Loss of Endothelial Pertussis Toxin–Sensitive G Protein Function in Atherosclerotic Porcine Coronary Arteries

Hiroaki Shimokawa, MD; Nicholas A. Flavahan, PhD; and Paul M. Vanhoutte, MD

Pertussis toxin, an irreversible inhibitor of some G proteins, inhibits endothelium-dependent relaxations to certain agonists in porcine coronary arteries. In the present study, the effects of the toxin were examined on endothelium-dependent and independent relaxations of hypercholesterolemic and atherosclerotic porcine coronary arteries to assess the functional state of the endothelial pertussis toxin–sensitive G protein. Male Yorkshire pigs were maintained on either a regular diet (control group, n = 7) or a 2% high-cholesterol diet (cholesterol-fed group, n = 7) for 10 weeks. After the initial 2 weeks of maintenance, animals in both groups underwent balloon catheter removal of the endothelium of the left anterior descending or left circumflex coronary arteries. Endothelium-dependent responses were examined in vitro after 10 weeks of maintenance; at this time, a full lining of endothelial cells in both left coronary arteries was confirmed histologically. In arteries with endothelium of the control group (normal responses), pertussis toxin significantly inhibited the endothelium-dependent relaxations to serotonin, UK14304 (a selective α2-adrenergic receptor agonist), and thrombin but not those to ADP, bradykinin, or the calcium ionophore A23187. In previously denuded arteries of the control group (effects of endothelial regeneration alone) or intact arteries of the cholesterol-fed group (effects of hypercholesterolemia alone), the relaxations to serotonin, UK14304, and thrombin were impaired significantly; those relaxations were impaired further in previously denuded arteries of the cholesterol-fed group (effects of atherosclerosis). The inhibitory effects of pertussis toxin were significantly reduced after endothelial regeneration and in hypercholesterolemia and were almost absent in atherosclerosis. Direct relaxations of coronary vascular smooth muscle evoked by nitric oxide or sodium nitroprusside were not significantly affected by either hypercholesterolemia or atherosclerosis. These results indicate that the function of endothelial pertussis toxin–sensitive G protein is impaired in regenerated endothelial cells or hypercholesterolemia and is almost absent in atherosclerosis, accounting in part for the endothelial dysfunction under those pathological conditions. (Circulation 1991;83:652–660)

Atherosclerosis impairs endothelium-dependent relaxations mainly because of the resulting reduced production (synthesis or release) of endothelium-derived relaxing factor.1–6 However, the cellular mechanisms for the endothelial dysfunction in atherosclerosis remain to be elucidated. Pertussis toxin, which is an irreversible inhibitor of certain G proteins, inhibits endothelium-dependent relaxations to serotonin and UK14304, which is a selective α2-adrenergic receptor agonist, but not relaxations to ADP, bradyki-
Methods

Animal Preparations

Fourteen male Yorkshire pigs, 6–8 weeks of age (19.0±0.6 kg), were used. They were randomly divided into two groups and were fed either a regular chow (0.09% cholesterol, Hog Finisher, Bedtk Brothers Feed and Seed Co., Dover, Minn.) (control group, n=7) or a 2% high-cholesterol diet (TD 86019 with 19% lard and 2% cholesterol, Teklad, Madison, Wis.) (cholesterol-fed group, n=7). Also, three pigs from the control group were used in a separate experiment for another study.8 After 2 weeks of feeding, animals in both groups underwent balloon removal of the endothelium of the first 4 cm of either the left anterior descending (four pigs) or left circumflex (three pigs) coronary artery.5,6,12 Afterward, they were fed for 8 weeks. This model allows the separate evaluation of the effects of endothelial regeneration (previously denuded artery in the control group), hypercholesterolemia (intact artery in the cholesterol-fed group), and atherosclerosis (previously denuded artery in the cholesterol-fed group).5,6,12 The plasma concentration of lipids was determined by enzymatic methods13 before and 10 weeks after the feeding. The animals were housed individually in temperature-controlled animal quarters. To prevent excessive weight gain, the daily food intake was limited to an amount equal to 3% of the body wt/day.5,6,12 In vitro experiments were performed after 10 weeks of feeding.

Organ Chamber Experiments

The pigs were anesthetized with ketamine hydrochloride (300 mg intramuscularly) followed by sodium pentobarbital (12.5 mg/kg intravenously).12 The hearts were removed, and both left coronary arteries were dissected free and immersed in cold modified Krebs–Ringer bicarbonate solution of the following composition (mM): 118.3 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, 25.0 NaHCO3, 0.016 Ca-EDTA, and 11.1 glucose (control solution). Because balloon removal of the endothelium was performed in the first 4 cm of the proximal portion of the coronary artery from the left coronary orifice, this portion of the previously denuded artery and the comparable portion of the intact artery were used. The rings (3–4-mm long) were numbered from the proximal to the distal direction, and rings with the same number from both coronary arteries were studied in parallel. They were cleaned of loose connective tissue, with care taken not to touch the intima. In some of the rings, the endothelium was removed deliberately by rubbing the luminal surface gently with a cotton swab wetted with control solution.12

The rings were then suspended horizontally between two stainless steel stirrups in organ chambers filled with 25 ml control solution (37°C, pH 7.4) gassed with 95% O2–5% CO2. One stirrup was anchored in the organ chamber, and the other was connected to a strain gauge (UC 2, Gould–Statham, Oxnard, Calif.) for the recording of isometric tension. The rings were then stretched progressively until the contractile response evoked by potassium chloride (20 mM) was maximal (optimal tension).12

After optimal tension had been achieved, the rings were equilibrated for 60 minutes. During this time, they were incubated with indomethacin (10−5 M) to prevent the formation of endogenous prostaglandins. Some of the rings were treated with pertussis toxin (100 ng/ml) for 90 minutes and indomethacin; rings with and without the toxin were examined in parallel. Relaxations were examined during a contraction caused by prostaglandin F2α (2×10−6 M) in the following orders: set A: 1) bradykinin, 2) serotonin, and 3) thrombin; set B: 1) UK14304, 2) ADP, and 3) A23187; set C: 1) nitric oxide and 2) ADP; and set D: sodium nitroprusside. When determining endothelium-dependent relaxations to serotonin, we treated rings with ketanserin (10−6 M) for 40 minutes to inhibit the direct 5-HT3-serotonergic activation of vascular smooth muscle by the monoamine.12

Drugs

The following drugs were used: ADP, bovine thrombin, bradykinin, A23187, 5-hydroxytryptamine creatinine sulfate (serotonin), indomethacin, pertussis toxin, potassium chloride, prostaglandin F2α, sodium nitroprusside (all from Sigma Chemical Co., St. Louis); ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium); and UK14304 (Pfizer Central Research, Sandwich, Kent, UK). All drugs were prepared daily with distilled water except for A23187 and indomethacin, which were dissolved in dimethylsulfoxide (1%) and Na2CO3 (10−5 M), respectively.

Solutions of nitric oxide were made as reported previously.14 Briefly, gas bulb fitted with a silicon injection septa was filled with nitric oxide from a cylinder (Union Carbide, Chicago). An appropriate volume (10–100 μl) was removed with syringe and injected into another gas bulb that had been filled with 100 ml distilled water, which had been gassed with helium for approximately 3 hours, giving stock solutions of nitric oxide of 4×10−5 and 4×10−6 M.

Morphology

The hearts were divided into five horizontal blocks and were examined macroscopically for the presence or absence of myocardial infarction.

The rings used in the organ chamber study were examined histologically by hematoxylin–eosin staining for determination of endothelial lining and general observation, by Sudan IV staining for examination of lipid deposition, and by Van Gieson’s elastic staining for determination of the thickness of the intima and the media.5,6 Morphometric determination was performed with a computer-assisted image analyzer (IBAS 2000, Kontron Electronics, FRG) to evaluate cross-sectional area of the intima and the media.5,6

Data Analysis

Results are expressed as mean±SEM. Unless otherwise specified, n refers to the number of animals.
Relaxations are expressed as percent changes from the contracted levels with prostaglandin F$_{2_{\alpha}}$. The negative logarithm of the effective molar concentration of agonist causing 50% (pIC$_{50}$) of the maximal relaxation was calculated for each concentration–response curve, and the means of these values are presented. The extent of the inhibition by pertussis toxin is expressed as percent inhibition of the area delineated by the concentration–response curves or of the maximal relaxation to the agonists. Statistical evaluation of the data was performed with Student’s $t$ test for paired observations. When more than two means were compared, an analysis of variance was used. If a significant $F$ value was found, Scheffé’s test for multiple comparisons was used to identify differences among groups. Values were considered significant when $p$ was less than 0.05.

Results

Baseline Data

After 10 weeks of feeding, body weight increased significantly in both groups (50.1 ± 2.4 kg in the control group and 53.0 ± 1.3 kg in the cholesterol-fed group). The plasma concentration of nitric oxide increased in the cholesterol-fed (from 93 ± 4 before to 587 ± 47 mg/dl after feeding) but not in the control animals (98 ± 7 before and 106 ± 5 mg/dl after feeding); the fractions of low-density lipoproteins increased in the cholesterol-fed group (from 53 ± 4 before to 454 ± 48 mg/dl after feeding).

Morphology

No macroscopically visible regions of myocardial infarction were noted in the 14 hearts. The presence or absence of the endothelium was confirmed histologically in all rings used for organ chamber experiments. After balloon endothelium removal, the cross-sectional area of the intima and the media increased significantly in the control group and increased further in the cholesterol-fed group (Table 1). Hypercholesterolemia alone had no significant effects (Table 1). Lipid deposition was observed only in the thickened intima in the cholesterol-fed group.

Organ Chamber Experiments

Characteristics of the smooth muscle. There were no significant differences in optimal tension or contractions evoked by prostaglandin F$_{2_{\alpha}}$ (2 × 10$^{-6}$ M) among the four arteries with different origins (Table 2). Sodium nitroprusside (10$^{-9}$ to 10$^{-6}$ M) caused comparable concentration-dependent relaxations in rings without endothelium from the four different arteries. Similarly, relaxations in response to nitric oxide (10$^{-9}$ to 10$^{-7}$ M) did not differ significantly (Table 2).

Serotonin and UK14304. In the control group, serotonin (10$^{-9}$ to 3 × 10$^{-6}$ M) and UK14304 (10$^{-6}$ to 10$^{-5}$ M) caused concentration-dependent, endothelium-dependent relaxations in the intact artery (Figures 1 and 2). These relaxations were significantly reduced in the previously denuded artery (Figures 1 and 3, Table 3). In the cholesterol-fed group, the

### Table 1. Cross-Sectional Area of the Intima and the Media of Porcine Coronary Arteries

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Cholesterol-fed group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Denuded</td>
</tr>
<tr>
<td>Intima (mm$^2$)</td>
<td>0.02±0.01</td>
<td>0.31±0.04*</td>
</tr>
<tr>
<td>Media (mm$^2$)</td>
<td>1.06±0.06</td>
<td>1.36±0.09*</td>
</tr>
</tbody>
</table>

Data were obtained from 34 rings with endothelium in each group and are expressed as mean±SEM.

Intact, nondenuded control artery; denuded, previously denuded artery.

*p<0.05 vs. intact artery in the control group; †p<0.05 vs. previously denuded artery in the control group.

Table 2. Characteristics of Porcine Coronary Smooth Muscle

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Cholesterol-fed group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Denuded</td>
</tr>
<tr>
<td>Optimal tension (g)</td>
<td>8.6±0.3 (42)</td>
<td>8.3±0.2 (42)</td>
</tr>
<tr>
<td>Developed tension to 2×10$^{-6}$ M PGF$<em>{2</em>{\alpha}}$ (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without pertussis toxin</td>
<td>9.6±0.7 (14)</td>
<td>9.0±0.8 (14)</td>
</tr>
<tr>
<td>With pertussis toxin</td>
<td>9.8±0.6 (14)</td>
<td>9.4±0.6 (14)</td>
</tr>
<tr>
<td>Relaxation to sodium nitroprusside (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max relaxation (%)</td>
<td>112±2</td>
<td>110±2</td>
</tr>
<tr>
<td>pIC$_{50}$ (−log M)</td>
<td>7.75±0.10</td>
<td>7.80±0.10</td>
</tr>
<tr>
<td>Relaxation to nitric oxide (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max relaxation (%)</td>
<td>111±3</td>
<td>109±3</td>
</tr>
<tr>
<td>pIC$_{50}$ (−log M)</td>
<td>6.71±0.11</td>
<td>6.73±0.11</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. Numbers in parentheses are the numbers of rings tested in each group. For optimal tension, the combined data from rings with and without endothelium are presented because there was no difference in optimal tension between both groups.

Intact, control artery; denuded, previously denuded artery; PGF$_{2_{\alpha}}$, prostaglandin F$_{2_{\alpha}}$; pIC$_{50}$, effective concentration causing 50% of the maximal relaxation; max relaxation, maximal relaxation in percent response to prostaglandin F$_{2_{\alpha}}$ (2×10$^{-6}$ M).
relaxations to serotonin and UK14304 were signifi-
cantly reduced in intact arteries compared with those
from the control group (Figures 1 and 2, Table 3); the
relaxations were impaired further in previously de-
denuded arteries (Figures 1 and 2, Table 3).

Pertussis toxin (100 ng/ml) inhibited significantly
the relaxations to serotonin and UK14304 in the
intact artery in the control group (Figures 1–3). The
inhibitory effect of the toxin was significantly reduced
in previously denuded arteries of the control group or
in intact arteries of the cholesterol-fed group; it was
almost absent in previously denuded arteries of the
cholesterol-fed group (Figures 1–3). The levels of
relaxation achieved in the presence of pertussis toxin
were comparable among the different groups (Fig-
ures 1 and 2), except for the relaxations in the

previously denuded arteries of the cholesterol-fed
group in response to serotonin which were signifi-
cantly less (Figure 1).

Thrombin. Thrombin (0.001–1.0 units/ml) caused
concentration-dependent, endothelium-dependent
relaxations of control arteries (Figure 4). The
response to thrombin was significantly reduced in intact
arteries of the cholesterol-fed group, was significantly
reduced even more in previously denuded arteries of
the control group, and was significantly reduced the
most in previously denuded arteries of the choles-
terol-fed group (Figure 4, Table 3). The relaxations to
thrombin were partially sensitive to pertussis toxin;
the inhibition by the toxin was significantly reduced
in the three diseased arteries (Figure 4). When
expressed as a percentage of the area under the

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**FIGURE 1.** Cumulative concentration–response
curves to serotonin in rings with endothelium during
contractions to prostaglandin F2α (2×10⁻⁶ M). All rings
were treated with indomethacin (10⁻⁵ M) and keta-
serin (10⁻⁶ M). Relaxations are expressed as percent
decrease in tension. Data shown as mean±SEM.

**FIGURE 2.** Cumulative concentration–response
curves to UK14304 in rings with endothelium during
contractions to prostaglandin F2α (2×10⁻⁶ M) in the
presence of indomethacin (10⁻⁵ M). Relaxations are
expressed as percent decrease in tension. Data shown as
mean±SEM.
curve, the inhibition by the toxin was 31±4% in the intact and 18±4% in the previously denuded arteries of the control group and 21±4% in the intact and 0% in the previously denuded arteries of the cholesterol-fed group. When the inhibition was expressed as a percentage of the maximal relaxation, similar results were obtained (data not shown).

**ADP, bradykinin, and A23187.** In intact arteries of the control group, ADP (10⁻⁸ to 10⁻⁶ M) caused concentration-dependent, endothelium-dependent relaxations, which were not significantly reduced by pertussis toxin (Figure 5). These relaxations were reduced significantly and to a comparable extent in the previously denuded artery of the control group and in intact arteries of the cholesterol-fed group and were reduced further in the previously denuded artery of the cholesterol-fed group (Figure 5, Table 3). Direct endothelium-dependent relaxations to ADP (in rings without endothelium) were comparable among the four groups; the mean pIC₅₀ value (−log M) ranged from 4.72 to 4.91, and the mean maximal relaxations ranged from 94% to 99% (n=7 in each group).

Bradykinin (10⁻¹₀ to 10⁻⁷ M) caused concentration-dependent, endothelium-dependent relaxations, which were not affected by pertussis toxin (Figure 6). These relaxations were reduced only in previously denuded arteries of the cholesterol-fed group (Figure 6, Table 3).

A23187 (10⁻⁹ to 10⁻⁶ M) caused concentration-dependent, endothelium-dependent relaxations,

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**Table 3. Endothelium-Dependent Relaxation of Porcine Coronary Arteries**

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=7)</th>
<th>Cholesterol-fed group (n=7)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Denuded</td>
</tr>
<tr>
<td>Serotonin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max relaxation (%)</td>
<td>80±5</td>
<td>37±9*</td>
</tr>
<tr>
<td>pIC₅₀ (−log M)</td>
<td>6.98±0.19</td>
<td>NA</td>
</tr>
<tr>
<td>UK14304</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max relaxation (%)</td>
<td>87±6</td>
<td>38±5*</td>
</tr>
<tr>
<td>pIC₅₀ (−log M)</td>
<td>7.00±0.07</td>
<td>6.60±0.14</td>
</tr>
<tr>
<td>Thrombin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max relaxation (%)</td>
<td>99±3</td>
<td>67±8*</td>
</tr>
<tr>
<td>pIC₅₀ (×10⁻² units/ml)</td>
<td>2.33±0.54</td>
<td>5.81±1.05*</td>
</tr>
<tr>
<td>ADP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max relaxation (%)</td>
<td>106±4</td>
<td>103±3</td>
</tr>
<tr>
<td>pIC₅₀ (−log M)</td>
<td>4.96±0.12</td>
<td>5.46±0.13</td>
</tr>
<tr>
<td>Bradykinin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max relaxation (%)</td>
<td>107±2</td>
<td>109±2</td>
</tr>
<tr>
<td>pIC₅₀ (−log M)</td>
<td>8.25±0.08</td>
<td>8.28±0.05</td>
</tr>
<tr>
<td>A23187</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max relaxation (%)</td>
<td>113±3</td>
<td>119±3</td>
</tr>
<tr>
<td>pIC₅₀ (−log M)</td>
<td>7.27±0.11</td>
<td>7.35±0.12</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. All rings were treated with indomethacin (10⁻⁵ M). In the experiments with serotonin, rings were treated with ketanserin (10⁻⁶ M) to inhibit the direct effects on vascular smooth muscle.

Intact, control artery; denuded, previously denuded artery; max relaxation, maximal relaxation in percentage of the response to prostaglandin F₂α (2×10⁻⁶ M); pIC₅₀, effective concentration causing 50% of the maximal relaxation; NA, not available, pIC₅₀ could not be calculated because no relaxation was achieved in one pig.

*p<0.05 vs. intact artery in the control group; †p<0.05 vs. previously denuded artery in the control group; ‡p<0.05 vs. intact artery in the cholesterol-fed group.
which were not affected by pertussis toxin (Figure 7). Although insignificant, these relaxations tended to be reduced in previously denuded arteries of the cholesterol-fed group (Figure 7, Table 3).

Discussion

The present study demonstrates that in porcine coronary arteries, the pertussis toxin–sensitive (G protein–dependent) endothelium-dependent relaxations are reduced prominently either after endothelial regeneration or hypercholesterolemia and are impaired further in atherosclerosis.

G Proteins and the Normal Endothelium

Pertussis toxin is an irreversible blocker of several G proteins, which modulate a variety of intracellular events.15–18 The present study confirmed previous findings with pertussis toxin in porcine coronary arteries.7,8 Thus, in coronary arteries with normal endothelium, pertussis toxin exerted a major inhibitory effect on endothelium-dependent relaxations to serotonin and UK14304, suggesting that endothelial 5-HT1–serotonergic and α2-adrenergic receptors may be closely coupled to effector systems by means of a pertussis toxin–sensitive G protein.

Pertussis toxin is capable of inhibiting the activity of several G proteins (Gp, Gi, and Gq).15–18 Gp couples receptors in an excitatory fashion to phospholipase C; Gi couples receptors in an inhibitory fashion to adenylyl cyclase; and the functional role of Gq is unclear. Bradykinin and ADP activate the endothelium by stimulating phospholipase C.19,20 The lack of inhibition of pertussis toxin against responses to those agonists suggests that, as occurs in other cell types,21 pertussis toxin does not inactivate the endothelial Gq protein. Similarly, an action of the toxin on Gq protein is unlikely because Gq protein does not appear to be present in endothelial cells.22 Activation of α2-adrenergic receptors on endothelial cells has been shown previously to reduce cAMP levels.23 Thus, the present results are consistent with this observation, suggesting that 5-HT–serotonergic and α2-adrenergic receptors are closely coupled to the effector systems by pertussis toxin–sensitive Gq protein. The endothelium-dependent relaxations to thrombin were partially sensitive to the toxin; this may reflect a dual action of thrombin to inhibit
adenylate cyclase and to activate phospholipase C as
has been reported with thrombin for other cell types
(e.g., platelets).21,24 In contrast, the endothelium-
dependent relaxations to ADP, bradykinin, or
A23187 were resistant to pertussis toxin, which con-
firms earlier observations.7,8 The toxin does not in-
hbit the relaxations to nitric oxide,7 which relaxes
the vascular smooth muscle by activating soluble
guanylate cyclase and which may be an endothelium-
derived relaxing factor.14,25,26 Thus, pertussis toxin
selectively inhibits the release of endothelium-de-
ferred relaxing factor to certain agonists (serotonin,
α2-adrenergic agonist, and thrombin), without af-
fected the ability of the vascular smooth muscle to
relax or the biological activity of endothelium-de-
ferred relaxing factor.

Endothelial G Protein in Atherosclerosis

In the present study, atherosclerotic lesions were
induced by the combination of endothelial denuda-
tion and a high-cholesterol diet.5,6,9-11 The study of
coronary arteries that underwent only the denuda-
tion procedure allows the definition of the effects of
the endothelial regeneration per se. The comparison
of intact coronary arteries from the control group
with those from the high-cholesterol group permits
the definition of the changes due to hypercholester-
olemia alone. The observations that the pertussis
toxin–sensitive, endothelium-dependent relaxations
were impaired most prominently in the chronic re-
genrated state, which confirms a previous study,8
and in the hypercholesterolemic state support the
concept that a dysfunction of the endothelial G

FIGURE 6. Cumulative concentration–response curves to
bradykinin in rings with endothelium during contractions to
prostaglandin F2α (2×10⁻⁶ M) in the presence of indometh-
acin (10⁻⁵ M). Relaxations are expressed as percent de-
crease in tension. Data shown as mean±SEM.

FIGURE 7. Cumulative concentration–response curves to
the calcium ionophore A23187 in rings with endothelium
during contractions to prostaglandin F2α (2×10⁻⁶ M) in the
presence of indomethacin (10⁻⁵ M). Relaxations are ex-
pressed as percent decrease in tension. Data shown as
mean±SEM.
protein underlies the impaired responses of these two states to 5-HT, serotonin and α-adrenergic activation. Intimal thickening probably does not affect the diffusion of pertussis toxin to the endothelium because the toxin directly reaches the endothelium from the luminal and cutting surfaces of the ring preparations and because the inhibitions by the toxin of the endothelium-dependent relaxations to serotonin and UK14304 were comparable in the four different arteries (except responses to serotonin in atherosclerotic arteries).

Quantitatively, the effects of endothelial regeneration were greater than those of hypercholesterolemia in terms of impairment of endothelium-dependent relaxations and of endothelial G protein function. However, under both conditions, pertussis toxin still inhibited some responses, suggesting that endothelial G protein function was partially retained. In contrast, in atherosclerotic arteries (combination of regenerating cells and high-cholesterol diet), the endothelium-dependent relaxations were impaired more than in either regenerated or hypercholesterolemic preparations, which confirms earlier observations.\(^5\),\(^6\) Moreover, in atherosclerotic arteries, the inhibitory effect of pertussis toxin on endothelium-dependent relaxation was practically absent, suggesting that endothelial G protein function is severely impaired by the atherosclerotic process. It is reasonable to assume that the extreme reduction of endothelial G protein function in atherosclerosis reflects the combined effects of endothelial regeneration and hypercholesterolemia and that the former may be more important than the latter in the present pig model.

**Other Pathogenetic Factors in Atherosclerosis**

The endothelium-dependent relaxations to ADP (which are relatively insensitive to pertussis toxin) were impaired in blood vessels with regenerating, hypercholesterolemic, and atherosclerotic endothelium. Those to bradykinin (which are resistant to the toxin) were impaired only in atherosclerotic arteries. Thus, mechanism(s) other than the dysfunction of endothelial pertussis toxin–sensitive G protein must be involved in the impaired endothelium-dependent relaxations under those conditions and in particular in atherosclerotic arteries. Because an impaired production or release of endothelium-derived relaxing factor is the main factor responsible for the impaired relaxations,\(^1\)–\(^6\) other receptor-coupled systems may be dysfunctional. The endothelium-dependent relaxations to A23187 in atherosclerotic arteries were slightly, but significantly, reduced in a previous series,\(^5\) and the tendency was similar in the present study. Thus, the final pathways (beyond receptor coupling) of production or release of endothelium-derived relaxing factor may be impaired also in some preparations with atherosclerosis. Because the half-life of endothelium-derived relaxing factor is short,\(^27\)\(^,\)\(^28\) a thickened intima may reduce its diffusion. However, endothelium-dependent relaxations to ADP were impaired in hypercholesterolemic coronary arteries without intimal thickening. Likewise, in the monkey, dietary treatment of atherosclerosis restores endothelium-dependent relaxations to acetylcholine and thrombin even in the presence of intimal thickening.\(^29\) The presence of an atheromatous plaque may have other consequences, such as the accumulation of white blood cells with production of oxygen-derived free radicals and deposition of lipids, which also may affect the biological activity of the endothelial factor.\(^29\)

The release of endothelium-derived contracting factor(s) should be considered also.\(^5\),\(^4\)\(^,\)\(^6\)\(^,\)\(^12\) However, because the endothelium-dependent contractures are attenuated by inhibitors or cyclooxygenase\(^5\) and because the relaxations were examined in the presence of indomethacin in the present study, this mechanism may not play a major role.

The present study confirms that the responses of vascular smooth muscle and, in particular, its sensitivity to sodium nitroprusside\(^30\) and nitric oxide\(^14\),\(^25\),\(^26\),\(^30\) (both of which induce relaxations through activation of soluble guanylate cyclase) do not endothelium-derived relaxing factor.\(^31\)–\(^33\) are unaltered by endothelial denudation, hypercholesterolemia, or atherosclerosis. However, it remains to be examined whether the responses of vascular smooth muscle to endothelium-derived relaxing factor per se are impaired in atherosclerosis.\(^6\)

**Pathophysiological Implications**

The present study demonstrates that endothelial pertussis toxin–sensitive G protein (most likely Gi protein) is closely linked to the inhibitory effects of the endothelium in response to several vasoactive substances (serotonin, α-adrenergic receptor agonist, and thrombin) and that its function is easily and prominently impaired during the atherosclerotic process. Thus, the present finding may help to explain why the vasoconstrictor responses to serotonin are augmented under atherogenic conditions and in atherosclerosis.\(^5\),\(^6\),\(^10\),\(^11\),\(^34\)–\(^36\) Endothelial G protein dysfunction may play an important role in the pathogenesis of coronary vasospasm.

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KEY WORDS: endothelium-derived relaxing factor, thrombin, pertussis toxin, regenerated endothelium, atherosclerosis, hypercholesterolemia, serotonin, α₁-adrenergic receptors, ADP, bradykinin, A23187, G proteins.
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