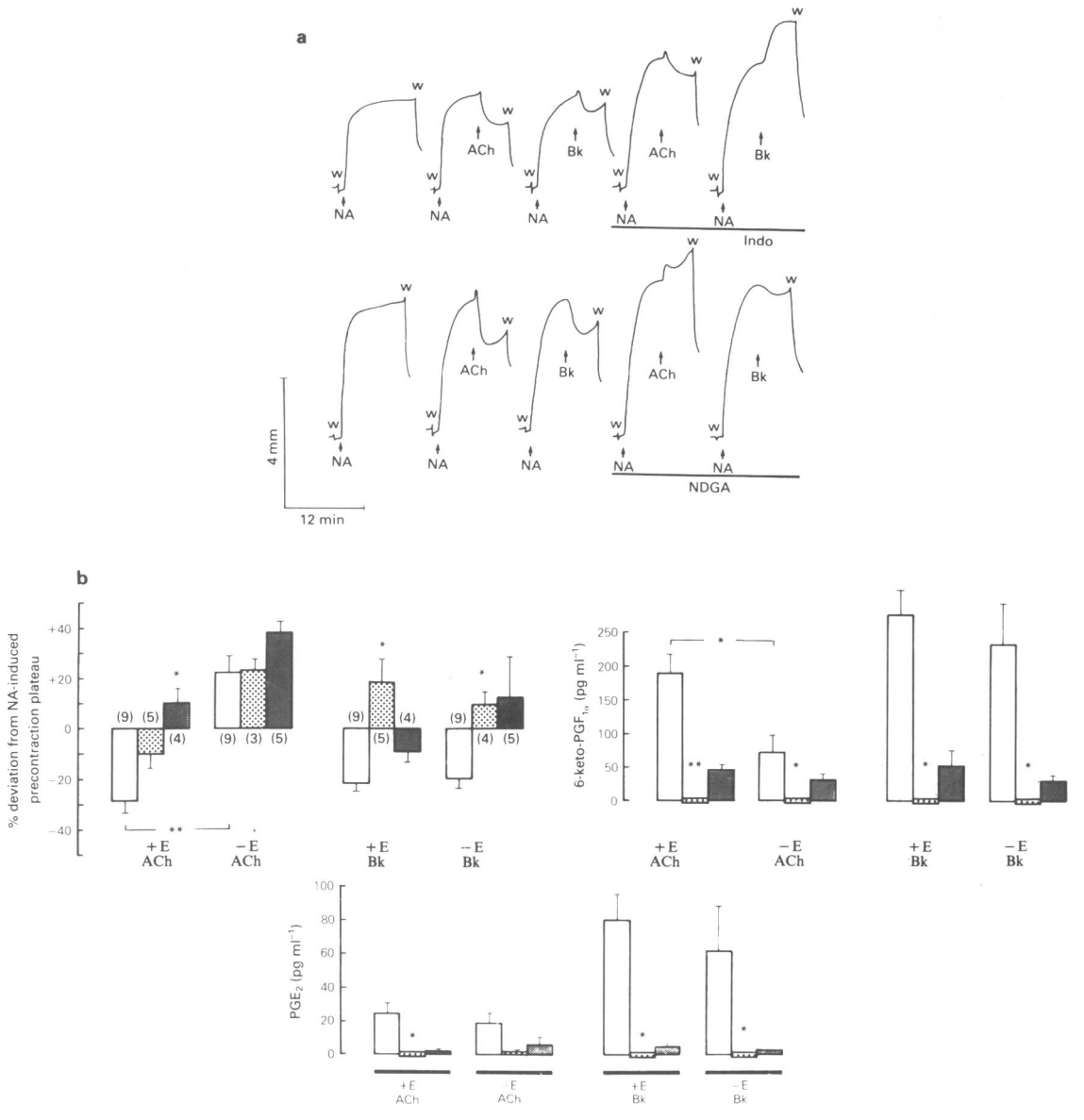

Figure 1 (a) Representative original recordings from strips of rabbit extrapulmonary artery with intact endothelium, made to contract with noradrenaline (NA, 10^{-7}M). Subsequently they were exposed to acetylcholine (ACh, 10^{-6}M) or bradykinin (Bk, 10^{-6}M). ACh and Bk were given again after treatment of the strips with indomethacin (Indo, 10^{-5}M, upper panel) or nordihydroguaiaretic acid (NDGA, 3×10^{-5}M, lower panel). The vertical axis indicates the actual shortening of the vascular strip. W: wash-out, change of bath medium. (b) Effects of ACh (10^{-6}M) and Bk (10^{-6}M) on the mechanical response of and 6-keto-$PGF_{1\alpha}$ and PGE_2 release by preconstricted strips of rabbit extrapulmonary artery with endothelium intact (+ E) and removed (− E). The control values are shown by the open columns. The same parameters were determined again after treatment of the strips with Indo (10^{-5}M, stippled columns) or with NDGA (3×10^{-5}M, heavily shaded columns). Asterisks indicate significant inhibition of relaxation or prostaglandin release compared to the respective controls (open columns: $P < 0.05$, $^{**}P < 0.01$). Columns without asterisks are not significantly different from controls. Significant differences in mechanical response and prostaglandin release between endothelium-intact and endothelium-denuded strips are also indicated. All data are means with vertical bars representing s.e.mean (the number of experiments indicated in parentheses is the same for mechanical response and prostaglandin release). Note the different ordinate scale for contractions/relaxations compared to Figures 2b and 3b.

showing equivocal reactions to 10^{-6}M ACh (e.g. only slight relaxations, $<10\%$ in the pulmonary artery, and $<30\%$ in the other two arteries) were discarded from the study, since unintentional damage of the intima could not be excluded (about 5% of total number). After ACh, different concentrations of bradykinin (10^{-9}–10^{-6}M) were added to the preconstricted strips at hourly intervals and in randomized order. Both ACh and bradykinin were dissolved and diluted in water and added to the bath in a volume of 50 µl. When the effect of indomethacin (10^{-5}M) or NDGA (3×10^{-5}M) on ACh- and bradykinin-induced relaxations and prostaglandin release were tested; the inhibitor was added to the bath fluid 36 min (3 wash-out periods) before the next contraction-relaxation period.

Radioimmunoassays

Bath fluids were collected separately after the total 12 min incubation periods and stored frozen until assayed for 6-keto-$PGF_{1\alpha}$ and PGE_2 content. These are the major cyclo-oxygenase products released by the tissues (Förstermann *et al.*, 1984a). The radioimmunoassays used for this purpose have been described previously (Jobke *et al.*, 1973; Machleidt *et al.*, 1981). The antibodies are highly specific for their respective antigens. Charcoal was used to separate free and antibody-bound fractions. The sensitivities of the assays (detection limit defined as 10% displacement of tracer binding) were 11 pg ml^{-1} for 6-keto-$PGF_{1\alpha}$ and 4 pg ml^{-1} for PGE_2.

Effect of exogenous prostaglandins

In these experiments the medium was changed every 6 min. The exogenous prostaglandins were added to the bath immediately after wash-out and the NA contraction was elicited 1 min later. The percentage changes in the resulting NA contraction amplitudes (obtained after 6 min) were taken as the inhibitory effects of the vasodilator prostaglandins. Indomethacin (3×10^{-6}M) was present in the bath fluid throughout to exclude indirect effects possibly caused by endogenous prostaglandins or thromboxane (Borda *et al.*, 1983). PGI_2 was dissolved (1 mg ml^{-1}) in 10 mM ice-cold Tris buffer (pH 10.0) and used within hours. PGE_2 was dissolved (1 mg ml^{-1} in 70% ethanol and diluted with 2 mM Na_2CO_3 before use. During control contractions the respective vehicles were present in the bath medium.

Calculations and statistical analysis

Prostaglandin release induced during the contraction-relaxation periods was calculated by subtracting basal prostaglandin concentrations (measured in the bath fluid sample obtained immediately before the addition of drugs) from the sample obtained during the contraction-relaxation period. Since both basal and stimulation-induced prostaglandin release had a tendency to decrease with time, means of the basal values determined 2 h and 3 h after the beginning of the experiments were always used for comparison between strips with an intact endothelium and those devoid of endothelial cells (Table 1).

Relaxations (−a) or further contractions (+a) starting from the NA-induced contraction plateau (b) are always expressed as a/b (%) in Figures 1–6, Table 2, and the text. When biphasic responses occurred (e.g. shortlasting initial contractions before the actual relaxations (Figure 1)), the final state reached at the end of the incubation period (12 min) was always compared to the NA plateau reached after 7 min.

All data are expressed as means ± s.e.mean. Differences in contractility and prostaglandin release between strips with intact and removed endothelium

Table 1 Basal release of 6-keto-$PGF_{1\alpha}$ and PGE_2 by three different rabbit arteries, intact and with endothelium removed, and the effect of indomethacin

	Extrapulmonary artery		*Coeliac artery*		*Mesenteric artery*	
	6-keto-$PGF_{1\alpha}$	*PGE_2*	*6-keto-$PGF_{1\alpha}$*	*PGE_2*	*6-keto-$PGF_{1\alpha}$*	*PGE_2*
Endothelium intact	147 ± 18 (20)	12 ± 2 (20)	220 ± 29 (20)	25 ± 4 (20)	285 ± 29 (20)	63 ± 10 (20)
Endothelium removed	120 ± 17 (20)	12 ± 1 (20)	126 ± 15** (20)	16 ± 4 (18)	210 ± 22* (20)	37 ± 7* (20)
Endothelium intact, + indomethacin	<11 (10)	<4 (10)	<11 (8)	<4 (8)	<11 (10)	<4 (10)
Endothelium removed, + indomethacin	<11 (7)	<4 (7)	<11 (10)	<4 (10)	<11 (10)	<4 (10)

Indomethacin (10^{-5}M) was added to the bath fluid at least 36 min before measurements. All values are given in pg ml^{-1} 12 min^{-1} incubation as means ± s.e.mean from the number of experiments indicated in brackets. Differences in prostaglandin release from preparation with intact and removed endothelium were tested for significance by Student's *t* test (*$P<0.025$; **$P<0.005$).

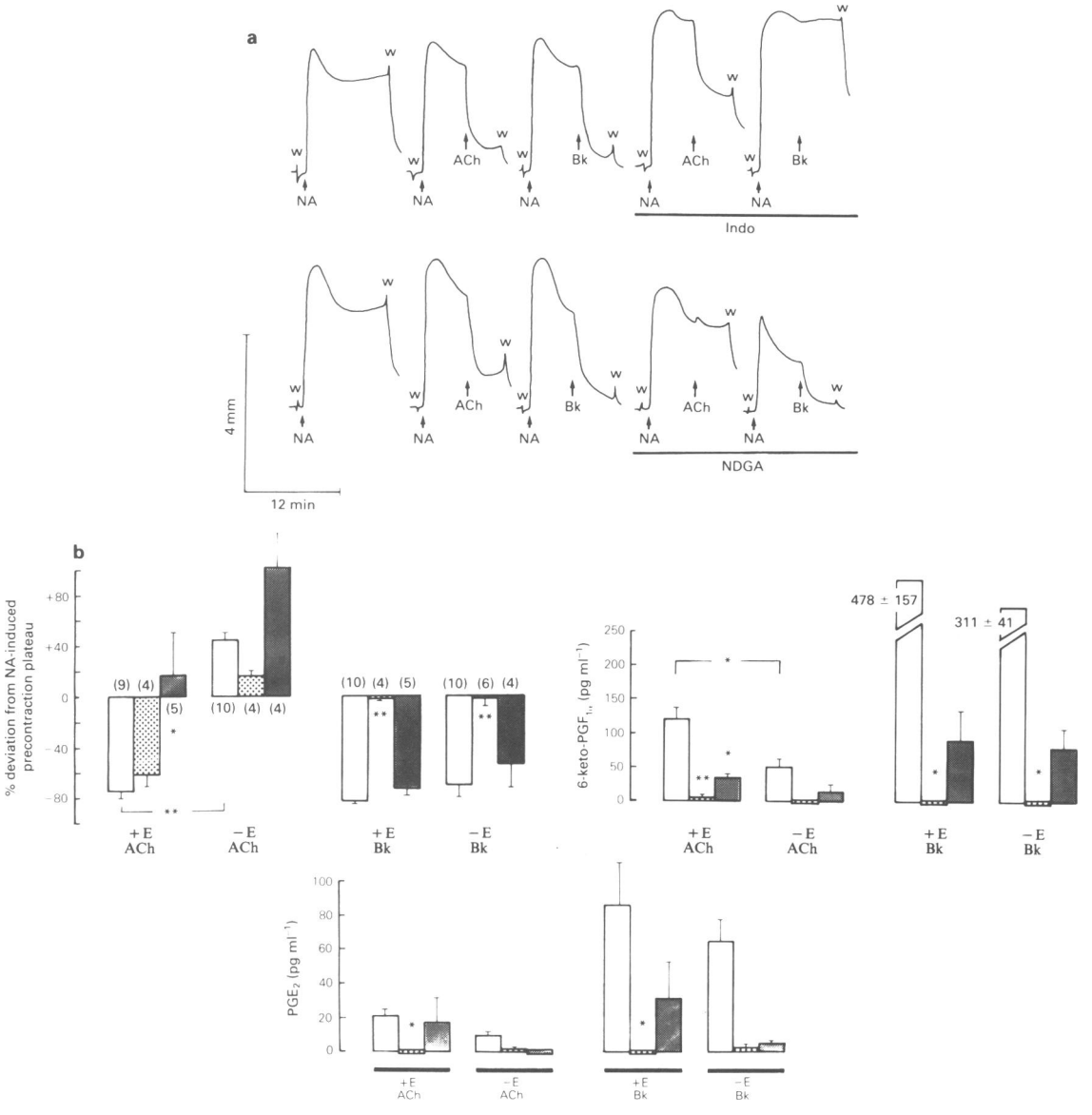

Figure 2 (a) Representative original recording from strips of rabbit coeliac artery with intact endothelium contracted with noradrenaline (NA, 3×10^{-7}M). Subsequently they were exposed to acetylcholine (ACh, 10^{-6}M) or bradykinin (Bk, 10^{-6}M). ACh and Bk were given again after treatment of the strips with indomethacin (Indo 10^{-5}M, upper panel) or nordihydroguaiaretic acid (NDGA, 3×10^{-5}M, lower panel). The vertical axis indicates the actual shortening of the vascular strip. W: wash-out, change of bath medium. (b) Effects of ACh (10^{-6}M) and Bk (10^{-6}M) on the mechanical response of and 6-keto-$PGF_{1\alpha}$ and PGE_2 release by preconstricted strips of rabbit coeliac artery with endothelium intact (+ E) and removed (− E). The control responses are shown in the open columns. The same parameters were determined again after treatment of the strips with Indo (10^{-5}M, stippled columns) or with NDGA (3×10^{-5}M, heavily shaded columns). Asterisks indicate significant inhibition of relaxation or prostaglandin release compared to the respective controls (open columns: $P < 0.05$; $^{**}P < 0.01$). Significant differences in mechanical response and prostaglandin release between endothelium-intact and endothelium-denuded strips are also indicated. All data are means with vertical bars representing s.e.mean (the number of experiments indicated in parentheses is the same for mechanical response and prostaglandin release).

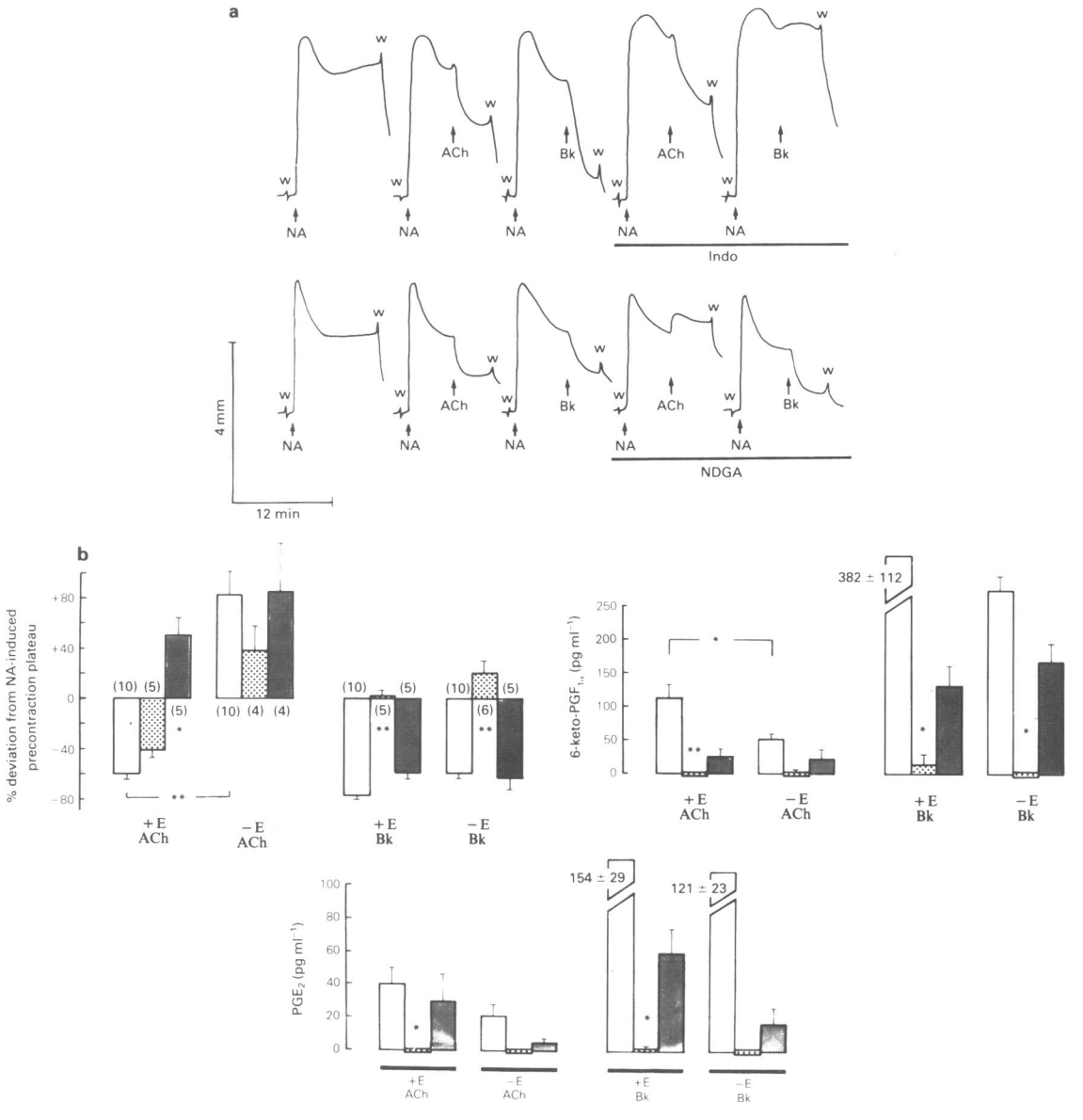

Figure 3 (a) Representative original recordings from strips of rabbit mesenteric artery with intact endothelium, contracted with noradrenaline (NA, 3×10^{-7}M). Subsequently they were exposed to acetylcholine (ACh, 10^{-6}M) or bradykinin (Bk, 10^{-6}M). ACh and Bk were given again after treatment of the strips with indomethacin (Indo 10^{-5}M, upper panel) or nordihydroguaiaretic acid (NDGA, 3×10^{-5}M, lower panel). The vertical axis indicates the actual shortening of the vascular strip. W: wash-out, change of bath medium. (b) Effects of ACh (10^{-6}M) and Bk (10^{-6}M) on the mechanical response of and 6-keto-$PGF_{1\alpha}$ and PGE_2 release by preconstricted strips of rabbit mesenteric artery with endothelium intact (+ E) and removed (− E). The control values are given in the open columns. The same parameters were determined again after treatment of the strips with Indo (10^{-5}M, stippled columns) or with NDGA (3×10^{-5}M, heavily shaded columns). Asterisks indicate significant inhibition of relaxation or prostaglandin release compared to the respective controls (open columns: $P < 0.05$; $^{**}P < 0.01$). Significant differences in mechanical response and prostaglandin release between endothelium-intact and endothelium-denuded strips are also indicated. All data are means with vertical bars representing s.e.mean (the number of experiments indicated in parentheses is the same for mechanical response and prostaglandin release).

or strips before and after indomethacin or NDGA treatment (data of Figures 1b–3b) were tested for statistical significance using one-way analysis of variance followed by the F-test. The Scheffé-test was used for comparison of different means (Scheffé, 1953). For comparison of two means (data of Table 2) Student's t test was used.

Linear regression was calculated between log [PGE_2] and the bradykinin relaxation of the extrapulmonary artery, and log [6-keto-$PGF_{1\alpha}$] and the bradykinin relaxation of the coeliac and mesenteric arteries respectively (data of Figures 4–6).

Drugs

Noradrenaline HCl, acetylcholine HCl, bradykinin, PGE_2, and nordihydroguaiaretic acid were purchased from Sigma, Munich, FRG. Indomethacin was donated by MSD-Sharp and Dohme, Munich, FRG. Prostacyclin sodium salt was a generous gift of Dr J. Pike, Upjohn, Kalamazoo, Mi., U.S.A. All concentrations given refer to the active bases or acids respectively. Radiolabelled prostaglandins for the radioimmunoassays [5,8,9,11,12,14,15-^{3}H]-6-keto-$PGF_{1\alpha}$, sp. act. 120 Ci mmol^{-1} and [5,6,8,11,12,14,15-^{3}H]-PGE_2, sp. act. 160 Ci mmol^{-1}) were purchased from New England Nuclear, Dreieich, FRG.

Results

Basal prostaglandin release

Under basal conditions all three types of vascular strips continuously released significant amounts of vasodilator prostaglandins into the bath medium. PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) was the predominant compound, PGE_2 was found in smaller quantities (Table 1). During the initial hour after the rubbing procedure all endothelium-denuded arteries released larger quantities of prostaglandins than the respective endothelium-intact control-vessels. Prostaglandin measurements in sample groups of e.g. 5 coeliac

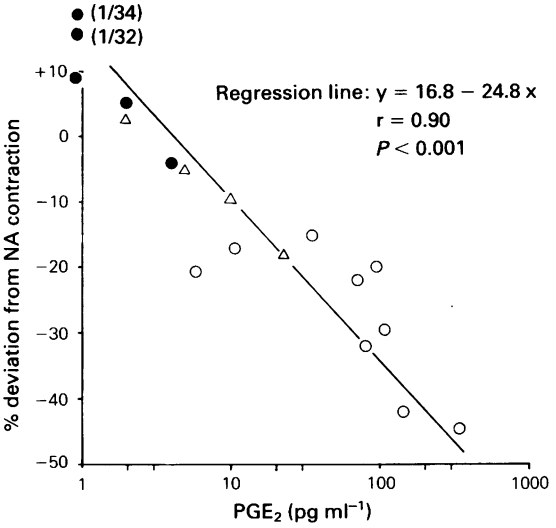

Figure 4 Scatter diagram showing the correlation between prostaglandin E_2 (PGE_2) release (log transformed data on the abscissa scale) from rabbit extrapulmonary artery and the mechanical response of the preparation (ordinate scale) after administration of bradykinin. The arteries were preconstricted with noradrenaline (NA, 10^{-7}M). Each point is a single observation from one vascular strip. Three different experimental conditions are shown: (○) bradykinin alone (control conditions); (●) bradykinin in the presence of 10^{-5}M indomethacin; (△) bradykinin in the presence of 3×10^{-5}M nordihydroguaiaretic acid. The regression line was calculated according to the least squares method (values of PGE_2 below 1 pg ml^{-1} were taken as 1 pg ml^{-1}).

Table 2 The effect of different concentrations of the vasodilator prostaglandins, PGI_2 and PGE_2, on noradrenaline (10^{-7}M or 3×10^{-7}M)-induced contractions of three different rabbit arterial strips

		Extrapulmonary artery	*Coeliac artery*	*Mesenteric artery*
Control		0	0	0
PGI_2	(10^{-8}M)	+2.7 ± 1.6 (6)	−26.2 ± 8.2 (6)	−8.7 ± 2.4 (4)
	(10^{-7}M)	−2.7 ± 2.9 (6)	−72.8 ± 15.4 (6)	−33.3 ± 8.8 (4)
	(10^{-6}M)	−14.7 ± 6.0 (6)	−94.7 ± 2.7 (6)	−82.0 ± 4.6 (4)
PGE_2	(10^{-8}M)	−15.4 ± 7.5 (5)	−21.8 ± 5.5 (5)	−17.7 ± 5.8 (3)
	(10^{-7}M)	−26.4 ± 10.1 (5)	−37.8 ± 8.9 (5)	−23.0 ± 7.5 (3)
	(10^{-6}M)	−30.2 ± 11.3 (5)	−59.2 ± 11.5 (5)	−36.7 ± 14.2 (3)

Data (means ± s.e.mean) are given as percentage deviation from the respective noradrenaline contraction in the presence of the respective prostaglandin vehicles (control). Indomethacin (3×10^{-6}M) was present in all these experiments. Numbers in parentheses represent the number of determinations.

arteries without endothelium and 5 endothelium-intact coeliac arteries 1 h after set up of the tissues yielded 668 ± 102 pg ml^{-1} 12 min^{-1} for 6-keto-PGF$_{1\alpha}$ and 79 ± 18 pg ml^{-1} 12 min^{-1} for PGE$_2$ in the rubbed tissues versus 290 ± 37 pg ml^{-1} 12 min^{-1} 6-keto-PGF$_{1\alpha}$ and 36 ± 15 pg ml^{-1} 12 min^{-1} for PGE$_2$ in the intact tissues. However, after two or more hours the basal formation of 6-keto-PGF$_{1\alpha}$ was slightly reduced in strips of extrapulmonary arteries without endothelium, but markedly and significantly diminished in endothelium-denuded coeliac and mesenteric arteries as shown in Table 1.

Effects of noradrenaline

All three types of arteries rapidly contracted in response to NA. Contractions of the coeliac and mesenteric arteries reached a maximum after about 2 min and then declined to a stable level (Figures 2a and 3a). In the extrapulmonary artery the plateau was attained directly (Figure 1a). In the concentration used here (10^{-7}M) the precontraction agonist, NA, released small amounts of 6-keto-PGF$_{1\alpha}$ (10–30 pg ml^{-1} 12 min^{-1}) from the extrapulmonary artery. Since this release was small compared to the effects both of ACh and, especially, bradykinin and was similar in all contraction-relaxation periods it was not taken into account when calculating the prostaglandin release induced by the two vasodilators. In coeliac and mesenteric arteries, NA (3 × 10^{-7}M) did not enhance prostaglandin release.

Effects of acetylcholine

ACh induced concentration-dependent relaxations of all three types of arteries between 10^{-8} and 10^{-6}M. Maximal relaxations (after 10^{-6}M ACh) of the extrapulmonary arteries were generally smaller (−20% to −30% of the precontraction level) than those of the other two arteries (−70% to −90% of the precontraction level) (Figures 1–3). In addition, ACh-induced relaxations of the extrapulmonary artery were often (but not always) preceded by a small transient initial contraction (Figure 1a). At concentrations >10^{-7}M, ACh also released 6-keto-PGF$_{1\alpha}$ and PGE$_2$ from all arterial strips. If the endothelium had been removed, blood vessel strips of all three types were further contracted by 10^{-6}M ACh (Figures 1b–3b)

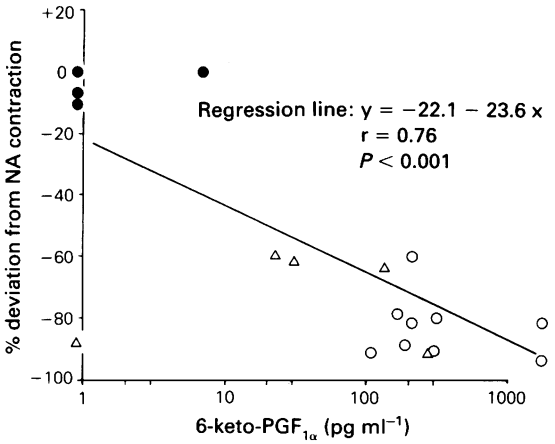

Figure 5 Scatter diagram showing the correlation between prostaglandin (PGI$_2$) release (measured as 6-keto-PGF$_{1\alpha}$: log transformed data on the abscissa scale) from rabbit coeliac artery and the mechanical response of the preparation (ordinate scale) after administration of bradykinin. The arteries were preconstricted with noradrenaline (NA, 10^{-7}M). Each point is a single observation from one vascular strip. Three different experimental conditions are shown: (○) bradykinin alone (control conditions); (●) bradykinin in the presence of 10^{-5}M indomethacin; (△) bradykinin in the presence of 3 × 10^{-5}M nordihydroguaiaretic acid. The regression line was calculated according to the least square method (values of 6-keto-PGF$_{1\alpha}$ below 1 pg ml^{-1} were taken as 1 pg ml^{-1}).

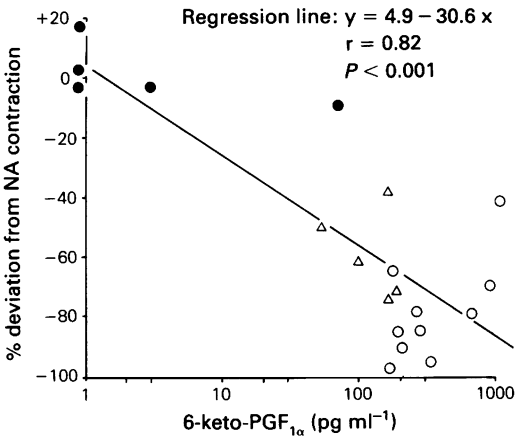

Figure 6 Scatter diagram showing the correlation between prostaglandin (PGI$_2$) release (measured as 6-keto-PGF$_{2\alpha}$; log transformed data on the abscissa scale) from rabbit mesenteric artery and the mechanical response of the preparation (ordinate scale) after administration of bradykinin. The arteries were preconstricted with noradrenaline (NA, 10^{-7}M). Each point is a single observation from one vascular strip. Three different experimental conditions are shown: (○) bradykinin alone (control conditions); (●) bradykinin in the presence of 10^{-5}M indomethacin; (△) bradykinin in the presence of 3 × 10^{-5}M nordihydroguaiaretic acid. The regression line was calculated according to the least squares method (values of 6-keto-PGF$_{1\alpha}$ below 1 pg ml^{-1} were taken as 1 pg ml^{-1}).

and the ACh-induced release of 6-keto-$PGF_{1\alpha}$ was significantly reduced (by about 60%) in all three vessel preparations (Figures 1b–3b). Strips without endothelium also formed slightly smaller quantities of PGE_2 than those with an intact intima; however, these differences were less pronounced and not statistically significant.

Effects of bradykinin

Addition of bradykinin (10^{-9}–10^{-6}M) to the Krebs-bicarbonate solution bathing the precontracted blood vessels resulted in concentration-dependent relaxations of all three types of vascular strips. As with ACh, the maximal relaxations attainable with bradykinin 10^{-6}M in preparations of the extrapulmonary artery were smaller (− 20% to − 30%) than those of the two other blood vessels (− 70% to − 90%). However, in contrast to ACh, bradykinin relaxed rabbit arteries without endothelium to a similar extent to those with an intact endothelium (Figures 1–3). In the extrapulmonary artery, bradykinin often induced small and shortlasting initial contractions before the actual relaxations occurred (Figure 1). Concomitantly with the relaxation, bradykinin at concentrations $>10^{-8}$M, stimulated the release of PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) and of PGE_2 from the preparations. The nonapeptide proved to be more potent than ACh at inducing prostaglandin formation (Figures 1b–3b). In none of the arteries tested and at no concentration of bradykinin was this enhanced prostaglandin release significantly smaller after removal of the endothelial cell layer, although the slight tendency of de-endothelialized strips to produce weaker relaxations was paralleled by a similar tendency to release slightly smaller amounts of both prostaglandins (Figures 1–3).

Effects of indomethacin

Indomethacin (10^{-5}M) reduced the basal concentrations of both prostaglandins to below the detection limits of the radioimmunoassays (11 pg ml^{-1} for 6-keto-$PGF_{1\alpha}$ and 4 pg ml^{-1} for PGE_2; Table 1). Similarly it prevented almost completely the formation of vasodilator prostaglandins in response to ACh (Figures 1b–3b). Relaxations induced by ACh were slightly reduced by indomethacin in all arteries; however, this inhibition did not attain statistical significance (Figures 1b–3b). ACh contractions of blood vessels without endothelium remained virtually unchanged after indomethacin.

The bradykinin-induced prostaglandin release was also blocked by indomethacin. Under these conditions bradykinin no longer induced relaxations (Figures 2b, 3b, 5 and 6) and in some of the extrapulmonary arteries it even produced moderate contractions (Figures 1b and 4).

Effects of nordihydroguaiaretic acid

NDGA (3×10^{-5}M) often also reduced the ACh-induced formation of vascular prostaglandin (in 21 out of 27 preparations). If this occurred, the ACh-induced relaxations were transformed into contractions. If the levels of the vasodilator prostaglandins were not affected by NDGA there was still a strong inhibition of the ACh-induced relaxation; however, a small relaxation often persisted.

NDGA also reduced the bradykinin-induced formation of vasodilator prostaglandins in several preparations (Figures 1b–3b). In the respective blood vessels a small attenuation of relaxation was seen. If NDGA did not inhibit bradykinin-induced prostaglandin release, relaxations were also not affected. In the extrapulmonary artery without endothelium a contractile component of the action of bradykinin was often unmasked by NDGA (as by indomethacin) (Figure 1).

Figures 4–6 demonstrate that in all three types of blood vessels with the endothelium intact there was a good correlation between the log concentration of dilator prostaglandins released and the degree of relaxation for each preparation with bradykinin. This held true for all three treatments used (control conditions, indomethacin and NDGA). For the coeliac and mesenteric arteries the correlation is shown between log [6-keto-$PGF_{1\alpha}$] and mechanical response. For the extrapulmonary artery the correlation is shown between log [PGE_2] and the mechanical response, since this blood vessel is more sensitive to PGE_2 (cf. Table 2). Qualitatively similar findings with bradykinin were obtained for preparations with the endothelium removed (not shown).

Effects of exogenous prostaglandins

In further experiments the effects of exogenous PGI_2 and PGE_2 on precontracted indomethacin-treated strips were tested. As can be seen from Table 2 the coeliac and mesenteric arteries were markedly relaxed by PGI_2 and to a lesser extent by PGE_2. In contrast, contractions of the extrapulmonary artery were only slightly reduced in the presence of even high concentrations of PGI_2, whereas PGE_2 inhibited the contractions in a concentration-dependent manner.

Discussion

Two or more hours after removal of endothelium basal values of 6-keto-$PGF_{1\alpha}$ release were significantly reduced in these blood vessel preparations. This is in accordance with findings showing that endothelial cells, in spite of their small relative mass, represent an important source of PGI_2 in the vascular wall (Mon-

cada et al., 1977; Herman et al., 1977; Ingerman-Wojenski et al., 1981). However, subendothelial components of the vessel wall also are capable of producing PGI_2 (Baenzinger et al., 1977; MacIntyre et al., 1978; Goldsmith, 1982). During an initial period after mechanical removal of endothelial cells the injury-induced prostaglandin release from these structures can even exceed the release from unperturbed vascular tissues. This phenomenon has been described by Goldsmith (1982) for calf aorta and qualitatively similar findings were obtained here. However, all comparative measurements of basal (Table 1) and stimulation-induced prostaglandin release (Figures 1b–3b) were performed after two or more hours when the release from tissues without endothelium had reached low, stable levels.

ACh increased the formation of PGI_2 and PGE_2 from all three blood vessels tested. The present results clearly demonstrate that this release originates mainly, although not exclusively, from endothelial cells (significant decrease by about 60% in all preparations without endothelium). Also in other arteries ACh has been found to release prostaglandins (Beetens et al., 1983; Busse et al., 1984; Förstermann & Neufang, 1984), and the ACh-induced PGI_2 formation was largely reduced after removal of the endothelium (Busse et al., 1984). In the prostaglandin-sensitive blood vessels investigated here, complete blockade of the ACh-induced prostaglandin formation slightly attenuated the relaxant effect of the muscarinic receptor agonist, but these effects were not statistically significant. Thus, in these blood vessels the major mechanism of relaxation is also independent of the concomitant prostaglandin formation. It is obviously mediated by endothelium-derived relaxing factor (EDRF) since the relaxations were completely inhibited by NDGA at a concentration which blocks EDRF-mediated relaxations (Furchgott & Zawadzki, 1980; Chand & Altura, 1981c; DeMey et al., 1982; Singer & Peach, 1983; Förstermann & Neufang, 1984). Nevertheless, the higher sensitivity of these blood vessels towards ACh reported by Furchgott (1984) may be due (at least partly) to the concomitant formation of vasodilator prostaglandins.

For the mechanism of bradykinin-induced relaxations in different blood vessels Cherry et al. (1982) have reported important species differences. Canine and some human arteries were relaxed by bradykinin in an endothelium-dependent manner. These relaxations were resistant to cyclo-oxygenase inhibition (Altura & Chand, 1981; Cherry et al., 1981; 1982). In contrast, in isolated arteries of cat and rabbit the vasodilator effect of bradykinin could still be elicited after rubbing of the intima, and the relaxations were inhibited by indomethacin (Aiken, 1974; Cherry et al., 1982). Our experiments with bradykinin on the latter type of arteries demonstrate that, in contrast to ACh, the prostaglandins released by bradykinin originate from non-endothelial structures, probably from smooth muscle cells, since removal of the endothelium did not significantly decrease their formation. Alexander & Gimbrone (1976) have demonstrated stimulation of prostaglandin formation in cultured vascular smooth muscle cells by bradykinin. We can confirm that these vasodilator prostaglandins represent the factor mediating the bradykinin relaxation in these blood vessels, since a concentration of indomethacin that completely suppressed the bradykinin-induced prostaglandin formation also completely inhibited the relaxation or transformed it into a contraction (Figures 1–3). Interestingly in the rabbit aorta, which is not relaxed by prostaglandins (Förstermann et al., 1984a) or by bradykinin (Cherry et al., 1982), bradykinin was almost ineffective in stimulating prostaglandin release. We found only 16 ± 5 pg 6-keto-$PGF_{1\alpha}$ ml^{-1} $12\,min^{-1}$ after 10^{-6}M bradykinin (mean $\pm$ s.e.mean., $n = 4$, unpublished observation).

NDGA at a concentration that blocks EDRF-mediated relaxations also inhibited the formation of PGI_2 and PGE_2 in several preparations. A similar inhibitory effect has recently been observed for the melittin-induced prostaglandin-release in rabbit aorta (Förstermann & Neufang, 1985b). Despite the fact that this effect of NDGA was not statistically significant in the present experiments it should be recognized since it indicates that NDGA is not a specific inhibitor of lipoxygenase, but can also exert inhibitory actions on either phospholipase or cyclo-oxygenase enzymes.

If NDGA reduced the bradykinin-induced prostaglandin release it also attenuated the bradykinin relaxation. This correlation between prostaglandin release and bradykinin relaxation (which was also seen during control conditions and indomethacin treatment; Figures 4–6) is further evidence for a causal relationship between the two parameters in these blood vessels.

The small prostaglandin-mediated components of the ACh-relaxation and the entire bradykinin relaxation of coeliac and mesenteric arteries can be explained by formation of endogenous PGI_2. However, the extrapulmonary artery is only slightly relaxed by PGI_2 (Table 2). In this artery the prostaglandin-mediated relaxant effects are probably mediated mainly by endogenous PGE_2, since the tissue is more sensitive to this prostaglandin (Table 2).

In conclusion, our data demonstrate that vascular prostaglandin formation is reduced to various degrees after removal of endothelial cells. The ACh-dependent formation of vasodilator prostaglandins occurs predominantly in endothelial cells, and makes only a very limited contribution to the vasodilator effect of

ACh in prostaglandin-sensitive blood vessels. The major mediating factor is probably EDRF. In contrast, the bradykinin-induced release originates preponderantly from sub-endothelial structures of the vessel wall and is entirely responsible for the relaxation of these rabbit arteries by bradykinin.

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