Different Activation of the Endothelial L-Arginine and Cyclooxygenase Pathway in the Human Internal Mammary Artery and Saphenous Vein

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The endothelium releases substances controlling vascular tone and platelet function. We investigated mediators of endothelium-dependent responses in human internal mammary arteries and saphenous veins. The inhibitor of nitric oxide formation, N⁶-monomethyl L-arginine, enhanced the sensitivity to norepinephrine (fivefold) and evoked more pronounced endothelium-dependent contractions in internal mammary arteries (19±6% of 100 mM KCl) than in saphenous veins (2±1%; p<0.005). In internal mammary arteries, N⁶-monomethyl L-arginine, but not indomethacin, markedly reduced endothelium-dependent relaxations to acetylcholine (from 95±2% to 39±7%; p<0.005) and prevented those to histamine (78±6% to 4±3%; p<0.005). In saphenous veins, endothelium-dependent relaxations to acetylcholine were weak (24±11%), while nitric oxide caused comparable relaxations (85±3%) as in internal mammary arteries (80±5%; NS). N⁶-Monomethyl L-arginine prevented the relaxations to acetylcholine and unmasked endothelium-dependent contractions (30±10%). Indomethacin and the thromboxane synthetase inhibitor CGS-13080 augmented relaxations of saphenous veins to acetylcholine from 24±11% to 46±9% (p<0.05). Histamine-evoked contractions were converted to endothelium-dependent relaxations by indomethacin and the thromboxane A₂/endoperoxide receptor antagonist SQ-30741 (38±3% and 40±6%; p<0.05) but not CGS-13080. Thus, 1) nitric oxide mediates endothelium-dependent relaxations in human arteries and veins; 2) internal mammary arteries release more nitric oxide than do saphenous veins, and 3) in saphenous veins, the effects of nitric oxide are reduced by endothelium-derived contracting factors originating from the cyclooxygenase pathway. (Circulation Research 1991; 68:52–60)

The endothelium is a source of several vasoactive substances that can affect vascular tone and platelet function.¹² Acetylcholine, bradykinin, histamine, and other agents can release endothelium-derived relaxing factors from endothelial cells in culture and from intact arteries with endothelium.³⁻⁹ In experimental animals, nitric oxide accounts at least in part for the biological activity of endothelium-derived relaxing factors released by acetylcholine and bradykinin.¹⁰⁻¹¹ In endothelial cells, nitric oxide is cleaved from its precursor, the amino acid L-arginine, by specific enzymes.¹²⁻¹⁴ By means of the methylated amino acid N⁶-monomethyl L-arginine (LNMMA), this pathway for the formation of nitric oxide can be inhibited.¹⁵⁻¹⁷ In addition to nitric oxide, the endothelium can produce other relaxing factors such as prostacyclin¹⁶⁻¹⁹ and yet unidentified substances such as endothelium-derived hyperpolarizing factor.²₀⁻²⁴ Furthermore, at least in blood vessels of dogs and rats, the endothelium can mediate contractions.²₅⁻₃₃ While the endothelium-derived contracting factor released during hypoxia is resistant to most pharmacological interventions,²₆ the contractions to arachidonic acid and acetylcholine can be prevented by inhibitors of cyclooxygenase.²₈⁻₃₁ The cyclooxygenase...
pathway can produce a variety of substances such as thromboxane A₂32 and other prostaglandins1,2,18 as well as superoxide anions33 that evoke contractions in isolated blood vessels.

Endothelium-dependent relaxations differ in arteries and veins.4,9,25 This difference could be related to the release of different amounts of endothelium-derived nitric oxide or other endothelium-derived relaxing factors,4,34,35 a different vascular responsiveness to the relaxing factors, or a different release of endothelium-derived contracting factors in the two blood vessels. This could have important consequences for the regulation of blood flow and that of platelet function12 in arteries and veins. In addition, this may contribute to functional differences of human arteries and veins when they are used as bypass grafts to treat patients with coronary artery disease.1,39,40

Thus, the present experiments were designed to characterize endothelium-dependent relaxations and contractions in the human internal mammary artery and saphenous vein and to delineate the mediators involved in these responses.

Materials and Methods

Preparation of Blood Vessels

Internal mammary arteries and saphenous veins were obtained from patients undergoing coronary bypass surgery for coronary artery disease. Care was taken during harvesting of the blood vessels not to touch the inner surface of the blood vessels. During surgical preparation of the saphenous vein, the -lateral procedure was avoided. Veins stained with methylene blue were discarded. Immediately after removal, the vessels were placed into modified Krebs-Ringer bicarbonate solution of the following composition (millimolar): 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). The blood vessels were dissected free, cut into rings of about 5 mm in length, and suspended in organ chambers filled with control solution (37°C, aerated with a gas mixture containing 95% O₂ and 5% CO₂).

Experimental Setup

The blood vessel rings were suspended in organ chambers filled with control solution, and changes in isometric force were recorded with force transducers (Statham Universal UC2, Gould, Inc., Cleveland, Ohio, or Showa Sokki, Rikadenki GmbH, Freiburg, FRG). Before the experiment, the vascular rings were stretched and exposed to norepinephrine (10⁻⁷ or 3×10⁻⁷ M in veins and arteries, respectively) at each level of stretch, until the optimal point of the length–tension relation was reached. Then the vessels were allowed to equilibrate for 45 minutes.

Endothelium Removal

The presence or absence of the endothelium was confirmed in each ring (contracted with norepineph-
contraction induced by norepinephrine. For analysis of the relaxations, the maximal relaxation, the negative log molar concentration of a given vasodilator exhibiting 50% relaxation (pD₂ value), was used. In certain experiments, the vessels were preincubated with drugs that interfere with the release of endothelium-derived vasoactive substances (i.e., LNMMA, indomethacin, and CGS-13080 for 30 minutes; SQ-30741 and superoxide dismutase for 15 minutes). Data are given as mean±SEM. In all experiments, n equals the number of patients from whom the blood vessels were obtained. Whenever possible, concentration–response curves of the tested agonists were performed in the presence and absence of the inhibitors in the same ring or in the rings obtained from the same patient. Because LNMMA by itself caused an increase in tension, the concentrations of agonists used to precontract the vessels were reduced to match the contractions obtained under control conditions. The t test for paired or unpaired observations and analysis of variance (ANOVA) followed by Scheffe’s test were used for statistical analysis. A two-tailed value of p<0.05 was considered to indicate a statistical difference.

FIGURE 1. Increase in tension caused by the inhibitor of nitric oxide formation, N⁶-monomethyl L-arginine (L-NMMA, 10⁻⁴ M in the presence of indomethacin 10⁻³ M) in the human internal mammary artery (IMA) and saphenous vein (SV) with endothelium. The contractions are expressed as percent of the increase in tension obtained with 100 mM KCl. *Statistically significant difference between the internal mammary artery and saphenous vein (p<0.005).

Results

Basally Released Endothelium-Derived Nitric Oxide

Endothelium-dependent contractions to LNMMA. In quiescent internal mammary arteries with, but not in those without, endothelium, LNMMA (10⁻⁴ M in the presence of indomethacin 10⁻³ M) evoked a contraction averaging 19±6% of the response to 100 mM KCl (Figure 1; p<0.001 as compared with rings without endothelium, n=4). In contrast, in the saphenous vein with endothelium, LNMMA (10⁻⁴ M in the presence of indomethacin 10⁻⁵ M) caused only a small contraction (2±1%; Figure 1; p<0.005 as compared with the artery, n=4).

Contractions to norepinephrine. Norepinephrine (10⁻⁹ to 10⁻⁵ M) caused concentration-dependent contractions in internal mammary artery. In vascular rings with endothelium, LNMMA (10⁻⁴ M) enhanced the sensitivity to norepinephrine (Figure 2, left panel; pD₂ control, 6.3±0.2; pD₂ LNMMA, 7.0±0.2; concentration shift, fivefold; p<0.05). In artery rings without endothelium, the methylated L-arginine analogue was without any effect (Figure 2, right panel; n=4).

FIGURE 2. Contractions induced by increasing concentrations of norepinephrine in internal mammary arteries with endothelium (left panel) or without endothelium (right panel). The inhibitor of nitric oxide formation, N⁶-monomethyl L-arginine (L-NMMA, 10⁻⁴ M), augmented the contractions in preparations with endothelium (left panel; concentration shift, fivefold, p<0.05), while it had no effect in arteries without endothelium (right panel).
L-NMMA does not affect the contractions to norepinephrine in the human saphenous vein.

Norepinephrine (10⁻⁹ to 10⁻⁴ M) caused contractions in saphenous veins with endothelium (Figure 3; pD₂ 6.3±0.01, n=4). LNMMA (10⁻⁴ M in the presence of indomethacin 10⁻³ M) did not augment the contractions to norepinephrine (Figure 3; n=4).

Stimulated Release of Endothelium-Derived Substances

Acetylcholine. In arteries with endothelium, acetylcholine (10⁻⁷ to 10⁻⁴ M) caused potent relaxations (Figure 4; n=8). The maximal response averaged 95±2%, and the pD₂ value was 7.7±0.1. Indomethacin (10⁻³ M), used to inhibit the production of prostacyclin, did not affect the sensitivity or the maximal response to the muscarinic agonist. LNMMA (10⁻⁴ M) markedly reduced the sensitivity and maximal response (39±7%) to acetylcholine (Figure 4; p<0.005 as compared with control). If indomethacin and LNMMA were added together, a further reduction of the response to 23±8% was achieved (Figure 4; p<0.05 as compared with LNMMA alone, n=5).

In saphenous veins, acetylcholine (10⁻⁹ to 10⁻⁴ M) caused only weak endothelium-dependent relaxations (Figure 5, left panel; n=5). The maximal response averaged 24±11% (p<0.05 as compared with rings without endothelium, n=4). The relaxation obtained was significantly less than in the mammary artery (p<0.005). LNMMA (10⁻⁴ M) prevented the relaxations to acetylcholine in the saphenous vein and unmasked endothelium-dependent contractions (Figure 6). The maximal contraction in LNMMA-treated rings with endothelium averaged 30±10% (Figure 5, left panel; p<0.05 as compared with rings without endothelium, n=4).

In contrast to the mammary artery, indomethacin (10⁻³ M) augmented endothelium-dependent relaxations to acetylcholine from 24±11% to 46±9% in the saphenous vein (Figure 5, left panel; p<0.05 as compared with control, n=5). The thromboxane synthetase inhibitor CGS-13080 (10⁻⁵ M) augmented the relaxations to acetylcholine to a comparable degree as indomethacin from 24±8% to 46±5% (Figure 5, right panel; p<0.05, n=8).

Histamine. Histamine (10⁻⁹ to 10⁻⁶ M in the presence of cimetidine 10⁻⁷ M and indomethacin 10⁻³ M) caused endothelium-dependent relaxations with a maximal response of 78±6% in internal mammary artery (Figure 7; pD₂ 7.0±0.2, n=4). LNMMA (10⁻⁴ M) completely prevented the relaxations induced by the monoamine (maximal response, 4±3%; p<0.005 as compared with control).

In rings of saphenous vein, histamine (10⁻⁹ to 10⁻⁶ M) did not cause significant endothelium-dependent relaxations under control conditions (i.e., in the absence of any inhibitor; maximal response, 9±5%) but evoked marked contractions at higher concentrations of the monoamine (3×10⁻⁷ to 1×10⁻⁶ M; maximal response, 72±16%; Figure 8, left panel; n=4). Endothelium-dependent relaxations were unmasked in the presence of both indomethacin (10⁻⁵ M) and the thromboxane A₂/endothoperoxide receptor antagonist SQ-30741 (3×10⁻⁶ M; Figure 8, right panel). The maximal relaxations obtained in the presence of indomethacin and SQ-30741 averaged...
The inhibitor of nitric oxide formation, N\textsuperscript{\textbf{G}}-monomethyl L-arginine (L-NMMA, 10\textsuperscript{-4} M), unmasks endothelium-dependent contractions to the muscarinic agonist. In contrast, the inhibitor of cyclooxygenase, indomethacin (10\textsuperscript{-5} M), significantly augments endothelium-dependent relaxations to acetylcholine (p<0.05). The inhibitor of thromboxane \(A_2\) synthetase, CGS-13080 (10\textsuperscript{-5} M), augments the relaxations induced by acetylcholine to a similar degree as indomethacin (p<0.05 as compared with control).

38±3% and 40±6%, respectively (p<0.005 as compared with control, \(n=4\) to 5). In contrast, the thromboxane synthetase inhibitor CGS-13080 (10\textsuperscript{-5} M) did not significantly affect the response.

The endothelium-dependent relaxations to histamine that occurred in the presence of indomethacin were abolished by L-NMMA (10\textsuperscript{-4} M; Figure 8, left panel; maximal response, 1±2%; p<0.005 as compared with indomethacin alone, \(n=5\)). In addition, L-NMMA (10\textsuperscript{-4} M) markedly enhanced the contractions to histamine (10\textsuperscript{-6} M; 200±64%; p<0.05 as compared with control, \(n=4\)). Superoxide dismutase (150 units/ml in the presence of LNMA 10\textsuperscript{-4} M) did not inhibit the response (209±10%; \(p=NS\) as compared with LNMA alone, \(n=4\)).

**Relaxations to Nitric Oxide**

Exogenous nitric oxide (10\textsuperscript{-9} to 3\times10\textsuperscript{-6} M) caused concentration-dependent relaxations of internal...
mammary arteries and saphenous veins. The maximal relaxation did not differ in the two blood vessels and averaged 85±3% in the artery and 80±5% in the vein (n=11 and 16, respectively; NS). The saphenous vein exhibited a slightly enhanced response to lower concentrations of nitric oxide (10⁻⁸ to 10⁻⁷ M) as compared with the mammary artery (p<0.05), while the pD₂ value did not differ statistically (6.7±0.2 and 7.0±0.2, NS; n=11 and 16, respectively).

**Discussion**

The present study demonstrates a differential activation of the endothelial l-arginine and cyclooxygenase pathway in the human internal mammary artery and saphenous vein. In the artery, potent endothelium-dependent relaxations were evoked by acetylcholine and histamine; the relaxations are mediated by endothelium-derived nitric oxide, while the cyclooxygenase products do not contribute to the response. In contrast, veins release less endothelium-derived nitric oxide in response to both autacoids than do arteries, and the nitric oxide effects are markedly inhibited by concomitantly released endothelium-derived contracting factors originating from the cyclooxygenase pathway.

The human internal mammary artery must continuously release nitric oxide since the inhibitor of nitric oxide formation, LNMMA, evoked endothelium-dependent contractions in this preparation. Similarly, LNMMA augmented the contractions induced by norepinephrine in arterial rings with, but not in those without, endothelium. This indicates that the basal release of endothelium-derived nitric oxide importantly modulates the effects of vasoconstrictor hormones in human arteries. In contrast to the arteries, the saphenous vein appears to form very little nitric oxide under basal conditions, since LNMMA evoked only weak endothelium-dependent contractions in quiescent preparations and had no effect on the concentration–response curve to norepinephrine.

The fact that acetylcholine and histamine evoked potent endothelium-dependent relaxations in the human internal mammary artery confirms previous observations. This study demonstrates that both agonists activate the endothelial l-arginine pathway and that nitric oxide fully accounts for the relaxations induced by histamine and in large part for that caused by acetylcholine. Indeed, the inhibitor of the formation of nitric oxide, LNMMA, prevented the endothelium-dependent relaxation to histamine. Since prostacyclin has been excluded, nitric oxide formed from l-arginine must be the only mediator of the response to histamine. Similarly, LNMMA markedly reduced endothelium-dependent relaxations to acetylcholine. In contrast to histamine, however, the muscarinic agonist still evoked a small relaxation in the presence of the inhibitor of the l-arginine pathway, indicating that other relaxing factors are released as well. Indeed, although the cyclooxygenase inhibitor indomethacin had no effect on endothelium-dependent relaxations to acetylcholine under control conditions, in the presence of LNMMA, the drug further inhibited the response. Because prosta-
cyclin is the major cyclooxygenase product of the internal mammary artery and a potent vasodilator in this preparation, this indicates that the prostanoid contributes to the endothelium-dependent relaxations only during inhibition of the L-arginine pathway. Indeed, in endothelial cells in culture, nitric oxide inhibits the formation of prostacyclin. Thus, it is likely that nitric oxide formed during stimulation with acetylcholine reduces the formation of the prostanoid under control conditions, while prostacyclin contributes to the endothelium-dependent relaxations during blockade of the L-arginine pathway. The fact that a small relaxation to acetylcholine persisted, even in the presence of LNMMMA and indomethacin, may be related to an incomplete inhibition of the L-arginine pathway or due to the release of a relaxing substance distinct from nitric oxide and prostacyclin such as endothelium-derived hyperpolarizing factor.

It is of interest that, although all patients from whom the internal mammary arteries were obtained suffered from coronary artery disease, the vessels exhibited potent endothelium-dependent relaxations to acetylcholine and histamine. Indeed, atherosclerosis is associated with a decreased release of endothelium-derived relaxing factor both in experimental animals and humans. Although it cannot be excluded that even better responses would be observed in normal subjects, this may be related to the known resistance of the human internal mammary artery against atherosclerosis. Even in patients with coronary artery disease, the incidence of atherosclerosis in the human internal mammary artery is less than 5%.

In line with previous observations from this laboratory, endothelium-dependent relaxations to acetylcholine and histamine were markedly less pronounced in the saphenous vein than in the mammary artery. In contrast to the artery, in the veins indomethacin caused a pronounced augmentation of the relaxations induced by acetylcholine and unmasked those to histamine. The relaxations to acetylcholine and histamine that occurred after inhibition of cyclooxygenase were prevented by LNMMMA, indicating that, in contrast to dogs, nitric oxide is the only relaxing factor released in human veins after stimulation with the autacoids. A reduced capacity of venous vascular smooth muscle to relax in response to nitric oxide can be excluded as a cause of the much weaker endothelium-dependent relaxations in veins as compared with arteries. Indeed, the relaxations induced by exogenous nitric oxide, as those to nitrovasodilators, were slightly enhanced in the human saphenous vein as compared with the internal mammary artery. Thus, veins must form less nitric oxide in their endothelial cells as compared with arteries.

In addition to nitric oxide, the saphenous vein concomitantly releases an endothelium-derived contracting factor that offsets, at least in part, the relaxing effects of the endogenous nitrate. Indeed, in the presence of LNMMMA, acetylcholine evoked endothelium-dependent contractions. Since both the inhibitor of cyclooxygenase, indomethacin, and that of thromboxane synthetase, CGS-13080, enhanced the endothelium-dependent relaxations to acetylcholine to a similar degree, thromboxane A2 must mediate the response. Similar conclusions have been reached for the basilar artery of dogs. In venous coronary bypass grafts studied in situ in the catheterization laboratory, selective infusion of acetylcholine does cause a contraction, while arterial grafts exhibit a dilatation in response to the muscarinic agonist. Thus, this response may also be operative in vivo. In contrast, indomethacin and the thromboxane A2/endothelium-derived contracting system may be important to limit blood loss during hemorrhage.

The present findings may have important consequences for coronary bypass surgery and graft function. In arterial grafts, endothelial damage during surgical preparation would augment the effects of vasoconstrictor hormones such as norepinephrine and serotonin and may in turn precipitate postoperative vasospasm. If the endothelial layer is preserved, however, the internal mammary artery has a potent endogenous nitrate system that can limit or prevent vasospasm, thrombus formation, and eventually graft occlusion. In contrast, in saphenous veins used as coronary bypass grafts, the endothelial L-arginine pathway is much less active and its effects are inhibited by concomitantly released endothelium-derived contracting factors. Particularly, thromboxane A2 may facilitate platelet adhesion and thrombus formation and may endanger local blood flow. In venous grafts, cyclooxygenase inhibitors favorably correct the imbalance between endothelium-derived nitric oxide and the contracting factors, and this may contribute to the beneficial effects of aspirin and aspirinlike drugs on venous graft function. A greater release of endothelium-derived nitric oxide in arterial grafts may not only protect against vasospasm and thrombus formation, but also against the development of atherosclerotic changes that commonly develop in venous grafts. Indeed, in vascular smooth muscle cells in culture, stimulation of cyclic GMP by nitric oxide and other nitrovasodilators inhibits mitogenesis and proliferation. Thus, the
differential biological properties of venous and arterial grafts may importantly contribute to graft function and at least in part explain their differential patency rate and clinical outcome.

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References


46. Brunkwall J, Bergqvist D, Stjernquist U: Prostacyclin and thromboxane release from the vessel wall: Comparison between an incubation and a perfusion model. Prostaglandins 1987;34:467–476


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