Platelets, endothelium, and vasospasm

P. M. Vanhoutte, M.D., and D. S. Houston, M.D.

The vascular endothelium synthesizes prostacyclin, a potent antiaggregatory substance with vasodilator properties. It also activates (e.g., angiotensin I) or inactivates (e.g., bradykinin, serotonin) a number of vasoactive substances present in the blood (figure 1). Thus, it is far from being a passive diffusion barrier, but it can actively alter the amounts of vasoactive substances reaching the deeper layers of the blood vessel wall. In addition, endothelial cells can modulate powerfully the responsiveness of the vascular smooth muscle of the tunica media. Indeed, Furchgott and Zawadzki first noted that rings of rabbit aorta, contracted with norepinephrine, would relax upon exposure to acetylcholine if they were handled carefully to preserve the endothelial layer; if the endothelium was simply mechanically rubbed off, no relaxation was seen. Similar findings have been obtained in a host of other blood vessels, including coronary arteries (figure 2), and with a variety of agents (figure 3). The prototypic response to acetylcholine probably is only a pharmacologic curiosity since endothelial cells are not innervated by cholinergic neurons, and any acetylcholine that would reach the blood would be degraded at once by circulating cholinesterases. However, the finding that a number of other substances present in the blood under normal or pathologic conditions can cause potent endothelium-dependent responses implies that such responses may play a role in health and disease. This article briefly discusses this possibility in regard to the interaction between platelets and endothelial cells (see references 7 and 8).

Contractile responses to aggregating platelets. Cohen et al. observed that isolated rings of canine coronary artery contract if autologous platelets aggregate in the organ chamber in which the rings are suspended. Such contractions are markedly enhanced if the endothelium is removed (figure 4). If the rings are first contracted with prostaglandin F2α, a definite relaxation to aggregating platelets is observed in rings with endothelium, but only further contraction is observed in denuded rings (figure 5). In segments of canine coronary artery perfused with physiologic salt solution, relaxation in response to platelets is observed only if the platelets are added intraluminally and if the segment still contains endothelial cells. If the platelets aggregate intraluminally after removal of the endothelium, or are added to the bath surrounding the outside of the blood vessel, strong contractions ensue (figure 6). Thus, aggregating platelets release substances that trigger potent endothelium-dependent inhibitory responses of the smooth muscle in the media. Of the other canine blood vessels studied (femoral artery, femoral vein, and saphenous vein, pulmonary artery, saphenous artery), only the coronary artery displays a sustained or consistent relaxation response to platelets aggregating in the organ chamber (figure 7).

Mediators of the endothelium-dependent responses to platelets

Serotonin. Almost all of the serotonin (5-hydroxytryptamine) in the blood is contained in the platelets, which release the monoamine when they aggregate. Concentrations of exogenous serotonin comparable to those released from aggregated platelets cause contractions of isolated canine coronary arteries. Serotonin-induced contractions of the coronary artery of the dog are enhanced in vitro and in vivo by removal of the endothelium. In isolated coronary arteries contracted with prostaglandin F2α, serotonin can cause endothelium-dependent relaxation, albeit not as profound as that induced by acetylcholine. The contraction of the canine coronary artery caused by serotonin is antagonized by ketanserin, suggesting that it is mediated by S2-serotonergic receptors on the smooth muscle. By contrast, the endothelium-dependent relaxation in response to serotonin is not affected by ketanserin, but is abolished by methiothepin, suggesting the involvement of S1-serotonergic receptors (figure 8).

S2-Serotonergic antagonists reduce the contractions evoked by aggregating platelets. Thus, it seems likely that serotonin released from the platelets contributes to the contractile response of the coronary artery. However, endothelium-dependent responses to aggregating platelets persist in the presence of concentrations of...
methiothepin, preventing responses to serotonin (figure 5). Thus, it is unlikely that serotonin is solely responsible for platelet-induced endothelium-dependent relaxation in response to aggregating platelets.

**Thromboxane.** Thromboxane A₂ is produced by activated and aggregating platelets. It further stimulates platelet activation, and causes contraction of coronary smooth muscle. There are no reports of endothelium-dependent relaxation induced by thromboxane A₂. The

**With endothelium**

\[
\begin{align*}
\text{With} & \quad \text{4 g} \\
& \uparrow \uparrow \uparrow \uparrow \uparrow \\
\text{Without} & \quad \text{4 g} \\
& \uparrow \uparrow \uparrow \uparrow \uparrow \\
\end{align*}
\]

\[\text{PGF}_{2\alpha}, \quad 2 \times 10^{-6} \text{M} \quad \text{Acetylcholine}, \quad -\log \text{M}\]

**Without endothelium**

\[
\begin{align*}
\text{With} & \quad \text{4 g} \\
& \uparrow \uparrow \uparrow \uparrow \uparrow \\
\end{align*}
\]

**Platelets, 1.2 \times 10^8 / ml**

**FIGURE 2.** Tracing of isometric tension recording of isolated rings of canine coronary artery. Prostaglandin F₂α (PGF₂α) added to the organ bath causes a contraction. In rings that have been denuded of endothelium, cumulative addition of acetylcholine causes no change in tension, whereas in rings with intact endothelium, concentration-dependent relaxation results. (Reprinted, with permission, from Shepherd and Vanhoutte.)

**FIGURE 3.** Postulated mechanisms of endothelially mediated relaxation of vascular smooth muscle. A host of substances can induce endothelially mediated relaxation (see text). More than one endothelium-derived relaxing factor (EDRF) may be produced, or the formation of the same factor may result from a number of mechanisms. The action of EDRF(s) on the smooth muscle cell appears to involve stimulation of the membrane Na⁺/K⁺ pump, and to be mediated by increases in cyclic GMP in the smooth muscle cell. M = muscarinic receptor; BK = bradykinin receptor; SP = substance P receptor; Th = thrombin receptor; α₁ = α₁-adrenergic receptor; P₂ = P₂ purinergic receptor; V₁ = V₁ vasopressinergic receptor; PGI₂ = prostacyclin receptor; S₁ = S₁-serotonergic receptor; H₁ = H₁-histaminergic receptor; X = unknown intermediate; A23187 = calcium ionophore.

**FIGURE 4.** Traces of isometric tension recording of isolated rings of canine coronary with and without endothelium. Platelets added to the organ chamber promptly aggregate and evoke contraction (the time of exposure to platelets shown is approximately 20 min). The contractions produced in rings with intact endothelium are smaller and more transient than those in rings form which the endothelium has been removed, indicating that platelets not only directly stimulate smooth muscle contraction, but also initiate an endothelially mediated inhibition. (Data from Cohen RA, Shepherd JT, Vanhoutte PM: Inhibitory role of the endothelium in the response of isolated coronary arteries to platelets. Science 221: 273, 1983.)
thromboxane synthetase inhibitor dazoxiben reduces the ischemic response to atrial pacing in subjects with coronary artery disease, suggesting that thromboxane A2 may contribute to constriction of coronary vessels even in the absence of overt spasm. Thromboxane B2 can be measured in the fluid from organ chambers where the interaction between aggregating platelets and isolated blood vessels are studied. However, inhibitors of cyclooxygenase do not alter the response of coronary arteries to aggregating platelets. Thus, it seems unlikely that thromboxane A2 contributes to the endothelium-dependent response to the latter.

Adenine nucleotides. ADP and ATP are contained in large quantities in the dense granules of platelets and are released during aggregation. Both compounds cause similar endothelium-dependent relaxation in a

**FIGURE 5.** Left. If rings of canine coronary artery with and without endothelium are first contracted with prostaglandin F2α (PGF2α), addition of platelets (6.1 x 10⁷/ml) causes relaxation in rings with endothelium, but only further contraction in rings without endothelium. This highlights the potent, endothelium-mediated relaxing effect of platelets. A similar relaxation can be seen if the rings are contracted with norepinephrine instead of PGF2α. Right. Methiothepin, in a concentration that abolishes endothelially mediated relaxation in response to serotonin, does not affect the endothelium-dependent relaxation in response to aggregating platelets. (Reprinted, with permission, from Houston et al.²¹)

**FIGURE 6.** Further evidence of the obligatory role of endothelial cells in the relaxation of a canine coronary artery induced by platelets. A segment of canine coronary artery was perfused with physiologic saline solution; hooks in the vessel wall allowed the recording of wall tension (equivalent to tension measurement in the ring preparation). The vessel was constricted with prostaglandin F2α (PGF2α). If only the luminal surface is exposed to aggregating platelets by their addition to the perfusate, a relaxation occurs; if, however, the platelets are added to the organ bath surrounding the outside of the vessel, only further contraction occurs. (Reprinted, with permission, from Cohen et al.¹¹)

**FIGURE 7.** Responses of canine femoral arteries and femoral veins with and without endothelium to aggregating platelets. Vessels were contracted with norepinephrine before addition of platelets. Although small transient or occasional periods of relaxation in response to platelets may be observed in these vessels, the sustained major relaxation seen in the coronary artery does not occur. (Data from Houston et al.¹²)
number of isolated arteries, including the canine coronary artery. The endothelium-dependent relaxation in response to platelets is almost abolished by apyrase (an enzyme that hydrolyzes ATP and ADP to AMP and inhibits the endothelium-dependent relaxations induced by adenine nucleotides) (figure 9). If endothelial receptors are saturated with ADP, the platelets cause contraction only, even though acetylcholine is still capable of causing relaxation (figure 9). Thus, the endothelium-dependent relaxation of isolated coronary arteries in response to aggregating platelets is due mainly to the release of ADP and ATP.

Platelet-activating factor. Platelet-activating factor is a phospholipid released by leukocytes, mast cells, and platelets. Is is a potent aggregating agent at concentrations as low as 10^{-12} M in some species. In a number of animals, including the rat in which it does not activate platelets, platelet-activating factor causes profound hypotension. Both the platelet-stimulating and hypotensive effects of platelet-activating factor are antagonized by compound CV-3988. The precursor, 2-lyso-platelet-activating factor, is inactive. In the isolated aorta of the rat, platelet-activating factor causes relaxation that is abolished by removal of the endothelium.

In canine coronary and femoral arteries, relaxation induced by platelet-activating factor is observed only

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**FIGURE 8.** Concentration/effect relationships of canine coronary artery, with and without endothelium, contracted with prostaglandin F_{2a} (2 \times 10^{-8} M) to cumulative addition of serotonin. In rings with endothelium, lower concentrations of serotonin (up to 10^{-7}) produce a slight contraction; higher concentrations cause relaxation. In rings without endothelium a much stronger contraction is observed. The difference between the curves reflects the inhibitory effect of the endothelium stimulated by serotonin. Ketanserin, a selective S_{2}-serotonic agonist, reduces the contractile effect of serotonin and shifts both curves downward. Methiothepin, an S_{2}-serotonic agonist, abolishes the endothelium-dependent inhibitory effect. (Reprinted, with permission, from Houston et al. 21)

**FIGURE 9.** Evidence that adenine nucleotides play a major role in the endothelial-dependent relaxation of canine coronary arteries induced by aggregating platelets. Rings with intact endothelium that contracted with prostaglandin F_{2a} (PGF_{2a}) show a typical relaxation response to platelets (A); this relaxation is abolished and contraction is seen if apyrase (an enzyme that degrades ATP and ADP) is present in the bath (0.67 units/ml) (C). Canine coronary rings with intact endothelium and contracted with prostaglandin F_{2a} (PGF_{2a}) relax on exposure to cumulative concentrations of ADP; with the purinergic receptors saturated, aggregating platelets can no longer induce relaxation, although the endothelium-dependent response to acetylcholine (ACh) persists (B). W_o = washout. (Modified from ref. 20.)
FIGURE 10. Concentration-effect curves in canine coronary artery, with and without endothelium, contracted with prostaglandin F₂α (PGF₂α; 2 × 10⁻⁶M) in response to cumulative additions of platelet-activating factor (PAF) and its 2-lyso derivative (2-lyso-PAF) and the ethanol solvent used. Changes in tension are expressed as percent of contraction obtained with PGF₂α. Relaxation induced by PAF and 2-lyso-PAF occurs in rings with intact endothelium above 10⁻⁶M: this effect is endothelium dependent (*p < .005 comparing responses of intact and denuded vessels to 10⁻⁴M PAF) (top). The lack of a difference between responses to PAF and 2-lyso-PAF suggests that this relaxation is not a specific, receptor-mediated effect of PAF. Further evidence in support of this interpretation is the lack of effect of the PAF antagonist CV-3988 on the endothelium-dependent response to high concentrations of PAF (bottom). The lack of effect of indomethacin on this response (bottom) shows that production of prostacyclin by the endothelium is not responsible for the relaxation induced by PAF.

at high concentrations (figure 10). This relaxation is endothelium dependent, but it persists in the presence of CV-3988; 2-lyso-platelet–activating factor causes relaxation similar to that caused by platelet-activating factor. It seems unlikely, therefore, that endothelium-dependent relaxation is due to the interaction of platelet-activating factor with a receptor similar to those mediating platelet activation and hypotension. At the high concentrations required to observe it, a direct membrane effect of these lipids analogous to that observed with arachidonic and oleic acid is a more likely explanation. The synthesis of prostacyclin is not involved, since incubation of the blood vessels with the inhibitor of cyclooxygenase indomethacin does not block the response (figure 10). Unless very high local concentrations of platelet-activating factor (and 2-lyso-platelet–activating factor, which is also contained in platelets) are achieved at the sites of platelet aggregation, it is unlikely that these substances contribute directly to platelet-induced relaxation.

Vasopressin. Human platelets contain vasopressin, which evokes endothelium-dependent relaxation of canine coronary and cerebral arteries. This relaxation is blocked by the V₁-vasopressinergic antagonist d(CH₂)₅Tyr(Me)AVP. However, the antagonist does not affect endothelium-dependent relaxation in response to platelets.*

Potential role in health and disease. It is a giant leap from studies in organ chambers with artificial solution and containing healthy canine tissue to a diseased coronary artery on a pumping human heart, so that inferences about human pathology must necessarily be tentative. Nonetheless, one cannot but speculate that these observations in isolated canine blood vessels may be key to the understanding of coronary arterial spasm. It is appealing to propose that the body has evolved a particularly effective defense against thrombosis in a critical vascular bed with little collateral blood supply, namely the myocardium. Thus, one can imagine that if, for any reason, platelets began to aggregate in a normal coronary artery with an intact endothelium, the response of the smooth muscle of the blood vessel to the substances released from the platelets would be relaxation. This would be reinforced if the platelet aggregation were to set the coagulation cascade in motion, which would cause the formation of thrombin. Indeed, in peripheral, cerebral, and coronary arteries, thrombin causes endothelium-dependent

relaxation, which overcomes the direct constrictor effect that it has on smooth muscle (figure 11).\textsuperscript{23, 24, 35–37} The endothelium-dependent relaxation to thrombin is abolished but the direct contractile effect is augmented by hypoxia.\textsuperscript{36, 37} An endothelium-dependent dilatation triggered by the platelet products (and thrombin) would tend to flush away the beginning aggregate before it could occlude the vessel (figure 12). If, on the other hand, the endothelium were absent, damaged, or for some reason failed to function properly, the response of the vessel to platelet products and thrombin would be contraction, as observed in the rings denuded of endothelium in vitro (figure 12). Such contraction would further reduce the luminal area and increase the obstruction to blood flow.\textsuperscript{38} Clinical coronary arterial spasm may be just such a contraction.

A potential pivotal role of the endothelium becomes apparent when one recalls that disruption of the endothelium by an atheromatous plaque is a ubiquitous feature of coronary disease, and that such plaques and partial obstructions can initiate platelet activation. It has been demonstrated that patients with classic and Prinzmetal’s angina have increased coronary sinus levels of thromboxane B\textsubscript{2} (the metabolite of the potent vasoconstrictor prostanoid produced by activated platelets, thromboxane A\textsubscript{2} (TBA\textsubscript{2}), platelet-activating factor (PAF), and vasopressin (VP), and initiate the coagulation cascade with production of thrombin. Top. The presence of an intact endothelium prevents platelet aggregation through production of the platelet inhibitor, prostacyclin (PGI\textsubscript{2}); PGI\textsubscript{2} is also a vasodilator. Serotonin is taken up by endothelial cells and degraded by monoamine oxidase (MAO). PAF (in high concentrations), 5-HT, VP, adenine nucleotides, and thrombin can all stimulate the endothelial production of a relaxing factor or factors that have an inhibitory effect on smooth muscle. Relaxation and vasodilatation would tend to flush away any developing aggregate or thrombus. Finally, an intact endothelium and basement membrane probably serve as a diffusion barrier to prevent the above-mentioned substances from reaching the smooth muscle layers of the media. Bottom. Platelet aggregation is enhanced by contact with collagen in the exposed vessel wall. Depending on the blood vessel, the thrombin, VP, 5-HT, adenine nucleotides, and thromboxane produced all directly activate vascular smooth muscle. These effects would be unopposed if the endothelium were damaged or dysfunctional. Vasospasm may thus result in areas of endothelial abnormality.

platelet-induced spasm in a number of other ways, including (1) by acting as a diffusion barrier preventing vasoconstrictor substances from reaching the smooth muscle cells, (2) by metabolizing vasoactive platelet products such as serotonin and catecholamines with...
monoamine oxidase (figure 1), and (3) by producing the vasodilator and platelet inhibitor prostacyclin in response to (and even out of) substances released from platelets.41,42

We would like to thank Mrs. Janet Beckman for typing the manuscript and Mr. Robert Lorenz for preparation of the illustrations.

References

CIRCUSSION
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P M Vanhoutte and D S Houston

Circulation. 1985;72:728-734
doi: 10.1161/01.CIR.72.4.728

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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