Endothelium-Derived Relaxing Factor and Protection Against Constrictions Induced by Histamine and Serotonin in the Human Internal Mammary Artery and in the Saphenous Vein

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We investigated the release of endothelium-derived relaxing factor (EDRF) in response to serotonin and histamine in the human internal mammary artery and saphenous vein. The arteries and veins were obtained intraoperatively and were suspended in organ chambers to record isometric tension. In mammary arteries, histamine (10^{-8} to 3 	imes 10^{-6} M) induced relaxations in rings with (70 \pm 5\% , \text{IC}_{50}, 6.5 \pm 0.2) but not without endothelium (p < 0.005 for rings with compared with those without endothelium, n = 7–10). The response was inhibited by methylene blue or hemoglobin, but not meclofenamate, and, therefore, EDRF was delineated as the mediator. Because chlorpheniramine but not cimetidine inhibited the response, EDRF was released by the H_{1}-histaminergic receptor (n = 5–8). In contrast, in saphenous veins, histamine caused only weak or absent endothelium-dependent relaxations, but contractions were enhanced in rings with endothelium (p < 0.05, n = 6). Serotonin did not induce endothelium-dependent relaxations, but contractions were markedly greater in veins compared with arteries (p < 0.005, n = 6). The endothelium inhibited the maximal contraction to serotonin in arteries (p < 0.034) but not in veins. Thus, EDRF protects against contractions induced by histamine and serotonin in the mammary artery but not in the saphenous vein. This may be important for improved graft function and patency of the artery compared with that of the vein. (Circulation 1989;80:1041–1048)

An increased interaction between platelets and other circulating blood cells and the vessel wall may play an important role in ischemia and vascular occlusion.1–7 Platelet-derived substances and coagulation products can interact with both the vessel wall and hemostatic systems to promote vasospasm and thrombosis.3–7 Serotonin and histamine, which can be released from platelets, mast cells, and the endothelium, have been implicated in coronary vasospasm in humans.1,5,8–11 The blood vessel wall has protective mechanisms preventing the occurrence of platelet adhesion, platelet aggregation, and vasospasm under normal conditions.1,4–6 The endothelium appears to play an instrumental role by releasing endothelium-derived relaxing factor (EDRF), which evokes relaxation of vascular smooth muscle and inhibition of platelet function.1,12–16 Ischemia and vascular occlusion occur frequently in some but rarely in other vascular beds of the circulation. Similarly, arterial coronary artery bypass grafts have a higher patency and lower patient mortality than venous grafts.17–20 We previously showed that endothelium-dependent relaxations to acetylcholine, ADP, and thrombin are larger in human internal mammary arteries than in saphenous veins of patients undergoing coronary artery
bypass surgery. This study was performed to investigate whether or not platelet-derived products such as serotonin and histamine evoke different endothelium-dependent responses in human artery and veins used in bypass grafting and whether or not EDRF mediates the response. Different endothelium-dependent responses to platelet-derived products and to histamine may be important for graft function and patency.

Methods

Protocol

Internal mammary arteries and veins and saphenous veins were harvested intraoperatively from patients undergoing coronary artery bypass surgery for coronary artery disease. The internal mammary artery and vein were dissected free, leaving the vessels in their original surrounding on a pedicle of internal thoracic fascia as previously described.

The experiments were performed in rings of the internal mammary artery and veins and in the saphenous vein with and without endothelium. The blood vessels were dissected free and cleaned of adherent connective tissue under a dissection microscope (Wild and Leitz, Zurich, Switzerland) as reported. The preparations were then cut into rings (about 5 mm in length) and placed in cold modified Krebs-Ringer bicarbonate solution of the following millimolar composition: 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 presence or absence of functional vascular endothelium was confirmed in each ring at the beginning of the experiment by the presence or absence of a relaxation to acetylcholine (10−6 M) or bradykinin (10−6 M). In internal mammary artery rings with endothelium, the relaxation to acetylcholine averaged 90±4%; in saphenous veins with endothelium, the relaxation to bradykinin was 54±4%. The endothelium was removed by intraluminal perfusion of the vascular segment with 0.5% 3-[(3-cholamidopropyl)dimethylammonio]1-propanesulfonate (CHAPS) for 60 seconds from rings of the internal mammary artery and for 90 seconds from rings of the saphenous vein.

The rings were suspended between two stirrups in organ chambers filled with 25 ml control solution (37°C) aerated with 95% O2-5% CO2. The rings were connected to force transducers (Statham Universal UC2), and changes in isometric force were recorded. The ring preparations were progressively stretched and exposed to norepinephrine (3×10−7 M) at each level of stretch until the optimal point of the length-tension relation was reached. Thereafter, the rings were allowed to equilibrate for 45 minutes. In some experiments, the preparations were incubated for 30 minutes with meclofenamate (10−5 M) to inhibit the production of vascular prostaglandins.

Drugs

The following drugs were used (unless otherwise stated, all drugs were from Sigma Chemical, St. Louis, Missouri): acetylcholine chloride, bradykinin, CHAPS, cimetidine (Smith, Kline & French, Welwyn Garden, Herts, UK), chlorpheniramine, hemoglobin (human), histamine, ketanserin (Janssen Pharmaceuticals, Beersse, Belgium), meclofenamate sodium (Smith, Kline & French Laboratories), methylene blue, L-norepinephrine bitartrate, serotonin (5-hydroxytryptamine). The concentrations of the drugs are expressed as final molar concentrations in the bath solution. All drugs were dissolved in distilled water. Human hemoglobin was prepared from freshly drawn human blood.

Data and Statistical Analysis

Rings with and without endothelium of human internal mammary artery, internal mammary vein, and saphenous veins were used for study. Maximal contractions are expressed as percentage of the increase in tension induced by 100 mM KCl. The concentration of an agonist exhibiting 30% contraction of that increase in tension (EC30 value) was calculated for each ring separately and expressed as negative log M. In experiments in which endothelium-dependent relaxations were studied, the rings were contracted with the half-maximal concentration of norepinephrine. The relaxations are expressed as percent relaxation of that contraction. For analysis, the maximal relaxation (in percentage), the concentration exhibiting 50% relaxation (IC50 value), or the area under the concentration-response curve (in arbitrary units) was used. Data are given as mean±SEM. In all experiments, n is the number of patients from whom the blood vessels were obtained. The t test for paired or unpaired observations was used for statistical analysis. A two-tailed p value smaller than 0.05 was considered to indicate a statistical difference.

Histamine

Endothelium-dependent responses. In internal mammary arteries contracted by norepinephrine (3×10−7 M), histamine induced relaxations in rings with but not in those without endothelium (p<0.005 for the comparison between rings with and without endothelium, n=7, Figures 1 and 2). In rings with endothelium, the IC50 value of histamine averaged 6.5±0.2, and the maximal relaxation averaged 70±5% (Table 1). Meclofenamate (10−5 M), used to block the production of prostacyclin, did not affect the relaxations evoked by histamine (ED50, 6.3±0.2; maximal relaxation, 62±9%; n=6). In rings with endothelium that were previously relaxed by acetylcholine (10−6 M, 87±6%), pretreatment with methylene blue (10−3 M), used to block the production of cyclic GMP, completely prevented the relaxations but not the contractions induced by histamine (n=3). Human hemoglobin (10−2 M) added after maximal
relaxation to histamine completely reversed the response (n=3, Figure 1).

In internal mammary artery rings with endothelium, the H1-histaminergic receptor antagonist chlorpheniramine (10^-5 M) inhibited relaxations to histamine (Figure 3). The H2-histaminergic blocker cimetidine (10^-5 M) did not affect endothelium-dependent relaxations but did augment the contractile response occurring at the highest concentration of histamine (10^-3 M). Combined H1- and H2-histaminergic blockade prevented relaxations induced by histamine.

Figure 1. Recordings of effects of histamine in human internal mammary artery rings with and without endothelium contracted with norepinephrine (NE, original recording). Histamine induced relaxations only in the ring with endothelium (top panel) but not in the ring without endothelium (bottom panel). Meclofenamate, used to inhibit prostacyclin, did not significantly affect the response (second panel). Hemoglobin, an inhibitor of endothelium-derived relaxing factor, reversed the relaxation (third panel), and inhibition of the formation of cyclic GMP in vascular smooth muscle by methylene blue prevented the response (fourth panel).

Figure 2. Plots of effects of histamine in human internal mammary arteries (left panel) and saphenous veins (right panel) with and without endothelium. Note the potent relaxations in arterial rings with endothelium and the enhanced contractions in veins with endothelium (p<0.005 and 0.05, respectively).
In saphenous veins with endothelium contracted by norepinephrine (10^{-7} M) that was previously relaxed by bradykinin (10^{-6} M), histamine failed to induce significant relaxations (maximal relaxation, 0−24%) but did evoke pronounced contractions at higher concentrations (Figure 2). The maximal contraction evoked by histamine was enhanced in rings with (239±33%) compared with those without endothelium (150±17%, n=6, p<0.05, Figure 2). In contrast, contractions to 100 mM KCl did not differ between saphenous vein rings with and without endothelium (7.2±0.3 and 7.1±1 g, respectively, Table 2). Similarly, the contractions to a half-maximal concentration of norepinephrine (10^{-7} M) were identical in preparations with and without endothelium (2.7±0.19 and 2.9±0.19 g, respectively, NS).

In internal mammary veins, in contrast to the saphenous veins, histamine induced endothelium-dependent relaxations to an extent similar (77±6%) and a sensitivity comparable (IC_{50}, 6.8±0.1) to that of the mammary artery (n=5, data not shown).

### Table 1. Effects of Histamine and Histaminergic Receptor Antagonists in Human Internal Mammary Arteries With Endothelium Obtained From Patients Undergoing Coronary Bypass Surgery

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IC_{50}</th>
<th>Maximal relaxation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.5±0.2</td>
<td>70±5%</td>
</tr>
<tr>
<td>Chlorpheniramine (10^{-5} M)</td>
<td>&gt;4.0</td>
<td>29±4%*</td>
</tr>
<tr>
<td>Cimetidine (10^{-5} M)</td>
<td>6.4±0.4</td>
<td>80±9%</td>
</tr>
<tr>
<td>Chlorpheniramine (10^{-5} M) plus cimetidine (10^{-5} M)</td>
<td>&gt;4.0</td>
<td>9±2%*</td>
</tr>
</tbody>
</table>

Data are mean±SEM; n=5–8 experiments.
*Statistically significant difference from control (p<0.005).

### Table 2. Contractile Responses of Human Internal Mammary Arteries and Saphenous Veins Obtained From Patients Undergoing Coronary Bypass Surgery

<table>
<thead>
<tr>
<th>Agonist</th>
<th>With</th>
<th>Without</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin T_{max} (%)</td>
<td>50±6</td>
<td>64±5†</td>
</tr>
<tr>
<td>Serotonin ED_{50}</td>
<td>6.0±0.1</td>
<td>6.3±0.2</td>
</tr>
<tr>
<td>KCl 100 mM (g)</td>
<td>3.8±0.2</td>
<td>3.4±0.4</td>
</tr>
<tr>
<td>Histamine* (%)</td>
<td>98±14</td>
<td>153±11</td>
</tr>
</tbody>
</table>

Data are mean±SEM; n=5–10 experiments.
T_{max}, maximal tension in percentage of the contraction to 100 mM KCl.
*Contractions are expressed as percentage of an increase in tension evoked by a half-maximal concentration of norepinephrine (i.e., 3×10^{-7} M in arteries and 10^{-7} M in veins). The contractions to norepinephrine did not differ in preparations with and without endothelium in either blood vessel (n=6).
†Indicates statistically significant difference between rings with and without endothelium (p<0.034); ‡Indicates statistically significant difference between arteries and veins (p<0.035–0.005).

In saphenous veins with endothelium, the H_{1}-histaminergic receptor antagonist chlorpheniramine (10^{-5} M) completely inhibited contractions induced by histamine (Figure 4). In contrast, H_{2}-histaminergic blockade with cimetidine (10^{-5} M) did not affect the response. Accordingly, combined H_{1}- and H_{2}-histaminergic blockade evoked effects similar to those of H_{1}-histaminergic blockade alone.

**Endothelium-independent responses.** In internal mammary artery rings without endothelium, histamine evoked only contractions at higher concentrations (Figure 2). In rings treated with the H_{1}-
histaminergic antagonist chlorpheniramine, contractions induced by the amine were inhibited, and weak relaxations were unmasked (Figure 3). The addition of the H₂-histaminergic blocker cimetidine under these conditions completely blocked these relaxations.

In saphenous veins without endothelium, histamine evoked contractions similar to those in arterial rings without endothelium (Figure 2). The effects of histaminergic blockers were comparable to those observed in the mammary artery without endothelium (Figure 4).

**Serotonin**

*Endothelium-dependent responses.* In internal mammary artery rings with endothelium contracted by prostaglandin F₂α, serotonin failed to evoke endothelium-dependent relaxations in the presence or absence of ketanserin (10⁻⁶ M) to inhibit S₂-serotonergic receptors on vascular smooth muscle cells (Figure 5, n=6). In unstimulated mammary artery rings with endothelium, serotonin (10⁻⁸ to 10⁻⁴ M) evoked concentration-dependent contractions (n=6, Figure 6). The maximal response averaged 50±6% of the contraction to 100 mM KCl, and the EC₃₀ value averaged 6.1±0.1 (Table 2). Endothelium removal did not significantly affect the sensitivity to the monoamine (EC₃₀ value, 6.3±0.2), but removal did slightly enhance the maximal response (64±5%, n=6, p<0.034).

In the saphenous vein with endothelium, serotonin (3×10⁻⁸ to 10⁻⁴ M) evoked contractions that were not affected by endothelium removal (n=6 and n=5, respectively, Figure 6). The EC₃₀ value (7.5±0.1) and the maximal response (107±15%) to the monoamine were markedly greater in the vein compared with that of the artery (Table 2, p<0.005).

**Discussion**

The present study demonstrates potent release of EDRF in response to histamine and inhibition of serotonin-induced contractions by the endothelium in the human internal mammary artery but not in the saphenous vein. In the saphenous vein, histamine evoked endothelium-dependent contractions, and the contractile responses to serotonin were markedly greater than those obtained in the internal mammary artery.

Endothelium-dependent relaxations to histamine in the mammary artery were almost as pronounced as those previously described for acetylcholine despite direct vasoconstrictor effects of the amine. Because the response was unaffected by inhibitors of cyclooxygenase, but was completely prevented or reversed by the inhibitor of guanylate cyclase methylene blue and by hemoglobin, the results are consistent with the role of EDRF as the mediator. The slight increase in tension above that induced by norepinephrine after the addition of hemoglobin may be related to basally released EDRF. Similar conclusions regarding the mediator of endothelium-dependent relaxations have been reached under similar conditions with acetylcholine, ADP, and thrombin. The receptor on endothelial cells linked to the release of EDRF must be H₂-histaminergic in nature because chlorpheniramine, but not cimetidine, inhibited the relaxations. The H₂-histaminergic antago-
Histamine may be released from endothelial cells, mast cells, and platelets.9-11,31,32 Endothelial cells have a histidine decarboxylase system capable of synthesizing histamine from its precursor.1,8,9 The capacity of endothelial cells to form histamine is much greater than that of vascular smooth muscle cells.31 Shear stress and transmural pressure increase endothelial histamine synthesis.8,9,33 Thus, the endothelium of the mammary artery, but not that of the saphenous vein, may react to changes in local blood flow and pressure by synthesizing histamine and releasing EDRF by a paracrine pathway. For a graft vessel, this may be important in adapting to the flow requirements of the coronary circulation. These biologic advantages of arterial grafts may be jeopardized at sites of inappropriate surgical handling (i.e., distal anastomosis17-20).

Another source of histamine is mast cells, and an increased amount of vascular mast cells at the site of coronary spasm has been documented in a patient with variant angina.11 Histamine does evoke coronary vasospasm in patients with variant angina.34-36 In the pig, histamine elicits coronary vasospasm at the site of previous endothelial denudation.37 Thus, the release of EDRF in response to histamine in the mammary artery may protect against vasospasm and, in turn, local platelet activation. Platelets can also release histamine.32,38 In the porcine pulmonary artery, platelet-derived histamine contributes to endothelium-dependent relaxations induced by aggregating platelets (personal communication with T. Sellers and P.M. Vanhoutte).

In contrast to effects in the mammary artery, the contractile effects of histamine predominate and are enhanced in the presence of the endothelium in the saphenous vein. Atherosclerosis, which appears to develop more frequently in venous than in arterial grafts,18,39 may further augment that response. Indeed, human atherosclerotic coronary arteries
have an increased histamine content and exhibit enhanced contractions to the monoamine. In contrast to venous grafts, the mammary artery remains remarkably free of atherosclerotic changes both as a native vessel and as a graft. In contrast to effects in the canine and porcine coronary artery, serotonin did not evoke endothelium-dependent relaxations in the mammary artery in the presence or absence of ketanserin to block excitatory \( \alpha \)-serotonergic receptors on vascular smooth muscle cells. Similar results have been obtained in the human coronary and renal arteries, indicating that the human vascular endothelium does not express serotonergic receptors linked to the release of EDRF. However, in the mammary artery, but not in the saphenous vein, endothelial cells slightly inhibited the maximal contraction to serotonin. This would indicate that basal release of EDRF modulates vascular tone in the artery but is weak or absent in the vein. In addition, the contractions to the monoamine were markedly enhanced in the vein compared with those in the artery. This contrasts with the only minimally enhanced sensitivity of the saphenous vein to norepinephrine.

Serotonin is a major component of circulating platelets released during activation and aggregation. Marked contractions to the monoamine, as occur in the saphenous vein, would reduce local blood flow at sites where platelets are activated and would favor thrombus formation. Histamine, which is elevated after coronary thrombosis, would further evoke contractions and decrease local blood flow in venous grafts. In contrast to results in the saphenous vein, the sensitivity and potency of serotonin were much lower in the mammary artery, and the endothelium further inhibited the contractions. The release of EDRF in response to other platelet-derived products such as ADP and thrombin and to histamine in arterial grafts would prevent vasospasm and inhibit platelet function, thereby limiting and flushing away a nascent thrombus and maintaining local blood flow. In contrast, the reduced release of EDRF and the enhanced contractions to serotonin in venous grafts would favor vasospasm and thrombus formation. Potent contractions of implanted venous grafts have occurred in vivo and in vitro. Because platelets have been implicated in coronary artery bypass occlusion, the effective release of EDRF in the mammary artery may be important for better function and higher patency of arterial compared with venous grafts.

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References


**KEY WORDS** • acetylcholine • bradykinin • hemoglobin • coronary vessels • endothelium-derived relaxing factor • histamine • serotonin
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