Heterogeneity of Endothelium-Dependent and Endothelium-Independent Responses to Aggregating Platelets in Porcine Pulmonary Arteries

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Experiments were designed to determine the endothelium-dependent and endothelium-independent responses to aggregating platelets in porcine pulmonary arteries. Isolated rings with and without endothelium from large (5–7-mm-diameter) and small (2–3-mm-diameter) pulmonary arteries were suspended in modified Krebs-Ringer bicarbonate solution bubbled with 95% O₂-5% CO₂ in the presence of indomethacin. Aggregating platelets caused relaxations in rings with endothelium but contractions in rings without endothelium, both of which were significantly larger in small versus large pulmonary artery rings. Serotonin and ADP caused concentration-dependent endothelium-augmented relaxations that were unaffected by ketanserin. Methiothepin, but not apyrase, significantly decreased the platelet-induced endothelium-dependent relaxations; the residual relaxation was abolished when rings were incubated with methiothepin, apyrase, and theophylline but was unaffected if apyrase was absent, indicating that ADP is responsible for the residual relaxation caused by aggregating platelets. Quiescent rings, with and without endothelium, contracted in a dose-dependent manner to norepinephrine and histamine but not to serotonin or vasopressin. The contraction to aggregating platelets was blocked by methiothepin, pyrilamine, and diphenhydramine but was unaffected by phentolamine, ketanserin, or incubation of the platelets with dazoxiben. These data indicate that, in large and small porcine pulmonary arteries, serotonin and ADP are the major contributors to the endothelium-dependent relaxation caused by aggregating platelets, while histamine appears to be responsible for the contraction that platelets cause in rings without endothelium. (Circulation Research 1991;68:1437–1445)

Aggregating porcine platelets release a variety of vasoactive substances, among which are 5-hydroxytryptamine (serotonin), ADP, thromboxane A₂, histamine, vasopressin, and catecholamines.¹ ² The endothelium inhibits contractions to aggregating platelets in canine and porcine coronary arteries but does not do so in canine pulmonary arteries, where aggregating platelets cause contractions in vessels with and without endothelium.³–⁶ Microembolization and in situ aggregation of platelets have been implicated in pulmonary vascular constriction and pulmonary hypertension through the release of vasoactive mediators.⁷–¹³ The present study was designed to determine whether the pulmonary arterial endothelium curtails the contraction of vascular smooth muscle to the vasoactive agents released from aggregating platelets and to determine the mediators involved.

Materials and Methods

Male swine (30–45 kg) were anesthetized with ketamine hydrochloride (300 mg i.m.; Bristol Laboratories, Syracuse, N.Y.) and sodium pentobarbital (12 mg/kg i.v.; Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) and exsanguinated. The lungs were removed en bloc and transported in cold modified Krebs-Ringers bicarbonate solution. Large (third-order) and small (fifth-order) pulmonary arteries were carefully dissected free from the right lung and immersed in cold modified Krebs-Ringers bicarbonate solution of the following composition (mM): NaCl 118.3, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25.0, CaEDTA 0.016, and glucose 11.1 (control solution). Rings (3–4 mm long) from large

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(5–7 mm in diameter) and small (2–3 mm in diameter) pulmonary arteries were cleaned of connective tissue, with special care taken not to touch the luminal surface. In some rings, the endothelium was deliberately removed by gently rubbing the luminal surface with either a cotton swab wetted with control solution (large rings) or a thin (0.3 mm) wire (small rings). The rings were mounted horizontally between a fixed stirrup and a force transducer (model UTC2, Gould Inc., Cleveland, Ohio) in an organ chamber filled with 25 ml control solution that was bubbled with 95% O_2–5% CO_2 and maintained at 37°C, and isometric tension was recorded. The rings were then allowed to equilibrate for 30–40 minutes before further experimentation. Rings with and without endothelium from large and small arteries were studied in parallel.

**Platelets**

Autogenous blood (300–500 ml) was drawn from the left carotid artery of the pig into 50-ml plastic syringes filled with 5–6 ml citrate anticoagulant to yield final concentrations (mM) of sodium citrate 9.3, citric acid 0.7, and dextrose 14. The blood was centrifuged at 750 rpm at room temperature, and the platelet-rich plasma was pipetted into plastic test tubes. An equal volume of cold citrate anticoagulant solution containing (mM) sodium citrate 93, citric acid 7, dextrose 105, and KCl 5 brought to a pH of 6.5 was added to the platelet-rich plasma and centrifuged at 1,600 rpm. The supernatant was discarded, and the remaining platelet pellet was resuspended in a small volume (1/40 of the original blood volume) of the second citrate anticoagulant solution. A platelet count of this suspension was obtained (Coulter Electronics, Inc., Hialeah, Fla.), and the volume of the suspension was adjusted so that, when it was added to the organ chamber, the resulting platelet concentrations in the organ bath were 25,000/µl, 50,000/µl, and 75,000/µl. Platelet aggregation on exposure to the collagen within the blood vessel wall and the calcium-containing control solution was evidenced by clearing of the initially turbid solution, formation of visible platelet clumps, and detectable levels of serotonin, histamine, and thromboxane B_2 in the bath solution.

In some studies, the platelets were incubated in the presence of dazoxiben (6×10⁻³ M), a selective inhibitor of thromboxane A₂ synthetase for 60 minutes before addition to the organ bath. A comparable concentration of dazoxiben (10⁻⁴ M) in the organ bath did not cause a change in tension in the quiescent rings (data not shown). Samples of fluid (2 ml) for the determination of serotonin, histamine, and thromboxane B_2 were drawn from the chambers 10 minutes after the addition of platelets, and frozen until analysis.

**Protocol**

After equilibration, rings were incubated with indomethacin (10⁻³ M) for at least 30 minutes. In quiescent rings, with and without endothelium, concentration–response curves to histamine (10⁻⁹ to 10⁻⁵ M), serotonin (10⁻⁹ to 10⁻³ M), arginine-vasopressin (10⁻¹¹ to 10⁻⁷ M), ADP (10⁻⁸ to 10⁻⁵ M), and platelets (25, 50, and 75×10⁵/µl) were obtained. Some rings were incubated with ketanserin (10⁻⁶ M) for 40 minutes before their exposure to serotonin and platelets. Concentration–response curves to histamine and platelets were obtained in some rings without endothelium after incubation for 40 minutes with pyrilamine (3×10⁻⁷ M), diphenhydramine (3×10⁻⁶ M), methiothepin (10⁻⁶ M), phenolamine (10⁻³ M) and propranolol (5×10⁻⁶ M), or ketanserin (10⁻⁶ M). Concentration–response curves to platelets incubated with dazoxiben (6×10⁻³ M) were obtained in some rings without endothelium. Responses to 20 mM KCl were obtained in rings after exposure to the highest concentration of platelets (75,000/µl).

Relaxations were assessed in rings, with and without endothelium, contracted to a level equal to 40–60% of the maximal response to norepinephrine with prostaglandin F₂α (2 to 4×10⁻⁸ M) or phenylephrine (10⁻⁷ to 10⁻⁶ M) in the presence of indomethacin (10⁻⁵ M). Concentration–response curves to serotonin (10⁻⁹ to 10⁻⁵ M), adenosine and ADP (10⁻⁸ to 10⁻⁵ M), thrombin (0.01–1 unit), arginine-vasopressin (10⁻¹⁰ to 10⁻⁶ M), and platelets (25, 50, and 75×10⁵/µl) were obtained in rings contracted with prostaglandin F₂α; those to sodium nitroprusside (10⁻⁹ to 10⁻⁵ M) were obtained in rings contracted with phenylephrine. Early studies showed no significant difference in the magnitude of relaxation to various agonists in rings contracted with prostaglandin F₂α. However, prostaglandin F₂α was used in the majority of the experiments because many of the blocking agents used also inhibited α₁-adrenergically mediated contractions. Some rings were incubated with ketanserin (10⁻⁶ M) for 40 minutes before their exposure to serotonin and platelets.

To delineate the relative contributions of the vasoactive substances released from aggregating platelets to the relaxation evoked by the platelets, rings with endothelium were first incubated with several selective and nonselective antagonists for 40 minutes before their exposure to increasing concentrations of the platelets. The antagonists used were methiothepin, a combined 5-HT₁₉- and 5-HT₂-serotonergic antagonist; ketanserin, a selective 5-HT₂-serotonergic antagonist; apyrase, an ADPase and ATPase; theophylline (10⁻⁴ M), a purinergic P₁-receptor blocker and phosphodiesterase inhibitor; rolipram (5×10⁻⁶ M), a phosphodiesterase inhibitor without purinergic antagonist activity; cinmetidine (10⁻⁶ M), a selective H₂-histaminergic antagonist; phenolamine (10⁻⁵ M) and propranolol (5×10⁻⁶ M), to block α- and β-adrenergic activity; and combinations of methiothepin and apyrase and/or theophylline and/or rolipram and/or cinmetidine. Apyrase sometimes caused a transient relaxation that lasted 30–60 seconds; platelets were added only after the tension returned to its previously contracted level. The presence or absence of the endothelium was
confirmed by the presence or absence of relaxation to bradykinin (10^{-7} \text{M}).

**Drugs**

The following drugs were used: adenosine, ADP, apyrase (ATPase and ADPase, grade V from potato), arginine-vasopressin, bradykinin, diphenhydramine hydrochloride, histamine, indomethacin, L-phenylephrine, DL-propranolol, prostaglandin F_2\alpha, pyrilamine maleate, 5-hydroxytryptamine creatinine sulfate (serotonin), sodium nitroprusside, theophylline, and thrombin (Sigma Chemical Co., St. Louis); dazoxiben hydrochloride (Pfizer Laboratories, New York); ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium); methiothepin maleate (Roche Laboratories, Nutley, N.J.); phentolamine (CIBA-GEIGY Co., Summit, N.J.); and rolipram (Berlex Laboratories, Inc., Wayne, N.J.). All drugs were prepared daily using distilled water. Indomethacin was dissolved with Na_2CO_3 (10^{-3} \text{M}). Apyrase was prepared with distilled water immediately before addition to the organ chamber in a concentration of 75 units ADPase and 75 units ATPase activity per 25 ml (1 unit activity liberates 1 mol PO_4/min). Drugs were kept on ice and platelets were kept at room temperature during the experiments. Drug concentrations are expressed as final molar concentrations in the organ bath; apyrase is expressed as unit activity of ADPase per milliliter of organ bath solution.

**Serotonin**

Immediately after the experiment, 0.5 ml fluid collected from the organ bath was added to 120 \mu l cysteine (1% solution), and proteins were precipitated by adding ZnSO_4 and NaOH. This solution was centrifuged at 1,400g for 30 minutes. The supernatant was decanted and frozen at −70°C until analysis. Analysis was performed by filtering the supernatant through centrifugal microfilters (Bioanalytical Systems, West Lafayette, Ind.) with regenerated cellulose membranes (pore size, 0.1 \mu m). The amine was quantitated by reversed-phase high-pressure liquid chromatography with electrochemical detection. The interassay coefficient of variation was 5.6%.

**Thromboxane B_2**

Fluid (2 ml) from the organ bath was collected immediately after the experiment and frozen (−70°C) until analysis. The solution (1 ml) was centrifuged at 3,000g for 10 minutes at 40°C and brought to pH 3.5 with 1N hydrochloric acid. Thromboxane B_2 was extracted with octadeccylsil silica columns (Bond Elect C18, Analytic-chem International, Harbor City, Calif.). The samples were further purified by elution with 2 ml ethyl acetate onto silica columns (Bond Elect Si). After being washed with a 2-ml mixture of benzene/ethyl acetate (80:20), thromboxane B_2 was eluted with a 4-ml mixture of benzene/ethyl acetate/methanol (60:40:40) and evaporated to dryness in a 37°C water bath under nitrogen. The samples were redisolved in phosphate buffer (pH 7.4). Aliquots (100 \mu l) of standards and diluted samples were assayed by displacement of [3H]thromboxane B_2 from thromboxane B_2 antiserum in a total incubation volume of 30 \mu l at 4°C. After centrifugation (3,000g, 5 minutes, 4°C), the supernatant containing the antibody-bound [3H]thromboxane B_2 fraction was counted in a scintillation counter, and the concentration of thromboxane B_2 was estimated by comparison with a standard curve. The recovery of the cold standard averaged 94%. The interassay coefficient of variation was 5.6%, and the interassay variation was 9.6%.

**Histamine**

The histamine standards for the assay (62–989 ng/ml) were freshly prepared by diluting histamine phosphate (Sigma) in Krebs’ solution. These standards (“internal standards”) were carried through the entire extraction process with the unknown samples. A 1-ml sample of standard or test solution was added to 13×100-mm glass tubes containing 300 mg NaCl and 1.25 ml butanol, and while the tubes were vortexed, 0.1 ml of 3N NaOH was added, and the tubes were vortexed for an additional 2 minutes. After centrifugation for 5 minutes at 1,800 rpm, 1 ml of the butanol layer was transferred to tubes containing 0.7 ml of 0.12N HCl and 2 ml heptane, and the tubes were vortexed for 1 minute, then allowed to remain in the cold room overnight. The HCl layer (0.6 ml) was transferred to 10×75-mm glass tubes, to which were then added 0.25 ml of 0.75N NaOH and 0.075 ml o-phthalaldehyde solution (3 mg in 4.5 ml methanol), and the tubes were vortexed briefly and incubated in an ice bath for 40 minutes. The reaction was stopped by addition of 0.12 ml of 1.25 M phosphoric acid, after which the tubes were again vortexed and allowed to warm to room temperature. Tubes were read in a fluorometer at an excitation wavelength of 450 nm. The reading given by Krebs’ solution alone (blank) was subtracted from the reading given by unknown samples. The efficiency of extraction and conjugation of the histamine was calculated to be 50%, as measured by “external standards” of histamine in 0.12N HCl that were not carried through the extraction process.

**Statistical Analysis**

Results are expressed as mean±SEM. For platelet-induced contractions in quiescent rings without endothelium, the maximal contraction is expressed as a percentage of the contraction evoked by 20 mM KCl. Relaxations are expressed as a percent decrease in tension of the contraction evoked by prostaglandin F_2\alpha or phenylephrine (ED_{50} of the maximal response to norepinephrine). Unless otherwise stated, n represents the number of animals from which the rings were taken. Statistical analyses were performed using Student’s t test for paired and unpaired observations and a one-way analysis of variance for comparison of multiple means. Results were considered statistically significant for values of p<0.05.
Results

Platelets

In quiescent preparations, rings without endothelium contracted in response to aggregating platelets. The contractions were significantly larger in small compared with large arterial rings (Figure 1). Rings with endothelium did not contract in response to aggregating platelets, but rather exhibited no change or a decrease in tension. Quiescent rings without endothelium incubated with ketanserin or phentolamine and propranolol exhibited contractions similar to those observed in control rings. Methiothepin, pyrilamine, and diphenhydramine inhibited the contractions to aggregating platelets. Platelets incubated with dazoxiben, a thromboxane A2 synthetase inhibitor, caused contractions similar to those in control rings (Figure 2).

Aggregating platelets caused endothelium-dependent relaxations that were larger in small compared with large arterial rings (Figure 3). Incubation of the rings with ketanserin did not alter the magnitude of the relaxation (data not shown). Incubation of the rings with methiothepin and apyrase significantly lessened but did not abolish the relaxations, whereas relaxations in rings incubated with apyrase alone were not significantly different from relaxations in control rings (Figure 4). The addition of cimetidine to methiothepin or the combination of propranolol and phentolamine did not further reduce the relaxation (data not shown). Theophylline in combination with methiothepin and apyrase nearly abolished the platelet-induced relaxation, whereas the combination of rolipram, methiothepin, and apyrase did not cause a similar reduction in relaxation (Figure 4).

Serotonin

Serotonin caused endothelium-augmented relaxations that were larger in small compared with large arterial rings (Figure 5, left panel; Table 1). Incubation with ketanserin did not change the magnitude of relaxation in rings with or without endothelium (Figure 5, right panel). In quiescent rings, with or without endothelium, serotonin did not cause contractions, but rather caused small decreases in basal tension.

ADP

ADP caused endothelium-augmented relaxations in small arterial rings. The relaxations in large arterial rings were not significantly different in rings with or without endothelium. Relaxations were significantly larger in small compared with large rings with endothelium but not in rings without endothelium (Figure 6, Table 1). ADP did not alter the tension in

Figure 1. Effects of aggregating platelets (75,000/μl) in the presence of indomethacin (10^-5 M) on quiescent large (5-7 mm in diameter) and small (2-3 mm in diameter) porcine pulmonary rings without endothelium. Changes in tension are expressed as percent of the contraction evoked by 20 mM KCl in the same rings. Data are shown as mean ± SEM. *p<0.05.

Figure 2. Effects of methiothepin (MTP, 10^-6 M), ketanserin (KET, 10^-6 M), pyrilamine (PYR, 5x10^-7 M), diphenhydramine (DIPH, 5x10^-6 M), phentolamine (10^-5 M) plus propranolol (5x10^-6 M) (PHEN), and dazoxiben (DZXBN, 6x10^-3 M) on platelet-induced contractions in quiescent rings without endothelium from small (2-3 mm in diameter) porcine pulmonary arteries. Indomethacin (10^-5 M) was present in all experiments. Changes in tension are expressed as percent of the contraction evoked by 20 mM KCl in the same ring. Data are shown as mean ± SEM. Platelets=75,000/μl. *p<0.05 compared with controls.

Figure 3. Concentration-response curve to aggregating platelets in large (5-7 mm in diameter) and small (2-3 mm in diameter) porcine pulmonary arteries with and without endothelium. Rings were contracted with prostaglandin F2α (2-4x10^-8 M) to a contraction equal to 40-60% of the maximal norepinephrine contraction. Data are shown as mean ± SEM. All experiments were performed in the presence of indomethacin (10^-3 M).
Adenosine

Adenosine caused concentration-dependent relaxations in rings with and without endothelium (100% relaxation in all rings studied, n=4) that were attenuated significantly (maximal relaxation observed = 30% of the prostaglandin contraction) in rings incubated with theophylline, a P₁-receptor blocker (Figure 7). The presence of the endothelium did not affect the relaxation to the exogenous agonist.

Histamine

Quiescent rings without endothelium from large and small pulmonary arteries were significantly more sensitive to histamine (ED₅₀ [-log M±SEM]: small, -6.66±0.12; large, -6.20±0.02). Pyrilamine, diphenhydramine, and methiothepin inhibited the contractions evoked by histamine, whereas phenolamine plus propranolol and ketanserin had no effect on the contractile response (data not shown).

Thrombin

Increasing concentrations of thrombin caused dose-dependent decreases in tension in rings with, but not in those without, endothelium. Rings denuded of endothelium exhibited contractions to thrombin (Figure 8).

Vasopressin

Arginine vasopressin caused endothelium-dependent relaxations, but only at 10⁻⁶ M. No change in tension was observed in quiescent rings (data not shown).

Sodium Nitroprusside

Sodium nitroprusside caused relaxations in large and small arterial rings, with and without endothelium, that were similar in magnitude (ED₅₀ [-log M±SEM]: small, -7.51±0.10; large, -7.43±0.16).

Figure 4. Effects of methiothepin (10⁻⁶ M), apyrase (3 units/ml), theophylline (10⁻⁴ M), and rolipram (5×10⁻⁶ M) on platelet-induced relaxations in rings, with endothelium, contracted with prostaglandin F₂α (2–4×10⁻⁶ M, which elicits a contraction equal to 40–60% of the maximal response to norepinephrine). Rings were taken from small (2–3 mm in diameter) porcine pulmonary arteries; indomethacin (10⁻⁵ M) was present. Data are shown as mean±SEM. Platelets=75,000/µL. *p<0.05 compared with controls.

Figure 5. Left panel: Cumulative concentration–response curves to serotonin in rings, with and without endothelium, taken from large (5–7 mm in diameter) and small (2–3 mm in diameter) pulmonary artery rings contracted with prostaglandin F₂α (2–4×10⁻⁶ M, which caused a contraction equal to 40–60% of the maximal response to norepinephrine). Right panel: Similar experiments were performed in small porcine pulmonary artery rings, with and without endothelium, in the presence and absence of ketanserin (10⁻⁶ M). All experiments were performed in the presence of indomethacin (10⁻⁵ M). Data are shown as mean±SEM.
TABLE 1. Endothelium-Dependent Relaxations in Porcine Pulmonary Arteries

<table>
<thead>
<tr>
<th></th>
<th>ED50 (M)</th>
<th>Maximal relaxation (%)</th>
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<tbody>
<tr>
<td>Serotonin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large + E</td>
<td>-7.02±0.14*</td>
<td>100</td>
</tr>
<tr>
<td>Large - E</td>
<td>-6.67±0.13†</td>
<td>100</td>
</tr>
<tr>
<td>Small + E</td>
<td>-7.88±0.28*</td>
<td>100</td>
</tr>
<tr>
<td>Small - E</td>
<td>-6.24±0.24†</td>
<td>100</td>
</tr>
<tr>
<td>ADP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large + E</td>
<td>-5.61±0.09</td>
<td>33±8*</td>
</tr>
<tr>
<td>Large - E</td>
<td>-5.63±0.09</td>
<td>28±12</td>
</tr>
<tr>
<td>Small + E</td>
<td>-5.78±0.13†</td>
<td>62±8*†</td>
</tr>
<tr>
<td>Small - E</td>
<td>-5.48±0.04†</td>
<td>39±9†</td>
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Data are expressed as mean±SEM. ED50 concentration (expressed in -log M) that caused 50% of the maximal observed relaxation; maximal relaxation, expressed as percent relaxation of the prostaglandin F20-induced contraction (40–60% of the maximal response to norepinephrine); + E, with endothelium; − E, without endothelium.

* p<0.05, small vs. large pulmonary arteries (ANOVA).
† p<0.05, intact vs. denuded rings (paired t tests).

Products Released From Aggregating Platelets

Aggregating platelets released serotonin, thromboxane A2, and histamine into the bath solution. The concentration of serotonin (n=6) detected averaged 180±117 ng/ml (5×10^-6 M), while that of thromboxane (n=8) was 57 pg/ml or less (1.5×10^-10 M). Histamine was detected in concentrations of 234±107 ng/ml (1.3×10^-6 M, n=18).

Discussion

The present study demonstrates that 1) the endothelium reduces the contraction of pulmonary vascular smooth muscle evoked by the vasoconstrictor mediators released from aggregating platelets; 2) in the absence of endothelium, platelets cause contractions of porcine pulmonary arteries and platelet-derived histamine contributes importantly to the contraction; and 3) platelet-induced relaxations and contractions are more pronounced in distal than in proximal pulmonary arteries.

In the porcine pulmonary arteries aggregating platelets caused endothelium-dependent relaxations that appear to be mediated by serotonin and ADP as in canine and porcine coronary arteries.2–5 Indeed, although the serotonin and platelet-induced relaxations are unaffected by ketanserin (a selective 5HT2- serotoninergic antagonist), they were significantly inhibited by methiothepin, a nonselective 5HT1 and 5HT2 antagonist, suggesting that 5HT1-serotonergic receptors are implicated in the platelet-induced response.17–19 A similar conclusion has been reached for porcine coronary arteries and other blood vessels from other species.3–4,20–23 That histamine and catecholamines (vasoactive substances whose contractile effects we have found can be blocked by methiothepin) are not involved in the relaxation through H2-histaminergic or α1-, α2-, and β-adrenergic receptors is confirmed by the absence of change in the
platelet-induced relaxation in the presence of cimetidine, an H₂-histaminergic antagonist, and the combination of phen tolamine and propranolol.

ADP appears to be involved in the platelet-induced relaxation that remains (approximately 50%) after serotonergic blockade. However, unlike in the coronary arteries of dogs, this relaxation is not inhibited by the addition of apyrase alone, but can be inhibited almost entirely by the addition of apyrase and theophylline; apyrase cleaves the phosphate molecules converting ADP to AMP and adenosine, while theophylline blocks the P₁-receptor on the vascular smooth muscle, preventing the direct relaxation by adenosine.4,24

In the intact vessel and cell culture, ADP is converted to AMP and adenosine by endo-5'-nucleotidase within the endothelial cell. This conversion can also be accomplished outside of the endothelial cell by the surface enzyme ectonucleotidase. The extracellular adenosine can then be taken up by the endothelial cell and transported to the vascular smooth muscle, released endoluminally, or converted to adenine nucleotides or inosine (and its degradation products) by adenosine kinase and adenosine deaminase, respectively.25 Adenosine causes relaxation of vascular smooth muscle via stimulation of the A₂-receptor subclass of the P₁-receptor that activates adenylate cyclase, increasing intracellular cAMP levels in the vascular smooth muscle. This increase in intracellular cAMP and the subsequent relaxation can be antagonized by theophylline, a P₁-purinergic receptor blocker.24 In addition to relaxation, adenosine plays another important physiological role as an inhibitor of platelet aggregation and thus helps protect the vessel against platelet-induced vasoconstriction by two separate mechanisms.25 That both serotonin and ADP contribute to the platelet-induced relaxation in porcine pulmonary arteries is consistent with findings in systemic porcine arteries but different from canine coronary arteries in which ADP appears to be mainly responsible for platelet-induced endothelium-dependent relaxations.2,4-5,21,26

Thrombin, a product of the coagulation cascade, induced endothelium-dependent relaxations in the porcine pulmonary artery. This has also been observed in canine pulmonary arteries.27 Although it is probably not involved directly in the platelet-induced relaxation in vitro, thrombin plays a significant role in the interactions between the endothelium and the platelets in vivo.28

In rings without endothelium, aggregating platelets cause contractions that are inhibited by serotonergic and histaminergic antagonists. Serotonin is the vasoactive substance responsible for platelet-induced contractions in canine pulmonary and coronary arteries and porcine systemic vessels.2-6,26 However, it does not cause contractions in porcine pulmonary arteries. That platelet incubation with dazoxiben, a thromboxane synthetase inhibitor, before platelet exposure to the vascular rings failed to inhibit the platelet-induced contraction suggests that thromboxane A₂ is also not responsible for the contraction. This is confirmed by the low levels of thromboxane B₂ (the stable metabolite of thromboxane A₂) detected in the organ bath solution. However, platelet-induced contractions in this preparation were inhibited by three H₁-histaminergic antagonists, implicating histamine in the contraction by activating H₁-histaminergic receptors. The magnitude of the contractions induced by platelets is comparable to the level of contraction caused by the concentrations of histamine found in the organ bath solution. Although not thought to play a predominant role in the platelet-induced contractions in other porcine vessels, a partial role for histamine in the platelet-induced contraction has been described for porcine coronary arteries.1

The relaxations and contractions caused by aggregating platelets observed in this study were significantly larger in the smaller, more distal pulmonary arteries. Previous work described larger relaxations
in smaller vessels to other endothelium-dependent vasodilators such as acetylcholine and bradykinin but not the calcium ionophore A23187, indicating that the larger relaxations are secondary to increased number or affinity of the receptors or a tighter receptor coupling mechanism in the smaller vessels and not an increased ability of the endothelium of the distal vessels to synthesize or release endothelium-derived relaxing factor.\textsuperscript{29,30} Since the sodium nitroprusside-relaxing relaxations were similar in large and small arteries, it is unlikely that a difference in sensitivity of soluble guanylate cyclase or to cGMP explains the increased relaxations to platelets in the small vessels.\textsuperscript{31,32} The serotonin levels were similar in the organ chambers containing the large and small vessels; the corresponding concentration causes similar relaxations in both large and small vessels. Because the ADP-induced relaxations were larger in the small vessels at the highest concentrations, the larger relaxations observed to aggregating platelets in the smaller vessels could be explained by larger ADP-induced relaxations. This could be due to differences in number, affinity, or coupling of the purinergic receptors.\textsuperscript{29} However, a heterogeneity in the degradation or dephosphorylation of ADP by the endothelial cells in the more distal vessels is another possibility. The latter explanation would support the important role for adenosine in the relaxations evoked by aggregating platelets.

The larger contractions to aggregating platelets in the smaller vessels parallels that to histamine. Because the potassium-induced contraction (the standard against which the platelet-induced contraction was compared) was similar, any difference in the histamine-induced contraction between the two sizes of vessels could account for the difference in magnitude of the platelet-induced contractions.

Physiologically, the lungs act as a filter to circulating platelet microaggregates, helping to prevent systemic embolization. Most of these aggregates are filtered at the level of the precapillary and capillary vessels. Constriction of these vessels in response to vasoactive substances released from platelets may cause elevation of pulmonary artery pressures and resistance. Angiograms of cat lungs, which have been infused with platelet microaggregates, show tapering or a "cut off" appearance of the precapillary muscular vessels. The larger elastic vessels increased in size with the same treatment.\textsuperscript{33,34} These findings in vivo are consistent with the in vitro data obtained in this study. The musculoelastic arteries in our study dilated to the aggregating platelets. This dilation may be important in allowing the microaggregates to travel as far "downstream" as possible so as not to block a large vascular segment that would cause underperfusion of a larger segment of lung as opposed to underperfusion of the smaller cross-sectional area represented by small capillary beds. The endothelium may thus be important in protecting the lung vessels against the mechanical blockade of platelet aggregates, allowing for less ventilation and perfusion abnormalities.

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