Endothelium-Dependent Relaxation to Aggregating Platelets in Isolated Basilar Arteries of Control and Hypercholesterolemic Pigs

Hiroaki Shimokawa, Phyo Kim, and Paul M. Vanhoutte

The role of the endothelium was examined in the response to aggregating platelets in cerebral arteries from normal and hypercholesterolemic animals. Male Yorkshire pigs were fed either a normal diet or a 2% high-cholesterol diet for 10 weeks. Endothelium-dependent responses were examined in vitro. In rings of basilar arteries from control animals aggregating platelets caused endothelium-dependent relaxations, which were significantly inhibited by apyrase, an adenosine diphosphatase and triphosphatase, but were augmented by methiothepin, a combined S₁ and S₂-serotonergic blocker. In quiescent rings platelets induced contractions that were inhibited by the presence of the endothelium; these contractions were significantly inhibited by methiothepin, but not by ketanserin (an S₂-serotonergic blocker) or dazoxiben (a thromboxane-synthetase blocker) in the presence or absence of SQ29548 (a thromboxane-receptor blocker). Adenosine diphosphate but not serotonin caused endothelium-dependent relaxations. In cholesterol-fed animals the endothelium-dependent relaxations in response to aggregating platelets and adenosine diphosphate were impaired. These experiments indicate that 1) the endothelium inhibits the vasoconstrictor effect of aggregating platelets in porcine cerebral arteries; 2) platelet-induced relaxations are achieved mainly by a purinergic mechanism, while platelet-induced contractions are mediated by activation of S₂-serotonergic receptors with little contribution of thromboxanes; and 3) hypercholesterolemia impairs the endothelium-dependent relaxations in response to aggregating platelets due to the impaired responses to adenosine diphosphate.

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In vitro studies have demonstrated the important role of the endothelium in modulating the responsiveness of the underlying vascular smooth muscle.1-3 Especially, the endothelium appears to prevent the vasoconstrictions induced by aggregating platelets.4-7 In the cerebral circulation several vasoactive substances can cause endothelium-dependent relaxations.8-15 However, little information is available on endothelium-dependent responses to aggregating platelets and their products in cerebral arteries. Moreover, it is unknown how chronic atherogenic conditions, such as hyperlipidemia and hypertension, affect endothelium-dependent relaxations in the cerebral circulation. Augmented cerebral vasoconstrictor responses to serotonin (one of the major products of platelets) in atherosclerotic monkeys suggest that interactions between aggregating platelets and cerebral arteries are altered following atherosclerosis.16 Cerebral thrombosis is one of the most important causes of stroke and is frequently associated with atherogenesis.17,18

The present study was designed to examine, in porcine basilar arteries, whether aggregating platelets cause endothelium-dependent relaxation, and whether the responses are altered by hypercholesterolemia.

Materials and Methods

Twenty-five male Yorkshire pigs 6-8 weeks old (20.7 ± 0.5 kg) were used in this study. They were randomly divided into two groups and were fed either a regular chow (Hog Finisher, Bedtk Brothers Feed and Seed Co, Dover, Minnesota, 0.09% cholesterol) (control group, n = 15) or a 2% high-cholesterol diet (TD 86019 with 19% lard and

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2% cholesterol, Teklad, Madison, Wisconsin) (cholesterol-fed group, n = 10) for 10 weeks. The plasma concentrations of lipids (enzymatic method), heart rate, and blood pressure (under anesthesia as mentioned below) were determined before and after the 10-week period. To prevent excessive weight gain, the daily food intake was limited to an amount equal to 3% of the body wt/day. The pigs were housed individually in temperature-controlled quarters. In vitro experiments were performed after 10 weeks of feeding.

Organ Chamber Experiments

The pigs were anesthetized with ketamine hydrochloride (300 mg i.m.) and sodium pentobarbital (12.5 mg/kg i.v.). After collecting autologous blood (300 ml) from the left carotid artery into the citrate-anticoagulant for platelet preparation, the animals were exsanguinated and the brain was removed. The basilar artery was removed carefully under a microscope and immersed in cold, modified Krebs-Ringer bicarbonate solution of the following millimolar composition: 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). The artery was cleaned of loose connective tissue and cut into rings 3–4 mm long, with special care taken not to touch the luminal surface. Eight rings of basilar arteries were used in each experiment. In some of the rings, the endothelium was removed deliberately by rubbing the luminal surface gently with a watchmaker’s forceps on a paper towel wetted with control solution.20

The rings were mounted horizontally in organ chambers filled with 25 ml of control solution (37°C, pH 7.4) and gassed with 95% O2-5% CO2. The basilar artery was removed carefully under a microscope and immersed in cold, modified Krebs-Ringer bicarbonate solution of the following millimolar composition: 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). The artery was cleaned of loose connective tissue and cut into rings 3–4 mm long, with special care taken not to touch the luminal surface. Eight rings of basilar arteries were used in each experiment. In some of the rings, the endothelium was removed deliberately by rubbing the luminal surface gently with a watchmaker’s forceps on a paper towel wetted with control solution.20

The rings were mounted horizontally in organ chambers filled with 25 ml of control solution (37°C, pH 7.4) and gassed with 95% O2-5% CO2. The preparations were attached to a strain gauge (UC2, Gould Statham, Oxnard, California) and isometric tension was recorded. The rings were progressively stretched until the contractile response evoked by 20 mM potassium chloride was maximal (optimal tension).20 They were then allowed to equilibrate for 30 minutes before the experiments.

Protocol

Relaxations were examined in rings contracted with prostaglandin F2α (2 × 10−6 M) and contractions were examined in quiescent rings (n = 10 in each group). In the first set of experiments, relaxations of contracted rings were tested in response to: serotonin (10−9–10−3 M), adenosine diphosphate (ADP) (10−4–10−4 M), and platelets (25,000–100,000/μl). In the following two sets of experiments responses were examined after confirming the presence or absence of functional endothelial cells by bradykinin (10−8–10−7 M). In the second set quiescent rings were tested in response to: serotonin (10−8–10−5 M) and platelets (75,000/μl). In the third set the response to sodium nitroprusside (10−2–10−5 M, contracted rings) and to prostaglandin F2α (10−9–10−5 M, quiescent rings) was examined. Each contraction was followed by 20 mM KCl; preliminary experiments had shown that this concentration of KCl caused maximal contractions in rings of porcine basilar arteries without endothelium.

In the first set, in which endothelium-dependent relaxations were investigated, rings were treated with indomethacin (10−3 M) for 40 minutes before inducing contraction with prostaglandin F2α to prevent the synthesis of vasoactive prostanooids.20 Endothelium-derived relaxing factor(s) is not a product of cyclooxygenase.

In an additional five control pigs, the effects of apyrase (0.67 units/ml) or methiothepin (10−6 M) on the relaxations in response to ADP or bradykinin and the relaxing effects of vasopressin were tested.

After the experiments, the rings were examined histologically by hematoxylin-eosin staining for general observation and by Sudan IV staining for confirmation of lipid deposition in the blood vessel wall.

Drugs

The following drugs were used: ADP, apyrase (ADPase ATPase, Grade V from potato), arginine vasopressin, bradykinin, indomethacin, KCl, prostaglandin F2α; 5-hydroxytryptamine creatinine sulfate (serotonin), sodium nitroprusside (all from Sigma Chemical, St. Louis, Missouri), dazoxiben HCl (Pfizer, Groton, Connecticut), ketanserin tartrate (Janssen Pharmaceuticals, Beerse, Belgium), methiothepin maleate (Hoffmann-LaRoche, Nutley, New Jersey), and [1S-[1α, 20 (5), 3a, 4a]-7-[3-[(2-phenylamino) carbonyl]hydrazinomethyl]-7 oxabicyclo[2.2.1] hept-2-yl]-5-heptenoic acid (SQ29548; Squibb and Sons, Princeton, New Jersey). Unless otherwise specified, drugs were prepared daily in distilled water and kept on ice. Indomethacin was dissolved in an equal molar concentration of Na2CO3 (10−5 M). SQ29548 was dissolved in ethanol (final bath concentration, 4 × 10−4 M), diluted in 2 mM Na2CO3 (final concentration, 4.6 × 10−7 M), and then in distilled water.20 Inhibitors were added to the bath 40 minutes before experiments. Drug concentrations are reported as the final molar concentration in the bath solution.

A platelet-rich solution was prepared by centrifugation as described previously.20 The platelet suspension was kept at room temperature. Apyrase was suspended in control solution and added to the organ chambers 5 minutes before addition of platelets, in a concentration of 16.7 units ADPase and 12.5 units ATPase activity per 25 ml (as defined by the supplier, 1 unit activity liberates 1 μmol PO4/min).20 The concentration of apyrase is reported as the ADPase activity. The levels of serotonin or thromboxane A2 liberated from aggregating platelets are not different between the control and the cholesterol-fed groups (1 × 10−6–2 × 10−6 M and 6 × 10−10–9 × 10−10 M, respectively).24 In some cases, the platelets were incubated in the presence of dazoxiben (6.2 × 10−3 M), a selective inhibitor of thromboxane A2 synthetase, for 60 minutes before...
addition to the organ chamber. This treatment inhibits the synthesis of thromboxanes in platelets.\(^{19}\) A comparable concentration of dazoxiben in the organ chambers (10\(^{-4}\) M) caused no change in tension.

**Calculations and Statistical Analysis**

Results are expressed as mean ± SEM. In rings contracted with prostaglandin F\(_{2\alpha}\) responses are expressed as percent changes from the contracted levels, and in quiescent rings responses are expressed as percent changes of the contractions in response to 20 mM KCl. Unless otherwise noted, \(n\) is the number of animals from which rings were taken. Statistical evaluation of the data was performed by Student’s \(t\) test for paired or unpaired observations. When more than two means were compared, a two-way analysis of variance was used. If a significant value was found, Scheffe’s test for multiple comparisons was used to identify differences among groups. Values were considered to be statistically different at \(p<0.05\). For relaxations, the effective concentration of agonists causing 50% (IC\(_{50}\)) of the contractions in response to prostaglandin F\(_{2\alpha}\) was calculated from each concentration-response curve, and the means of these values were presented as the negative logarithm of the molar concentration. Maximal relaxation was expressed as percent of the response to prostaglandin F\(_{2\alpha}\) (2 x 10\(^{-6}\) M) irrespective of the concentration of the agonist; the means of these values are reported. For contractions evoked by prostaglandin F\(_{2\alpha}\) or serotonin, the effective concentration producing 50% (ED\(_{50}\)) or 30% (ED\(_{30}\)) of the contractions in response to 20 mM KCl was calculated.

**Results**

**Baseline Data**

Body weight increased significantly but in a comparable fashion in both groups during 10 weeks of maintenance; heart rate and blood pressure were unchanged in both groups (Table 1). The plasma concentration of cholesterol significantly increased (mainly in the fractions of low density and very low density lipoproteins) in the cholesterol-fed but not in the control group. In contrast, the concentration of triglyceride was unchanged in both groups.

**Light Microscopy**

The presence of an endothelial lining was confirmed histologically in those rings with but not

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**Table 1. Baseline Data in Control and Cholesterol Groups**

<table>
<thead>
<tr>
<th></th>
<th>Control Before</th>
<th>Control 10 weeks</th>
<th>Cholesterol Before</th>
<th>Cholesterol 10 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td>20.3 ± 1.0</td>
<td>51.4 ± 2.0*</td>
<td>21.3 ± 1.0</td>
<td>55.3 ± 3.3*</td>
</tr>
<tr>
<td><strong>Heart rate (beats/min)</strong></td>
<td>116 ± 5</td>
<td>110 ± 4</td>
<td>107 ± 4</td>
<td>106 ± 3</td>
</tr>
<tr>
<td><strong>Blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>147 ± 4</td>
<td>141 ± 4</td>
<td>140 ± 4</td>
<td>136 ± 4</td>
</tr>
<tr>
<td>Diastolic</td>
<td>96 ± 4</td>
<td>92 ± 3</td>
<td>98 ± 5</td>
<td>93 ± 4</td>
</tr>
<tr>
<td><strong>Cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>92 ± 6</td>
<td>103 ± 5</td>
<td>93 ± 6</td>
<td>535 ± 59*†</td>
</tr>
<tr>
<td>β-LDL</td>
<td>59 ± 4</td>
<td>65 ± 5</td>
<td>53 ± 4</td>
<td>378 ± 60*†</td>
</tr>
<tr>
<td>preβ-VLDL</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 0</td>
<td>70 ± 21*†</td>
</tr>
<tr>
<td>α-HDL</td>
<td>34 ± 2</td>
<td>37 ± 2</td>
<td>38 ± 3</td>
<td>74 ± 8*†</td>
</tr>
<tr>
<td>Triglyceride (mg/ml)</td>
<td>32 ± 3</td>
<td>35 ± 3</td>
<td>29 ± 4</td>
<td>40 ± 8</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Heart rate, blood pressure, and plasma concentrations of lipids are reported for 10 pigs in each group. Control, control group (\(n=15\)); Cholesterol, cholesterol-fed group (\(n=10\)); β-LDL, β-low density lipoprotein; preβ-VLDL, preβ-very low density lipoprotein; α-HDL, α-high density lipoprotein.

*\(p<0.05\) compared with before.
†\(p<0.05\) compared with control group.

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**Table 2. Characteristics of Vascular Smooth Muscle**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Optimal tension (g)</strong></td>
<td>2.9 ± 0.1 (119)</td>
<td>2.9 ± 0.1 (77)</td>
</tr>
<tr>
<td><strong>Contraction to 20 mM KCl</strong></td>
<td>3.2 ± 0.2 (119)</td>
<td>3.5 ± 0.2 (77)</td>
</tr>
<tr>
<td><strong>Contraction to PGF(_{2\alpha}) ((n=6))</strong></td>
<td>6.35 ± 0.32</td>
<td>6.42 ± 0.16</td>
</tr>
<tr>
<td>ED(_{50}) (−log M)</td>
<td>82 ± 6</td>
<td>78 ± 4</td>
</tr>
<tr>
<td><strong>Relaxation to sodium nitroprusside ((n=6))</strong></td>
<td>7.20 ± 0.12</td>
<td>7.17 ± 0.14</td>
</tr>
<tr>
<td>IC(_{50}) (−log M)</td>
<td>136 ± 10</td>
<td>135 ± 7</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Control, control group; Cholesterol, cholesterol-fed group; PGF\(_{2\alpha}\), prostaglandin F\(_{2\alpha}\); ED\(_{50}\), effective concentration producing 50% of the response to 20 mM potassium chloride (KCl); Contraction (10\(^{-3}\) M), contraction at 10\(^{-3}\) M of prostaglandin F\(_{2\alpha}\) in percent of the response to 20 mM KCl; IC\(_{50}\), effective concentration causing 50% inhibition of the contractions to prostaglandin F\(_{2\alpha}\) (2 x 10\(^{-6}\) M); Max relaxation, maximal relaxation in percent of the response to prostaglandin F\(_{2\alpha}\) (2 x 10\(^{-6}\) M).

Numbers in parentheses are the numbers of rings tested in each group. Since the contractions to KCl or prostaglandin F\(_{2\alpha}\) were not achieved in one ring in the control and in three rings in the cholesterol group, these rings were excluded from the experiments. For the contractions to prostaglandin F\(_{2\alpha}\) or relaxations to sodium nitroprusside, the data obtained in rings without endothelium are reported.
Endothelium, Platelets, and Basilar Arteries

Control group

Endothelium: with or without 25 50 75 100

Cholesterol group

Endothelium: with or without PQF-2x10^-6 M

FIGURE 1. Relaxations in response to aggregating platelets in basilar arteries taken from normal (top) and cholesterol-fed (bottom) pig in presence of indomethacin (10^-5 M). Rings first were contracted with prostaglandin F^2a (PGF^2a) (2x10^-6 M). W_o and dots, wash out with Krebs-Ringer bicarbonate solution.

Organ Chambers

Characteristics of the smooth muscle (Table 2). There was no statistically significant difference between the two groups in optimal tension or amplitude of the contractions evoked by 2x10^-6 M prostaglandin F^2a or 20 mM KCl. Similarly, prostaglandin F^2a (10^-2-10^-3 M) caused comparable concentration-dependent contractions in rings without endothelium in both groups. Sodium nitroprusside (10^-5-10^-3 M) caused comparable concentration-dependent relaxations in rings without endothelium in the two groups.

Endothelium-dependent relaxations. During a contraction in response to 2x10^-4 M prostaglandin F^2a, aggregating platelets (25,000-100,000/μl) caused concentration-dependent, endothelium-dependent relaxations in the control group (Figures 1 and 2). These relaxations were significantly reduced in the cholesterol-fed group (Figures 1 and 2). In both groups, the platelet-induced relaxations were augmented by methiothepin (a combined S^- and S^2-serotonergic blocker) and were significantly inhibited by apyrase (an ADPase and ATPase).

In the control group, ADP-induced relaxations were significantly more pronounced in rings with endothelium than those without endothelium (Figure 3). The endothelium-dependent component of the relaxation evoked by ADP was significantly blocked by apyrase (0.67 units/ml) but not by methiothepin (10^-6 M); the IC_{50} value (-log M) and the percent of maximal relaxation in control rings with endothelium in the presence of apyrase or methiothepin were 6.03±0.14, 5.24±0.08, and 6.23±0.05 (IC_{50} value), and 129±9, 48±4, and 128±8 (maximal relaxation), respectively (n=5). In contrast, the endothelium-independent component of the relaxation (rings without endothelium) was not significantly altered by apyrase or methiothepin; the IC_{50} value (-log M) and the percent of maximal relaxation in the three conditions mentioned above were 5.55±0.11, 5.52±0.08, and 5.65±0.14 (IC_{50} value), and 45±4, 38±4, and 46±4 (maximal relaxation), respectively (n=5). The endothelium-dependent component of the relaxation evoked by ADP was significantly reduced in the cholesterol-fed group (Figure 3, Table 3). In contrast, the relaxations in rings without endothelium were unaltered (Table 3).

Serotonin caused contraction from 10^-8-3x10^-7 M, followed by relaxations with higher concentrations of the monoamine; no significant differences were noted between rings with and without endothelium, or between control and cholesterol-treated arteries (Figure 4).

FIGURE 2. Relaxations in response to aggregating platelets during contraction evoked by prostaglandin F^2a (PGF^2a) (2x10^-4 M) in rings of basilar arteries taken from control (left) and cholesterol-fed (right) pigs in presence of indomethacin (10^-5 M). Responses are expressed as percent change in tension from contraction level evoked by prostaglandin F^2a (2x10^-4 M). Data shown as mean±SEM. Filled symbols represent statistically significant difference (p<0.05) from untreated rings with endothelium (open circles). *Statistically significant difference (p<0.05) from untreated rings with endothelium in control group.
Adenosine diphosphate, \(-\log M\)

**FIGURE 3.** Cumulative concentration-response curves of porcine basilar arteries to ADP during contraction by prostaglandin F2a (PGF2a) (2 \times 10^{-6} M) in presence of indomethacin (10^{-5} M). Relaxations are expressed as percent decrease in tension from contraction evoked by prostaglandin F2a (2 \times 10^{-6} M). Data shown as mean±SEM. Statistical analyses are reported in Table 3.

Bradykinin caused comparable endothelium-dependent relaxations in both groups (Table 3). These relaxations were not affected by apyrase (0.67 units/ml) or methiothepin (10^{-6} M) in the control group; the IC_{50} value (\(-\log M\)) and the maximal relaxation (%) in untreated condition, in the presence of apyrase or methiothepin were 8.09 ± 0.06, 8.14 ± 0.08, and 8.09 ± 0.07 (IC_{50} value), and 144 ± 5, 136 ± 6, and 146 ± 10 (maximal relaxation), respectively (n = 5).

Vasopressin caused no endothelium-dependent or independent relaxations in basilar arteries from control animals (n = 5, data not shown).

**Contractions.** In the control group, aggregating platelets caused contractions in quiescent rings; those contractions were significantly inhibited in rings with endothelium compared with those without (Figure 5). The platelet-induced contractions were significantly inhibited by methiothepin but not by ketanserin (an S_2-serotonergic blocker). Dazoxiben, a thromboxane synthetase blocker, failed to inhibit the platelet-induced contractions even when combined with SQ29548, a thromboxane-receptor blocker (Figure 5). When dazoxiben was combined with methiothepin, the degree of the inhibition was the same as with methiothepin alone. In rings with endothelium, the platelet-induced contractions were significantly potentiated by apyrase (Figure 5).

In the cholesterol-fed group, the platelet-induced contractions in rings with endothelium were significantly greater compared with the control group, while comparable contractions were induced in rings without endothelium (Figure 5). The different inhibitors affected the contractions in the same manner as in the control group (Figure 5).

In quiescent rings without endothelium, serotonin caused contractions followed by relaxations at higher concentrations (Figure 6). These contractions were not significantly affected by ketanserin but were significantly inhibited by methiothepin in both groups (Figure 6); in the control group, the ED_{50} value (\(-\log M\)) and maximal contraction (as expressed in percent of that to 20 mM KCl) in untreated and ketanserin-treated rings were 7.73 ± 0.22 and 7.39 ± 0.31, and 59 ± 7 and 49 ± 6, respectively. Similarly, those values in the cholesterol-fed group in untreated and ketanserin-treated rings were 7.75 ± 0.13 and 7.67 ± 0.20, and 66 ± 6 and 54 ± 6, respectively. There also was no significant difference between the control and the cholesterol-fed group in the ED_{50} value or maximal contraction.

**Discussion**

The present study was initiated because no information was available on the endothelium-dependent responses to aggregating platelets in the cerebral circulation, under normal or pathologic conditions. The major findings in the present study were 1) in porcine basilar arteries, aggregating platelets cause relaxations in the presence of endothelium and contractions in its absence; 2) the endothelium-dependent relaxations in response to aggregating platelets are achieved mainly by activation of purinergic receptors with a minimal contribution of serotonin, but the endothelium-independent contractions are achieved mainly by activation of S_1-

**Table 3. Endothelium-Dependent Relaxations of Porcine Basilar Arteries**

<table>
<thead>
<tr>
<th>Endothelium</th>
<th>IC_{50} (IC_{15}) ((-\log M))</th>
<th>Max relaxation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Cholesterol</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>ADP (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With</td>
<td>5.79 ± 0.15</td>
<td>5.20 ± 0.16*</td>
</tr>
<tr>
<td></td>
<td>(5.78 ± 0.27)</td>
<td>(5.51 ± 0.18)</td>
</tr>
<tr>
<td>Bradykinin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With</td>
<td>8.21 ± 0.10</td>
<td>8.08 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM.

IC_{50} or IC_{15}, effective concentration causing 50% or 15% inhibition of the contractions to prostaglandin F_{2a} (2 \times 10^{-6} M); Max relaxation, maximal relaxation in percent of the response to prostaglandin F_{2a} (2 \times 10^{-6} M). ... indicates the response did not attain the IC_{50} or IC_{15} level.

* p<0.05 compared with control.

†p<0.05 compared with rings with endothelium.
serotonergic receptors with a minimal contribution of thromboxanes; and 3) the inhibitory actions of the endothelium are significantly impaired by hypercholesterolemia at a time when the ability of the underlying smooth muscle to relax or contract is unchanged.

In the cerebral circulation, endothelium-dependent relaxations have been demonstrated, in vitro and/or in vivo, in response to acetylcholine (cat, mouse), bradykinin (mouse, dog), thrombin (dog), vasopressin (dog), and oxytocin (dog). It is likely that endothelium-dependent relaxations play a physiological role in the regulation of the cerebral circulation. The contribution of a given endothelium-dependent response must depend on the presence of the substance in the immediate vicinity of the endothelial cells. Therefore, the presence or absence of endothelium-dependent responses to aggregating platelets and their products are potentially important as they can easily reach the cerebral blood vessels. Indeed, the present study demonstrated that aggregating platelets cause pronounced endothelium-dependent relaxations in porcine basilar arteries. Aggregating platelets cause varying degrees of endothelium-dependent relaxations in systemic porcine arteries. The extent of the relaxations produced by aggregating platelets in basilar arteries observed in the present study are second to those seen in coronary arteries, but much greater than those observed in carotid or femoral arteries in pigs.

Endothelium-dependent responses to aggregating platelets are the global expression of the changes in tension induced by several released platelet products and of their interactions. In the present study, the platelet-induced, endothelium-dependent relaxations in porcine basilar arteries were almost abolished by apyrase, while they were augmented rather than inhibited by methiothepin. The endothelium-dependent relaxations in response to ADP were significantly inhibited by apyrase but were not augmented by methiothepin. Vasopressin caused no endothelium-dependent or independent relaxations. These results indicate that the platelet-induced relaxations are due mainly to the adenine nucleotides, while the serotonin released by the platelets contributes only to direct activation of vascular smooth muscle. This interpretation is supported further by the observations that ADP caused pronounced relaxations, which depended on the presence of endothelial cells, while serotonin caused no endothelium-dependent relaxations. The present study also revealed that there is a

![Figure 4](http://circres.ahajournals.org/)

**Figure 4.** Cumulative concentration-response curves to serotonin of porcine basilar arteries during contraction evoked by prostaglandin F2α (PGF2α) (2×10⁻⁶ M) in presence of indomethacin (10⁻⁵ M). The relaxations are expressed as percent change in tension from contraction evoked by prostaglandin F2α (2×10⁻⁶ M). Data shown as mean±SEM. No significant differences were observed between experimental groups.

![Figure 5](http://circres.ahajournals.org/)

**Figure 5.** Effects of aggregating platelets (75,000 μl) in quiescent rings of basilar arteries taken from control (top) and cholesterol-fed (bottom) pigs. Contractions are expressed as percent increase in tension obtained with 20 mM KCl. Data shown as mean±SEM. Concentrations of blockers used were as follows: apyrase (0.67 U/ml), ketanserin (10⁻⁶ M), methiothepin (10⁻⁶ M), dazoxiben (6.2×10⁻⁷ M), and SQ29548 (10⁻⁶ M). *p<0.05 compared with untreated rings; tp<0.05 compared with rings without endothelium; tp<0.05 compared with rings in control group.
large species difference between pigs and dogs in vasopressin-induced vascular responses of basilar arteries; in the latter, vasopressin causes pronounced endothelium-dependent relaxations. In contrast, the platelet-induced contractions were significantly inhibited by methiothepin but not by ketanserin, dazoxiben, or SQ29548. These results indicate that the platelet-induced contractions are achieved mainly by activation of S<sub>1</sub>-serotonergic receptor rather than by thromboxanes. Serotonin-induced contractions also were inhibited significantly by methiothepin but not by ketanserin. These findings are in agreement with observations in cat pial arterioles where the platelet-induced contractions are achieved mainly by a serotonergic mechanism (S<sub>1</sub>-receptor in this case) with little contribution of thromboxanes. The observation that apyrase potentiated the platelet-induced contractions in quiescent rings with endothelium indicates that purinergic activation contributes to the inhibitory effects of the endothelium against platelet-induced contractions. The fact that aggregating platelets cause endothelium-dependent inhibitory effects by activation of purinergic receptors on the endothelium in porcine basilar arteries while they exert direct excitatory effects by activation of S<sub>1</sub>-serotonergic receptors on the smooth muscle contrasts with their effects in the coronary arteries of the same species where purinergic and S<sub>1</sub>-serotonergic mechanisms are activated by the endothelium and S<sub>1</sub>-serotonergic receptors on the smooth muscle. The reason for such heterogeneity is unclear. Major differences in endothelium-dependent responsiveness between the cerebral and coronary artery have also been reported in the dog. Human platelets generate greater amounts of thromboxanes than pig or dog platelets. Therefore, the results of the present study may not necessarily apply to the role of thromboxanes in platelet-induced contractions in the human cerebral circulation.

The endothelium-dependent relaxations caused by platelets were reduced by hypercholesterolemia. Since the endothelium-dependent relaxations in response to ADP also were impaired and serotonin-induced contractions were unaltered, it is logical to assume that the impaired endothelium-dependent relaxations in response to ADP account for the reduced relaxations in response to aggregating platelets. In contrast, in atherosclerotic monkeys, cerebral vasoconstrictor responses to serotonin are augmented. It would be important to examine whether these augmented contractions are due to the altered responsiveness of the endothelium and/or the vascular smooth muscle.

Several possibilities could explain an impaired endothelium-dependent relaxation in response to ADP in hypercholesterolemic animals. First, the characteristics of smooth muscle cells and in particular their sensitivity to endothelium-derived relaxing factor(s) may be changed. This possibility is not likely because in rings without endothelium the relaxations in response to sodium nitroprusside, which induces relaxations through activation of guanylate cyclase as does endothelium-derived relaxing factor(s), may be impaired. This possibility is unlikely because there was no inimal thickening as a possible mechanical barrier for a factor(s) with a very short half-life and because endothelium-dependent relaxations in response to bradykinin were not affected by hypercholesterolemia. Third, the production and/or release of the endothelium-derived relaxing factor(s) may be depressed. Hypercholesterolemia injures endothelial cells, resulting in increased turnover rate. A previous study demonstrated that regenerated endothelial cells exhibit reduced endothelium-dependent responsiveness to aggregating platelets and serotonin in porcine coronary arteries 4 weeks after endothelial denudation. In the more chronic regenerated stage (8 weeks after the denudation), endothelium-dependent relaxations in response to ADP also are depressed (authors’ unpublished observation). If this were the case for basilar arteries, this third possibility would be most likely. In addition, it was recently shown that acutely administered low density lipoproteins nonspecifically inhibit endothelium-dependent relaxation in the rabbit aorta. However, it remains to be examined why the response to bradykinin is not affected. In pial arterioles of the cat, the endothelium may release a different type of relaxing factor(s) in response to acetylcholine and bradykinin.
The present study has several important clinical implications. In the porcine cerebral circulation, the endothelium exerts inhibitory actions against aggregating platelets. This finding is important because large arteries in the cerebral circulation contribute to the total vascular resistance and circulating platelets can aggregate at the site of endothelial injury. Further, the platelet-induced contractions are mainly achieved by a serotoninergic mechanism with little contribution of thromboxanes. These contractions caused by serotonin, together with thrombus formation, could play a role in the pathogenesis of cerebral ischemia, giving a clue for the prevention of the disorder. The species difference in the subtype of serotoninergic receptor responsible for the platelet-induced contractions (S1 receptor in the pig) and S2 receptor in the cat makes studies on human cerebral arteries necessary before a final conclusion can be reached as to the type of serotoninergic blocker that may be clinically useful. Finally, the observation that the inhibitory actions of the endothelium against aggregating platelets are impaired by hypercholesterolemia may help to explain why this condition is a risk factor for the occurrence of (cerebral) atherosclerosis. Although hypercholesterolemia impairs endothelium-dependent relaxations in response to aggregating platelets in a generalized manner in porcine arteries, the impairment is more prominent in coronary and basilar arteries (this study). Atherothrombotic infarction is prone to occur in patients with hypertension, diabetes mellitus, and hyperlipidemia. Acute severe hypertension (for 15 minutes) impairs endothelium-dependent dilatation in response to acetycholine in cat cerebral arterioles. These pathological conditions injure endothelial cells, which is one of the initiating events in atherosclerosis. Therefore, impaired interactions between platelets and the blood vessel wall in the presence of endothelial dysfunction would favor the occurrence of platelet aggregation and platelet-induced contractions of cerebrovascular smooth muscle, leading to ischemic events such as cerebral thrombosis and cerebral vasospasm.

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