Selective Attenuation of Endothelium-Mediated Vasodilation in Atherosclerotic Human Coronary Arteries

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This study was undertaken to determine whether atherosclerosis impairs relaxations mediated by endothelium-derived relaxing factor (EDRF) in human coronary arteries. Epicardial coronary arteries were obtained from the hearts of cardiac transplantation patients with or without histologically documented coronary atherosclerosis (atherosclerotic arteries were from patients aged 42–55 years, nonatherosclerotic arteries were from patients aged 14–24 years). Transverse strip preparations were mounted in organ baths for isometric tension recording. Tension was induced with prostaglandins F₂o. Indomethacin (10⁻⁶ M) was present to prevent possible interference from endogenously formed prostaglandins. The EDRF-mediated relaxations in response to substance P (10⁻⁷ to 10⁻⁴ M), bradykinin (10⁻⁷ to 10⁻² M), and Ca²⁺-ionophore A23187 (10⁻⁷ to 10⁻³ M) were significantly attenuated in atherosclerotic arteries. In deendothelialized tissues these compounds had no effect. In contrast, endothelium-independent relaxations induced by isoprenaline (10⁻⁷ to 10⁻⁴ M) were not affected by atherosclerosis. Atherosclerotic arteries showed also normal relaxations with high concentrations of glyceryl trinitrate (10⁻⁴ to 10⁻⁷ M), but reduced relaxations with a lower concentration of the compound (10⁻⁷ M). Acetylcholine (10⁻⁷ to 10⁻⁴ M) only produced endothelium-dependent relaxations in 8 of 60 arterial preparations (with or without atherosclerosis). In most of the arteries, it was a direct vasoconstrictor (which may have masked EDRF release in many cases). Omission of indomethacin from the bath solution increased the incidence of moderate acetylcholine-induced relaxations (9 of 16 preparations). It is concluded that atherosclerosis attenuates EDRF-mediated vasodilation in epicardial human coronary arteries and that this impairment could promote coronary vasospasm and myocardial ischemia. (Circulation Research 1988;62:185–190)
All preparations included in Group A were free of atherosclerosis although some showed moderate intimal thickening or minor fibrotic changes. Arteries in this group were free of fibrous and lipid plaques; fatty streaks and debris were not found in the intima. Preparations of Group B showed typical complicated atherosclerotic lesions including elevated lipid-laden fibrous intimal lesions encroaching the lumen (eccentric or concentric plaques), lipid and calcific debris in intima, fragmentation of internal elastic lamina. Severely calcified parts of Group B arteries were not used in the organ bath experiments. In all arteries tested the endothelial cell layer was preserved by more than 70% with no major differences between arteries of Groups A and B as indicated by en face light microscopy after silver staining (method modified from Poole et al10).

Organ Bath Studies

During the preparation of the arterial strips any contact with the luminal surfaces was avoided to preserve endothelial integrity. Strips were suspended in 5 ml organ baths filled with oxygenated (95% O2-5% CO2) modified Krebs' solution (pH 7.4, 37° C) of the following composition (mM): Na+ 145.0, K+ 5.95, Ca2+ 1.7, Mg2+ 1.2, Cl- 128.15, HCO3- 25.0, H2PO4- 1.2, SO42- 1.2, glucose 10.7, and disodium EDTA 0.025. To prevent the synthesis of vascular prostaglandins and to investigate the "pure" EDRF mechanism, the buffer contained indomethacin (10^-5 M) unless indicated otherwise (data of Table 1). This concentration of indomethacin completely inhibits vascular prostanoid production in animal arteries.12 The vascular preparations were connected to force transducers for isometric tension recording; resting force was 2 g. The preparations were connected to force transducers for isometric tension recording; resting force was 2 g. The strips were contracted with prostaglandin F2a (PGF2a, 3 x 10^-7 to 3 x 10^-6 M) to induce a tension of 1-2 g. After the tension had stabilized (plateau usually reached after 10 minutes) cumulative concentrations of one of the following compounds were added to the organ bath: substance P, bradykinin, A23187, or acetylcholine (as endothelium-dependent vasodilators) and glyceryl trinitrate or isoproterenol (as endothelium-independent vasodilators) (cf. Furchgott*).

| Table 1. Effect of Indomethacin (10^-5 M) on Acetylcholine (ACH)-Induced Relaxations of Human Coronary Artery Strips |
|-----------------|-----------------|-----------------|
| ACH (M)         | Response without indomethacin (%) | Response with indomethacin (%) |
| 10^-3           | -6 ± 2           | +1 ± 1**        |
| 10^-7           | -10 ± 4          | +1 ± 1**        |
| 10^-6           | -21 ± 7          | +12 ± 10*       |

Sixteen strips from four different patients were tested: one artery from Group A, three from Group B. Nine strips showed moderate relaxations in response to acetylcholine. Only these nine preparations are included and their response is shown in normal Krebs' solution and after addition of indomethacin (10^-5 M). In the presence of indomethacin, only one preparation relaxed, all others showed no effect or additional contractions. Response (mean ± SEM) is given as percent deviation from prostaglandin F2a concentration plateau (-relaxation, + additional contraction). Asterisks indicate significant difference from response in the absence of indomethacin: *p<0.05, **p<0.01.

Most of the coronary preparations showed rhythmic myogenic activity (especially after exposure to PGF2a). In about 20% of the arteries, however, this rhythmicity was so strong that a clear contraction plateau could not be defined. These arteries were discarded from the study.

In some experiments, the vascular strips were taken out of the organ baths after a first exposure to vasodilators and the endothelium was carefully removed with the blunt side of a scalpel (rubbing procedure). After resuspension and a 30-minute re-equilibration period, the strips were again exposed to the same agents. The completeness of endothelial removal by this method was confirmed in sample preparations by en face light microscopy after silver staining (method modified from Poole et al10).

Materials

Substance P and bradykinin (Sigma) were dissolved 1 mg/ml in phosphate buffer containing 0.1% gelatine, A23187 (Sigma) 1 mg/ml in dimethyl sulfoxide, prostaglandin F2a-tromethamine (Upjohn) 1 mg/ml in 70% ethanol, glyceryl trinitrate (10% triturated mixture in lactose) (Mack) (1 mg/ml) in distilled water. Solutions of isoproterenol were prepared (1 mg/ml) in 1 mM HCl containing ascorbic acid (1 mg/ml). Prostacyclin sodium salt (Upjohn) was dissolved (1 mg/ml in Tris buffer (10 mM, pH 10.0). Dilutions were made immediately before the experiment in Tris buffered saline (pH 8.5). Appropriate dilutions of all compounds were added to the organ bath in 5 μl. Indomethacin (Merck Sharp & Dohme) was dissolved in phosphate buffer (1 mg/ml) and diluted into the Krebs' solution.

Calculations and Statistics

Response to vasodilators is expressed as percent deviation (of the maximum effect) from the PGF2a-induced contraction plateau (−relaxation, + additional contraction). All data are given as mean ± SEM. Differences in response between intact and atherosclerotic arteries were tested for statistical significance using one way analysis of variance followed by the Fisher least-significant-difference test.13 Data of Table 1 were analyzed by Student's t test for paired values. Significance was always accepted at the 0.05 level of probability.

Results

Endothelium Dependent and Independent Relaxations

Substance P (10^-10 to 10^-4 M), bradykinin (10^-8 to 10^-7 M), and Ca2+ ionophore A23187 (10^-9 to 10^-7 M), as well as glyceryl trinitrate (10^-6 to 10^-2 M) and isoprenaline (10^-7 to 10^-5 M) induced concentration-dependent relaxations in Group A arteries (Figures 1-7). After removal of the endothelial cell layer, relaxations in response to substance P, bradykinin, and A23187 were completely abolished (data not shown). In contrast, relaxations by glyceryl trinitrate and isoprenaline were not significantly changed in endothelialized arteries.
Effect of Atherosclerosis on Relaxations

The endothelium-dependent vasodilators substance P, bradykinin, and A23187 all produced significantly smaller relaxations in atherosclerotic arteries (Group B) compared to arteries of Group A (Figures 1 and 3-5). In contrast, the relaxing effect of high concentrations of glyceryl trinitrate (>10^-5 M) was not different in nonatherosclerotic and atherosclerotic arteries (Figures 2 and 6). However, at 10^-5 M glyceryl trinitrate, relaxations were significantly smaller in atherosclerotic arteries (Group B). The β-adrenoceptor agonist isoprenaline induced the same degree of relaxation in Group A and Group B arteries (Figure 7).

Effects of Acetylcholine

Acetylcholine is the "prototype" of endothelium-dependent vasodilators in animal arteries. Of 60 human coronary strip preparations exposed to acetylcholine (in indomethacin-containing solution), the muscarinic agonist induced relaxations in only eight preparations. Two of these tissues were from the heart of a 14-year-old boy and clearly belonged to Group A; the other six were from the hearts of 52- and 53-year-old men whose arteries were clearly classified as atherosclerotic (Figures 2 and 6). However, at 10^-5 M glyceryl trinitrate, relaxations were significantly smaller in atherosclerotic arteries (Group B). The β-adrenoceptor agonist isoprenaline induced the same degree of relaxation in Group A and Group B arteries (Figure 7).
nonatherosclerotic (Group A) and atherosclerotic (Group B). In all other preconstricted preparations that relaxed in response to one or more endothelium-dependent vasodilators, acetylcholine had either no effect or produced moderate additional contractions. There were no appreciable differences in contraction between arteries of Groups A and B.

In the same arteries under basal tension, acetylcholine was about equipotent to prostaglandin F$_{\alpha}$ in inducing contractions (Figure 8). Interestingly, contractions induced by acetylcholine could be relaxed in an endothelium-dependent fashion by Ca$^{2+}$-ionophore A23187 ($n=12$), bradykinin ($n=4$), and substance P ($n=4$) (Figure 8).

**Effect of Indomethacin on Acetylcholine and Substance P-Induced Relaxations**

Acetylcholine is known to stimulate the production of prostacyclin in different animal arteries$^{12,14,15}$ and in isolated endothelial cells.$^{16}$ The prostacyclin formed could be relevant to the relaxation, since human coronary arteries are highly sensitive to this prostanoid (cf. below). Therefore, in 16 strip preparations from four human coronary arteries (1 belonging to Group A and 3 belonging to Group B), preconstricted with prostaglandin F$_{\alpha}$ (PGF$_{\alpha}$). Indomethacin ($10^{-5}$ M) was present in organ bath. Induced tension prior to addition of A23187 was 1.6 ± 0.2 g in nonatherosclerotic and 1.3 ± 0.1 g in atherosclerotic groups. Relaxations were significantly smaller in atherosclerotic arteries (17 strips from 6 patients) than in nonatherosclerotic arteries (16 strips from 5 patients). $^p<0.05$. 

**Effect of Endothelium-Dependent Relaxant A23187 on nonatherosclerotic and atherosclerotic human coronary arteries preconstricted with prostaglandin F$_{\alpha}$ (PGF$_{\alpha}$).** Indomethacin ($10^{-4}$ M) was present in organ bath. Induced tension prior to addition of A23187 was 1.6 ± 0.2 g in nonatherosclerotic and 1.3 ± 0.1 g in atherosclerotic groups. Relaxations were significantly smaller in atherosclerotic arteries (17 strips from 6 patients) than in nonatherosclerotic arteries (16 strips from 5 patients). $^p<0.05$. 

**Effect of Isoprenaline (whose relaxing effect is not mediated by endothelial cells) on nonatherosclerotic and atherosclerotic human coronary arteries preconstricted with prostaglandin F$_{\alpha}$ (PGF$_{\alpha}$).** Indomethacin ($10^{-5}$ M) was present in organ bath. Induced tension prior to addition of isoprenaline was 1.5 ± 0.3 g in the nonatherosclerotic and 1.3 ± 0.1 g in the atherosclerotic groups. Relaxations of nonatherosclerotic preparations (6 strips from 3 patients) and atherosclerotic preparations (20 strips from 6 patients) were not significantly different. 

**FIGURE 5.** Effect of endothelium-dependent relaxant A23187 on nonatherosclerotic and atherosclerotic human coronary arteries preconstricted with prostaglandin F$_{\alpha}$ (PGF$_{\alpha}$). Indomethacin ($10^{-4}$ M) was present in organ bath. Induced tension prior to addition of A23187 was 1.6 ± 0.2 g in nonatherosclerotic and 1.3 ± 0.1 g in atherosclerotic groups. Relaxations were significantly smaller in atherosclerotic preparations (17 strips from 6 patients) than in nonatherosclerotic arteries (16 strips from 5 patients). $^p<0.05$.

**FIGURE 6.** Effect of glyceryl trinitrate (whose relaxing effect is not mediated by endothelial cells) on nonatherosclerotic and atherosclerotic human coronary arteries preconstricted with prostaglandin F$_{\alpha}$ (PGF$_{\alpha}$). Indomethacin ($10^{-5}$ M) was present in organ bath. Induced tension prior to addition of glyceryl trinitrate was 1.5 ± 0.3 g in nonatherosclerotic and 1.4 ± 0.1 g in atherosclerotic preparations. Relaxations of nonatherosclerotic preparations (13 strips from 4 patients) and atherosclerotic preparations (23 strips from 8 patients) were not significantly different at $10^{-4}$ and $10^{-5}$ M. At $10^{-4}$ M, however, relaxations were significantly smaller in atherosclerotic arteries. $^p<0.05$.

**FIGURE 7.** Effect of isoprenaline (whose relaxing effect is not mediated by endothelial cells) on nonatherosclerotic and atherosclerotic human coronary arteries preconstricted with prostaglandin F$_{\alpha}$ (PGF$_{\alpha}$). Indomethacin ($10^{-5}$ M) was present in organ bath. Induced tension prior to addition of isoprenaline was 1.5 ± 0.3 g in the nonatherosclerotic and 1.3 ± 0.1 g in the atherosclerotic groups. Relaxations of nonatherosclerotic preparations (6 strips from 3 patients) and atherosclerotic preparations (20 strips from 6 patients) were not significantly different.

**FIGURE 8.** Effect of acetylcholine (ACh) on three different preparations of nonatherosclerotic human coronary arteries. First, the intima of the arteries was intact (+ ENDO); later, it was purposely rubbed to remove the endothelium (− ENDO). Indomethacin ($10^{-5}$ M) was present in organ bath. ACh-induced contractions were relaxed in endothelium-dependent fashion by Ca$^{2+}$-ionophore A23187 (preparation from a 14-year-old patient), by bradykinin (Bk) (preparation from a 24-year-old patient), and by substance P (SP) (different preparation from the same 24-year-old patient). All concentrations are expressed as logarithms of molar concentrations. W, wash, change of bath medium.
prostaglandin F<sub>2α</sub>, the effect of acetylcholine was tested before and after inhibition of prostaglandin synthesis with indomethacin. As indicated in Table 1, both the frequency and extent of relaxation in response to acetylcholine were significantly reduced by indomethacin. In contrast, relaxation by substance P (10<sup>−8</sup> M) was the same, irrespective of the absence or presence of the cyclooxygenase inhibitor (−82 ± 5% versus −85 ± 4%, mean ± SEM, n = 8). Preconstricted human coronary artery strips (in the presence of 10<sup>−5</sup> M indomethacin) proved to be highly sensitive to exogenous prostacyclin: 10<sup>−8</sup> M prostacyclin produced −81% ± 6% relaxation (mean ± SEM, n = 8) and 10<sup>−7</sup> M prostacyclin completely relaxed all preparations tested (−100%).

**Discussion**

The present study demonstrates that atherosclerotic lesions lead to a selective attenuation of endothelium-dependent vasodilation in human coronary arteries. This defect was observed with all endothelium-dependent vasodilators tested. In contrast, the endothelium-independent relaxations by isoprenaline were unchanged, and also relaxations by glyceryl trinitrate were attenuated only at the lowest concentration (10<sup>−3</sup> M) of the compound (Figure 6). As indicated by previous pharmacological studies, endothelium-dependent relaxations of human coronary arteries in response to the above compounds are mediated by EDRF.⁴

Acetylcholine, the "prototype" of endothelium-dependent vasodilators in animal arteries⁶ only rarely produced relaxation in human coronary arteries. A clear relation between the lack of acetylcholine relaxation and atherosclerotic lesions could not be demonstrated on the basis of the present data. We can confirm the recent report by Bossaller et al.¹⁷ that this lack of effect is specific for relaxations mediated by muscarinic receptors, as other receptor-operated relaxations could still be elicited in atherosclerotic arteries, albeit to a reduced degree (Figures 1, 3, and 4). Even when nonatherosclerotic arteries were preconstricted with acetylcholine, EDRF-mediated relaxation could be induced with A23187, Bradykinin, and substance P (Figure 8).

The most obvious reason why acetylcholine-induced relaxations were rarely observed in human coronary arteries is the marked, direct vasoconstrictor effect of the compound on human coronary arterial smooth muscle (Ginsburg et al.¹⁸ Figure 8). This constrictor potency is more pronounced in human coronary arteries than in various animal arteries including canine coronary arteries (U. Förstermann, unpublished observation). None of the other endothelium-dependent vasodilators tested (substance P, bradykinin, or A23187), produced direct constriction of human coronary smooth muscle. Thus, the effects of acetylcholine-induced EDRF release may be often masked by the potent constrictor effects of the compound. However, it cannot be excluded that the EDRF production was also smaller with acetylcholine than with the other endothelium-dependent relaxants.

Acetylcholine has also been shown to enhance the formation of the vasodilator prostacyclin in the endothelium of different arteries¹²,¹₄,¹₅ and in cultured endothelial cells.¹⁶ Accordingly, in human coronary arteries, the small relaxing effect of acetylcholine, if apparent, seems to have an indomethacin-sensitive component (presumably mediated by prostacyclin; Table 1) as well as a cyclooxygenase-independent component (mediated by EDRF; 8 of 60 preparations relaxed in the presence of indomethacin). On the other hand, cyclooxygenase products seem to play a negligible role in the marked endothelium-dependent relaxations induced by other agents, like substance P (this study).

Pharmacological concentrations of acetylcholine necessary to stimulate the EDRF mechanism in vitro are unlikely to occur at the endothelial cell. Therefore, other stimulators of EDRF could be much more important. These include vasoactive peptides, which are among the most potent stimulators of EDRF production,⁴,¹⁶ and adenine nucleotides, which may occur in vivo in concentrations necessary to induce EDRF-mediated relaxations.¹⁹ Adenosine diphosphate (10<sup>−9</sup> to 10<sup>−7</sup> M) did relax intact human coronary arteries; more than 60% of this response was mediated by endothelial cells (n = 8, U. Förstermann, unpublished observation).

In their recent study, Bossaller et al.¹⁷ have reported that in atherosclerotic coronary arteries, relaxations were completely preserved with the Ca<sup>2+</sup>-ionophore A23187. In our experiments, we found abnormal responses to both receptor agonists and the Ca<sup>2+</sup>-ionophore. We have no explanation for this discrepancy at present. Our data suggest, however, that abnormalities of endothelium-dependent relaxation are not solely related to receptor defects but also seem to involve the underlying effector mechanism.

Three explanations are conceivable for the impairment of endothelial relaxation in atherosclerotic arteries: 1) a decreased ability of the endothelium of atherosclerotic preparations to produce EDRF, 2) an impaired diffusion of EDRF (due to the thickened and sclerotic intima), and 3) an attenuated response of vascular smooth muscle. Whether decreased production or impaired diffusion of EDRF is mainly responsible for the attenuation of relaxation cannot be decided from the experiments in this study. At present, the relative importance of these two mechanisms in animal models of experimental atherosclerosis is debated. Using bioassay procedures for EDRF estimation, Verbeuren et al.²⁰ reported normal EDRF production in rabbit abdominal aortas moderately affected with cholesterol-induced atherosclerosis. In rabbit thoracic aortas that were apparently more affected by the atherosclerotic process, a reduced production of EDRF was found.²¹

Glyceryl trinitrate and EDRF (nitric oxide)³ are likely to have a similar mechanism of action in vascular smooth muscle. Both compounds lead to guanylate cyclase stimulation²¹,²² and to increased levels of cyclic guanosine monophosphate (for review, see Ignarro and
Kadowitz and Murad et al. In atherosclerotic human coronary arteries, relaxation in response to a low concentration of glyceryl trinitrate (10^{-9} M) was attenuated, but no inhibition of relaxation was found after higher concentrations (10^{-4} to 10^{-3} M) of the nitrovasodilator (Figure 6). This reduced potency, but unimpaired efficacy of glyceryl trinitrate has also been reported in atherosclerotic rabbit aortas. These data suggest that atherosclerosis can also affect smooth muscle responsiveness to stimulators of guanylate cyclase, including EDRF (nitric oxide). Vasodilators acting by other cellular mechanisms (like isoprenaline) seem to retain their relaxing potency in atherosclerosis (Figure 6).

In conclusion, the attenuation of EDRF-mediated relaxations observed in the present in vitro study may be one causal factor for the clinical observation that patients with atherosclerosis have an increased incidence of coronary vasospasm. This impairment of a potent vasodilator mechanism may promote coronary vasospasm and myocardial ischemia.

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References


Key Words: human coronary artery • endothelium-derived relaxing factor • atherosclerosis • substance P • bradykinin • A23187 • acetylcholine • glyceryl trinitrate • isoprenaline

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