Interaction Between Endothelin-1 and Endothelium-Derived Relaxing Factor in Human Arteries and Veins

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Endothelin-1 is a 21–amino acid endothelial vasoconstrictor peptide that may be the physiological antagonist of endothelium-derived relaxing factor (EDRF). Endothelin-1 (10^{-11} - 3 \times 10^{-7} M) evoked potent contractions of isolated internal mammary arteries, internal mammary veins, and saphenous veins, which were enhanced in internal mammary veins as compared with internal mammary arteries (concentration shift, 6.3-fold; \( p<0.05 \)) but not in the saphenous veins. Endothelial removal augmented the response to the peptide (at \( 3 \times 10^{-7} M \)) in internal mammary arteries (\( p<0.05 \)) but not in veins. In the artery, EDRF released by acetylcholine or bradykinin reversed endothelin-1–induced contractions; in saphenous veins, both agonists were much less effective compared with the artery and veins contracted with norepinephrine (\( p<0.005-0.01 \)). This inhibition of endothelium-dependent relaxations in veins occurred at half-maximal contractions but was most prominent at maximal contractions to the peptide. Nitric oxide similarly inhibited contractions to endothelin-1 and norepinephrine in internal mammary arteries, whereas in veins that were contracted with endothelin-1 but not with norepinephrine, the relaxations were blunted (\( p<0.005 \)). The nitric oxide donor SIN-1 and sodium nitroprusside induced complete relaxations of internal mammary arteries but were less effective in veins contracted with endothelin-1 (\( p<0.005 \)). Thus, in normal human arteries, EDRF inhibits endothelin-1–induced contractions, whereas the peptide specifically attenuates the effects of EDRF and nitrovasodilators in veins. This may be important in pathological conditions associated with increased levels of endothelin-1 and in veins used as coronary bypass grafts.

(Circulation Research 1990;66:1088–1094)

Endothelin-1 (human or porcine endothelin) is a 21–amino acid peptide hormone that is formed and released by endothelial cells after stimulation with thrombin, transforming growth factor \( \beta \), norepinephrine, phorbol ester, and the calcium ionophore A23187.\(^1\)–\(^3\) In experimental animals, the peptide has a greater vasoconstrictor potency than any other known vasoactive hormone.\(^4\)–\(^10\) Endothelin-1 is likely to be the physiological antagonist of endothelium-derived relaxing factors (EDRFs).\(^11\)–\(^15\) One of the factors, nitric oxide, is a potent vasodilator and inhibitor of platelet function and most likely accounts in large parts for endothelium–dependent relaxations evoked by acetylcholine and bradykinin.\(^16\)–\(^18\) The interaction and relative potency of endothelium–derived contracting and relaxing factors may be important in the regulation of local blood flow in health and disease.

The function of the endothelium and vascular smooth muscle differs in arteries and veins.\(^12\)–\(^13\),\(^19\)–\(^22\) Endothelium-dependent relaxations are less pronounced in veins compared with arteries,\(^19\)–\(^20\),\(^22\) whereas endothelium–dependent contractions predominate in veins.\(^23\) However, the heterogeneity of endothelium–dependent responses among species\(^13\) limits the results obtained in animal blood vessels and necessitates experiments in human vascular tissue.\(^20\),\(^22\)–\(^24\) The present study was designed to investigate the vascular effects of endothelin-1 in human blood vessels and its interaction with EDRF released under basal conditions and after stimulation with acetylcholine and bradykinin.
Materials and Methods

Internal mammary arteries and veins and saphenous veins were obtained intraoperatively. All patients (mean age, 59 ± 1 years; age range, 42–79 years; 84% males, 16% females) underwent bypass surgery for coronary artery disease. The vessels were dissected, cut into rings (5 mm in length), and placed into modified Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl 118, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25.0, edetate calcium disodium 0.026, and glucose 11.1 (control solution).

During harvesting, extreme care was taken not to touch the inner surface of the blood vessels. During surgical preparation of the saphenous veins, the dilatation procedure was avoided to preserve the endothelial layer. The presence or absence of the endothelium was confirmed at the beginning of the experiment in each ring (contracted with 10^{-7} – 3 \times 10^{-7} M norepinephrine) by a relaxation to acetylcholine or bradykinin (10^{-6} M). In some rings, the endothelium was removed by intraluminal perfusion of the vessel with 0.5% 3-[3-cholamidopropyl]dimethylamino]-1-propanesulfonate (CHAPS) for 60 seconds (mammary artery) or 90 seconds (saphenous vein) or mechanically. The rings were suspended in organ chambers filled with 25 ml control solution (37°C) aerated with 95% O2-5% CO2 and connected to force transducers (Statham Universal UC2, Gould, Cleveland, Ohio). Changes in isometric force were recorded. Before the experiment, the preparations were stretched to their optimal point of the length-tension relation.

The following drugs were used (unless otherwise indicated, the drugs were obtained from Sigma Chemical, St. Louis, Missouri): acetylcholine chloride, bradykinin, CHAPS, endothelin-1 (Peptide, Osaka, Japan), L-norepinephrine bitartrate, SIN-1 (the active metabolite of molsidomine; Hoechst Pharmaceutical, Paris, France), and sodium nitroprusside. The concentrations of the drugs are expressed as final molar concentrations in the bath solution.

Nitric oxide gas (99.9% pure) was obtained from L’Air Liquide Belge, Antwerp, Belgium. A glass bulb (125 ml in volume) was flushed with helium to remove oxygen. The flask was then filled with nitric oxide gas and closed. Separate glass bulbs were filled with 100 ml double-distilled water deoxygenated with helium over 4 hours. Appropriate volumes of nitric oxide gas were then injected into the water-filled gas bulbs, and the concentration of nitric oxide in the fluid was calculated, taking into account its solubility in water and air. All gas bulbs were interconnected in a closed gas-tight system to prevent any entry of oxygen (Arbor Ventil and Fitting AG, Niederrohrdorf, Switzerland). Nitric oxide was injected with gas-tight syringes (Hamilton, Kontron AG, Zurich, Switzerland) into the organ chamber near the suspended vessels.

Whenever possible, rings with and without endothelium of mammary arteries and veins and saphenous veins obtained from the same patients were studied in parallel. When concentration response curves were constructed, the drugs were added in a cumulative fashion except for nitric oxide gas (because of rapid breakdown and evaporation of the substance in the aerated organ chambers). Contractions are expressed as percent of the increase in tension induced by 100 mM KCl. The negative molar concentration of an agonist exhibiting 50% of the contraction to KCl (EC_{50} value) was calculated for each ring separately. To study endothelium-dependent relaxations, the rings were contracted with a maximal or half-maximal concentration of norepinephrine or endothelin-1, respectively. The relaxations are expressed as percent relaxation of the contraction induced by either norepinephrine or endothelin-1. The concentration of an agonist exhibiting 30% or 50% relaxation of that contraction was calculated for each ring and expressed as negative log molar (pD_{2}). For analysis, the maximal relaxation (in percent), the pD_{2} value, or the area under the concentration-response curve (in arbitrary units ranging from 0 to 1,000; i.e., the smaller the area, the greater the relaxation) was used. Data are given as mean±SEM. In all experiments, n equals the number of patients from whom the blood vessels were obtained. For statistical analysis, the t test for paired or unpaired observations and analysis of variance followed by Scheffe’s test were used. A two-tailed value of p < 0.05 was considered to indicate a statistical difference.

Results

Endothelin-1–Induced Constrictions

In internal mammary arteries with endothelium, endothelin-1 (10^{-11} – 3 \times 10^{-7} M) evoked concentration-dependent contractions (Figure 1). Endothelial removal enhanced the maximal response (3 \times 10^{-7} M) but not the sensitivity (EC_{50}) to the peptide (Figure 2; n = 9 and 8, p < 0.05). The internal mammary vein exhibited a markedly enhanced sensitivity and maximal response to endothelin-1 (Figure 1; n = 8). EC_{50} averaged 6.3 \times 10^{-10} M in the vein and 2.5 \times 10^{-9} M in the artery (concentration shift, 6.3-fold; p < 0.05); the maximal response was 123±8% and 87±5% of 100 mM KCl, respectively (p < 0.01). In the saphenous vein, endothelin-1 evoked similar contractions as in the artery (Figure 1; EC_{50}, 4 \times 10^{-9} M; n = 9; p = NS). The maximal response averaged 101±6% of 100 mM KCl. In the veins, endothelium removal did not affect the response to the peptide (Figure 2; n = 9 and 8).

Endothelin-1 and EDRF

Internal mammary artery. In rings maximally contracted with endothelin-1, acetylcholine (10^{-4} – 10^{-4} M) completely reversed the contractions induced by the peptide (Figure 3; n = 4). The maximal relaxation was 90±4%, and the pD_{2} value was 7.3±0.4. Brady-
Exogenous nitric oxide relaxed mammary arteries with a similar potency as endogenous EDRF irrespective of whether the rings were contracted with a maximal or half-maximal concentration of endothelin-1 (Figure 5; n=5 to 6). The response to nitric oxide did not differ, whether the arteries were precontracted with a half-maximal concentration of endothelin-1 or norepinephrine (Figure 6; n=5).

In mammary artery rings that were contracted with norepinephrine (3X10^{-7} M), endothelin-1 (10^{-13}-10^{-7} M) added on top of that contraction did not induce endothelium-dependent relaxations (n=3; data not shown).

**Veins.** In saphenous veins that were maximally contracted with endothelin-1, the relaxations induced by bradykinin (14±9% at 10^{-5} M) or acetylcholine (3±2% at 10^{-4} M) were severely blunted as compared with the artery (Figure 3; n=3 and 4, respectively; p<0.005 to 0.01). When contracted with a half-maximal concentration of endothelin-1, the relaxations to bradykinin were still markedly inhibited compared with preparations contracted with a half-maximal concentration of norepinephrine (Figure 4; pD_{50} 6.0±0.1 and 7.1±0.3; concentration shift, 13-fold; p<0.01; n=6).

Exogenous nitric oxide evoked potent relaxations of saphenous veins contracted with a half-maximal concentration of norepinephrine (Figure 6; n=5). Indeed, the response was slightly enhanced compared with the artery (p<0.01). In contrast, in saphenous and internal mammary veins contracted with a maximal or a half-maximal concentration of endothelin-1, the response to exogenous nitric oxide was severely blunted compared with those contracted with norepinephrine (Figures 5 and 6; n=4 and 5, respectively; p<0.005).

**Endothelin-1 and Nitrovasodilators**

The nitrocompound SIN-1 (10^{-9}-10^{-5} M), the active metabolite of molsidomine, inhibited maximal contractions induced by endothelin-1 in the internal mammary artery (pD_{50} 8.0±1.0; maximal response, 100±0%; Figure 7; n=4). The potency (48±11%)...
and the sensitivity to the nitrate (pD30, 4.2±1.0) were diminished in the saphenous vein compared with the artery (n=4; p<0.005). In both blood vessels, the response to SIN-1 was similar to that obtained by bradykinin under the same conditions (Figure 3). In contrast, in preparations contracted with norepinephrine (3×10⁻⁷ M), the relaxations induced by SIN-1 were enhanced in the saphenous veins compared with the mammary artery (concentration shift, eightfold; p<0.005; data not shown).

Sodium nitroprusside (10⁻⁸–10⁻⁴ M) was equally effective in internal mammary arteries irrespective of whether endothelin-1 or norepinephrine was used as a contractile stimulus (data not shown). In contrast, the veins, the nitrovasodilator was significantly less effective during contractions induced by endothelin-1 compared with those induced by norepinephrine (n=4; p<0.005).

Discussion

The present study demonstrates that endothelin-1 is a very potent vasoconstrictor hormone in human blood vessels. The internal mammary vein was more sensitive to the peptide than the artery; the saphenous vein was not. In the internal mammary artery, endothelin-1–induced vasoconstriction was completely inhibited by EDRF as well as by exogenous nitric oxide and nitrovasodilators, demonstrating the potency of the endogenous nitrate in normal human arteries. In contrast, endothelin-1 attenuated the effects of EDRF and nitrovasodilators in human veins.

The mammary vein exhibited more pronounced contractions to endothelin-1 than its anatomically adjacent artery. This finding confirms previous studies demonstrating that in experimental animals veins are more sensitive to the peptide than are arteries.8,26

Note that in arteries endothelium-derived relaxing factor (EDRF in normal human arteries. In contrast, endothelin-1 attenuated the effects of EDRF and nitrovasodilators in human veins.

The mammary vein exhibited more pronounced contractions to endothelin-1 than its anatomically adjacent artery. This finding confirms previous studies demonstrating that in experimental animals veins are more sensitive to the peptide than are arteries.8,26
The saphenous vein, however, was much less sensitive to the peptide than the mammary vein. This may indicate a heterogeneity of the responsiveness to endothelin-1 not only between arteries and veins, but also within the human venous system.

The fact that endothelin-1 dominates EDRF in the veins could be related to greater effects of endothelin-1 on venous vascular smooth muscle, to a reduced release of EDRF in the veins, and/or to a reduced responsiveness of veins to endogenous or exogenous nitrovasodilators. Differences in the contractile state of arteries and veins are unlikely to play a role, since endothelin-1 inhibited endothelium-dependent relaxations in saphenous veins both at maximal and half-maximal contractions to the peptide. In addition, the response to exogenous nitric oxide was also blunted in the internal mammary vein, which exhibited an enhanced responsiveness to endothelin-1, as much as in the saphenous vein, which responded in a manner similar to the artery. The fact that comparable increases in tension evoked by norepinephrine instead of endothelin-1 did not inhibit the effects of EDRF in veins further excludes differences in the contractile state of arteries and veins as a possible explanation.

A blunted release of EDRF is suggested by weaker endothelium-dependent relaxations to acetylcholine in the saphenous vein compared with the mammary artery.20 This, however, cannot explain the differences observed in this study, since EDRF released by
bradykinin was capable of inducing potent relaxations if the veins were contracted with norepinephrine instead of endothelin-1. A different interaction of endothelin-1 at the level of the endothelial cells (i.e., release of ED Raf27) is also unlikely, since in the human blood vessels studied the peptide did not release sufficient amounts of ED Raf to evoke a relaxation. Thus, endothelin-1 must interfere with the capability of venous vascular smooth muscle to relax in response to ED Raf. In line with this interpretation is the observation that endothelin-1 inhibited the relaxations induced by exogenous nitric oxide to a similar degree as those induced by ED Raf released by either bradykinin or acetylcholine. The experiments in which the blood vessels were contracted with norepinephrine instead of endothelin-1 further indicate that this must represent a specific property of endothelin-1. Indeed, in venous preparations contracted with the catecholamine, the sensitivity and potency of nitrates (i.e., nitric oxide, SIN-1, and sodium nitroprusside20,28–30) were similar or slightly augmented compared with the arteries. Thus, endothelin-1 must interfere with the effects of compounds that activate cyclic GMP28,31 in venous smooth muscle, whereas in the mammary artery, this pathway remains fully operative. The blunted effects of ED Raf and nitrovasodilators in veins exposed to endothelin-1 may be related to a reduced formation of cyclic GMP or to a decreased effectiveness of the second messenger. Indeed, in the dog, endothelin-1 induces a more pronounced depolarization of venous than arterial smooth muscle,26 a phenomenon that is associated with a decreased potency of ED Raf and nitrovasodilators.32

Although all blood vessels were obtained from patients with coronary artery disease who often exhibit atherosclerotic changes in other parts of the arterial circulation, it is likely that similar responses occur in healthy subjects of the same age. Indeed, the mammary artery stays remarkably free of atherosclerotic changes in the vast majority of patients undergoing coronary bypass surgery.33,34 The functional integrity of the endothelial layer of the mammary arteries studied is demonstrated by a full relaxation to acetylcholine, a response that is severely blunted in atherosclerotic human arteries.35,36

In human blood vessels, endothelin-1 induced most sustained contractions. Therefore, the availability of a potent endogenous antagonist such as ED Raf for prevention of vasoconstriction and eventually vascular occlusion appears important.13,14,37 Endothelin-1 is released by thrombin and transforming growth factor β, which can be formed or released at sites where platelets are activated.1–3 If the production of endothelin-1 is locally stimulated in response to these platelet-derived substances, its effects would be fully antagonized by ED Raf in the mammary artery but not in veins.20,38,39 Indeed, thrombin and ADP, which is also released from aggregating platelets, can liberate ED Raf in healthy human arteries; in veins, much less of the endogenous nitrate is released under these conditions.20,22 Similarly, in atherosclerotic arteries in which the release of ED Raf is blunted,35,36,40–42 endothelin-1 would be unopposed by the endogenous nitrate. The imbalance of the effects of ED Raf and endothelin-1 in veins may be of great clinical importance for coronary bypass surgery. Increased platelet–vessel wall interaction has been implicated in vein graft occlusion, and platelet inhibitors are effective in increasing the patency of venous bypasses.43 Indeed, the dominance of ED Raf in the mammary artery may contribute to the better short-term and long-term patency and lower patient mortality of arterial grafts compared with venous grafts.44–47 Moreover, this study would indicate that nitrates are more efficacious in arterial grafts than in venous bypasses as therapeutic interventions aimed at antagonizing endothelin-1–induced vasoconstriction.

Acknowledgments

The authors wish to thank E. Weber, B. Libs, and A. de S. Pinto, Department of Research, University Hospital Basel, and D. Popovic and S. Distel, Operating Room Laboratory, University Hospital Zurich, for technical assistance.

References


KEY WORDS: acetylcholine, bradykinin, internal mammary artery, internal mammary vein, saphenous vein, nitric oxide, nitric oxide donor SIN-1, sodium nitroprusside
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doi: 10.1161/01.RES.66.4.1088

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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