Involvement of 5-HT$_2$ Receptors in Chronic Endothelial Dysfunction After Balloon Injury of Porcine Coronary Arteries

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Background Endothelium-dependent, pertussis toxin-sensitive relaxation to 5-hydroxytryptamine (5-HT) is impaired selectively after balloon injury of porcine coronary artery, followed by regeneration of the endothelial cells. The present study was designed to test the hypothesis that 5-HT, released from aggregating platelets, affects the progression of the endothelial dysfunction.

Methods and Results Yorkshire pigs were assigned randomly to three groups: control group (standard diet), denudation group (high-cholesterol diet plus balloon denudation of the endothelium of the left anterior descending coronary artery under fluoroscopy), and DV-7028–treated group (denudation group plus chronic treatment with the selective 5-HT$_2$ receptor antagonist DV-7028, given from the first day on after balloon denudation). Four weeks after the denudation, quantitative angiography revealed that 5-HT injected into the coronary artery decreased the luminal diameter of the left anterior descending coronary artery at the denuded site in the denudation group but not in the control or the DV-7028–treated group. Then, animals were killed so we could study the endothelium-dependent responses of their coronary arteries in conventional organ chambers. The arteries from the denudation group exhibited less relaxation to 5-HT and sodium fluoride (a stimulant of G proteins) than those of the control group. Relaxations to 5-HT and sodium fluoride were greater in arteries from the DV-7028–treated group than in those from the denudation group. In contrast, the endothelium-dependent, pertussis toxin–insensitive relaxations to bradykinin and thrombin and the endothelium-independent relaxations to sodium nitroprusside and isoproterenol were not affected significantly by chronic treatment with DV-7028.

Conclusions These results suggest that 5-HT$_2$ receptors are involved in the chronic progression of endothelial dysfunction after balloon denudation in the porcine coronary artery. (Circulation. 1994;89:1776–1785.)

Key Words • endothelium • 5-hydroxytryptamine • G proteins

5-Hydroxytryptamine (5-HT) is a major contracting substance released from aggregating platelets that activates 5-HT$_2$ receptors of the vascular smooth muscle of porcine coronary arteries. This monoamine also induces potent endothelium-dependent relaxations via activation of 5-HT$_1D$ receptors, which are coupled to pertussis toxin–sensitive G proteins and mediate the release of nitric oxide. Earlier studies have demonstrated that regenerated endothelial cells, after balloon denudation of the porcine coronary artery, selectively lose the G protein–coupled responses. This is associated with an impairment of the protective role of the endothelium against the action of vasoconstrictor products released from platelets. This impairment may explain why 5-HT causes coronary spasm at the sites of endothelial injury in the pig. 5-HT has a dilation effect on normal human coronary arteries but causes constriction in arteries of patients with coronary artery disease. Thus, dysfunction in endothelium-dependent responsiveness may contribute to the pathogenesis of several cardiovascular diseases. In addition, 5-HT, by activation of 5-HT$_2$ receptors, reduces the proliferation and migration of endothelial cells, and it also inhibits the induction by cytokines of nitric oxide synthase in smooth muscle cells. Stimulation of 5-HT$_2$ receptors on the platelets augments platelet aggregation, which favors the formation of thrombi and the release of other vasoconstrictor substances and mitogens, including 5-HT itself. The intimal migration of smooth muscle cells is activated by several platelet factors, and this may impair the regeneration of endothelial cells. Taken in conjunction, these observations suggest that 5-HT, released from aggregating platelets, is involved in the progression of chronic endothelial dysfunction. In the present study, the effects of a chronic treatment with DV-7028 were studied on endothelium-dependent responsiveness after balloon denudation of the porcine coronary artery.

Methods

Animals Eighteen Yorkshire pigs (body weight, 20 to 25 kg; University of Texas Health Science Center, Science Park, Bastrop) were assigned to one of three groups (each consisting of six animals) after an observation period of at least 1 week in individual quarters. The first group consisted of control animals given a standard diet (control group). The second group of animals was fed a high-cholesterol diet including 2% cholesterol and 19% lard (Purina Mills Test Diet Lab, Richmond, Ind) and underwent balloon denudation of the endothelium of the left anterior descending coronary artery (LAD).
Balloon denudation of endothelium (LAD)

2 weeks 4 weeks

standard or cholesterol (2%) plus lard (19%) diet

DV-7028 (10 mg/kg, p.o./day)

DV-7028

Balloon denudation of the Endothelium

The animals were anesthetized with Telazol (100 mg IM per animal; a mixture of tiletamine hydrochloride, arylaminoclo-alkanone, and zolazepam hydrochloride) and atropine (0.4 mg IM per animal) followed by inhalation of halothane (1.5 L/min). The left carotid artery was dissected free, a guide catheter (hockey stick, 7F) was inserted into the artery, and blood was collected for cholesterol measurement. The ECG (lead 3) and arterial blood pressure were monitored during the procedure. A guide catheter was introduced into the left coronary ostium under fluoroscopy. Then, a balloon dilation catheter (Medtronic Inc, Minneapolis, Minn; 2.5 or 3 mm according to the size of the coronary artery) was introduced into the LAD through the guide catheter, and the endothelium of 3 cm of the proximal portion of the LAD was denuded three times for 30 seconds. 5-HT (10 μg/kg, at a volume of 0.1 mL/kg) was infused for 1 minute into the coronary arteries, and changes in the ECG and the luminal diameter of the coronary arteries by cineangiography were recorded to confirm the successful denudation of the endothelium.

Quantitative Coronary Angiography

Angiography was performed at balloon denudation and at the end of the treatment. Cineangiography was undertaken before and 30 seconds after completion of injection of 5-HT (10 μg/kg) into the coronary arteries. Iohexol (10 mL) was injected into the coronary arteries, and exposures were made at 85 to 95 kV and 300 mA for 3 seconds. The average changes in luminal diameter of the coronary arteries at the proximal 3 cm of the LAD were analyzed in a blinded manner using a digital radiography system (ADAC Laboratories, Milpitos, Calif).

Organ Chamber Experiments

The LAD was removed from the heart of the animals, and 3 cm of the proximal denuded portion was used for organ chamber studies. The arteries were rinsed in modified Krebs-Ringer bicarbonate solution containing (in mmol/L): NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 25, glucose 11.1, and Ca-EDTA 0.026 (control solution); cleaned of connective tissue; and cut into rings (3 to 4 mm in length). In some rings, the endothelium was removed by inserting the tip of a small forceps into the lumen and rolling the preparation back and forth on a paper towel wet with cold control solution. The rings were suspended by two stainless-steel stirrups in organ chambers filled with control solution, aerated with 95% O2-5% CO2 (pH 7.4), and maintained at 37°C. One of the stirrups was anchored to the bottom of the chamber, and the other was connected to a transducer (Statham UC2, Los Angeles, Calif) to measure isotropic force. The rings were stretched to the optimal point of their active length-tension curve (6 to 8 g; based on preliminary studies measuring contractions to 40 mmol/L KCl).

Protocol

After 1 hour of equilibration, the rings were contracted with prostaglandin F2α (2×10−4 mol/L), and the responses to brady-
kinin (10^{-6} to 3 \times 10^{-4} \text{ mol/L}) were examined to confirm the absence or presence of endothelium. The rings were assigned to three experimental sets. Set 1 (endothelium-dependent relaxations) consisted of relaxations to 5-HT (10^{-9} to 10^{-5} \text{ mol/L}), in the presence of 10^{-5} \text{ mol/L} DV-7028), thrombin (10^{-10} to 3 \times 10^{-3} \text{ U/mL}) and sodium fluoride (10^{-2} to 10^{-2} \text{ mol/L}, in the presence of 10^{-5} \text{ mol/L} AlCl3). Set 2 (endothelium-independent relaxations) consisted of relaxations to sodium nitroprusside (10^{-5} to 3 \times 10^{-3} \text{ mol/L}) and isoproterenol (10^{-9} to 3 \times 10^{-2} \text{ mol/L}). Set 3 (contractions) consisted of contractions to 60 mmol/L KCl reference contractions), 5-HT (10^{-9} to 10^{-5} \text{ mol/L}), and histamine (10^{-5} to 3 \times 10^{-5} \text{ mol/L}). Set 1 consisted of four rings obtained from each animal: ring 1 was the control, ring 2 was in the presence of 3 \times 10^{-5} \text{ mol/L} nitro-L-arginine (an inhibitor of nitric oxide synthase21), ring 3 was in the presence of 100 ng/mL pertussis toxin,\textsuperscript{45} and ring 4 was without endothelium. Set 2 consisted of one ring without endothelium. Set 3 consisted of three rings: ring 1 was control, ring 2 was in the presence of 3 \times 10^{-5} \text{ mol/L} nitro-L-arginine, and ring 3 was without endothelium. In sets 1 and 2, the rings were contracted with 2 \times 10^{-6} \text{ mol/L} prostaglandin F\textsubscript{2a}, and the effects of cumulatively increasing concentrations of the agonists were tested in the presence of indomethacin (10^{-5} \text{ mol/L}; to prevent the formation of vaso-active prostanoids). In set 3, quiescent rings were contracted with cumulatively increasing concentrations of the agonists.

**Morphology**

The coronary rings used to determine endothelium-dependent relaxations in organ chamber studies (set 1) were prepared for morphological analysis at the end of the experiments. The rings with endothelium were fixed in 10\% formaldehyde in phosphate buffer, and paraffin sections were examined after staining with hematoxylin and eosin. Nine paraffin sections were examined per coronary artery; three paraffin sections were obtained from each of three rings that were cut from the coronary artery.

**Calculations and Statistical Analysis**

Results are shown as mean±SEM. Unless otherwise specified, n refers to the number of animals. Relaxations are expressed as a percentage of the initial contractions to prostaglandin F\textsubscript{2a} (2 \times 10^{-6} \text{ mol/L}). The IC\textsubscript{50} values, defined as the negative logarithm of the concentration of agonists inducing 50\% inhibition of the contractions to prostaglandin F\textsubscript{2a}, were calculated for each concentration-response curve. Contractions are expressed as the percentage of the reference contraction to 60 mmol/L KCl. The EC\textsubscript{50} values, defined as the negative logarithm of the concentration of 5-HT or histamine causing 50\% of the maximal contractions to the amines, were calculated for each individual concentration-response curve. Statistical comparisons were performed by Student’s t test for paired observations and an ANOVA followed by Scheffe’s test when more than two groups were compared. Values of P<.05 were considered to indicate statistically significant differences between groups.

**Materials**

DV-7028 was obtained from Daiichi Pharmaceuticals (Tokyo, Japan); bradykinin acetate salt, cholesterol diagnostic kit, Formalin diagnostic solution, l-isoproterenol bitartrate, histamine hydrochloride, 5-HT–createine sulfate complex, indomethacin, pertussis toxin, and sodium nitroprusside were obtained from Sigma Chemical Co (St Louis, Mo); aluminum chloride, nitro-L-arginine, and sodium fluoride were obtained from Aldrich Chemical Co (Milwaukee, Wis); iohexol was obtained from Winthrop Pharmaceuticals (New York, NY); Telazol was obtained from A.H. Robins (Richmond, Va); and halothane was obtained from Ayerst Laboratories Inc (New York, NY).

**Results**

The body weight of animals increased significantly in the three groups. The final body weight averaged 28±1, 31±1, and 32±2 kg (n=6) in the control, denudation (balloon denudation plus high-cholesterol diet), and DV-7028-treated (balloon denudation, high-cholesterol diet plus DV-7028) groups, respectively. There was no significant difference in blood pressure between the denudation and DV-7028–treated groups. At the time of ballooning, systolic and diastolic blood pressures of the denudation and DV-7028–treated groups were 119±4/80±7 and 122±10/82±8 mm Hg (n=6), respectively. At the end of treatment, the blood pressures of control, denudation, and DV-7028 groups were 133±7/88±8, 132±3/90±3, and 137±5/91±7 mm Hg (n=6), respectively.

**Serum Cholesterol Level**

The serum cholesterol levels of the animals in the denudation and DV-7028–treated groups were fourfold to fivefold higher than those in the control group. At the time of ballooning, the cholesterol levels of the denudation and DV-7028–treated groups were 325±37 and 326±46 mg/dL (n=6), respectively. At the end of treatment, the cholesterol levels of control, denudation, and DV-7028 groups were 86±6, 428±61, and 449±64 mg/dL (n=6), respectively. There was no significant difference in serum cholesterol level between the denudation and DV-7028–treated groups.

**Quantitative Coronary Angiography**

Immediately after ballooning, 5-HT (10 \mu g/kg, intra-coronary) significantly decreased the luminal diameter of the LAD, at the site of endothelial denudation, to 81±3\% and 83±5\% of the initial value (before injection of 5-HT) in the denudation and DV-7028–treated groups, respectively. There was no significant difference between these two groups. Four weeks after ballooning, 5-HT decreased the luminal LAD diameters to 87±4\% of the initial value in the denudation group. However, 5-HT did not significantly change the diameters in the control (111±5\% of initial value) and DV-7028–treated (94±6\% of initial value) groups.

**Organ Chamber Studies**

There were no significant differences among the three groups regarding the contraction to prostaglandin F\textsubscript{2a} (2 \times 10^{-6} \text{ mol/L}) of the coronary rings, with and without endothelium, used in sets 1 and 2 (Table 1). The relaxations of coronary arteries to vasoactive substances are shown in Table 2.

**Set 1: Endothelium-Dependent Relaxations**

Bradykinin (10^{-6} to 3 \times 10^{-4} \text{ mol/L}) caused concentration-dependent, endothelium-dependent relaxations that were not inhibited by 100 ng/mL pertussis toxin (Fig 2). In the control solution, there was no difference in the responses to bradykinin among the three groups (Fig 2). In the presence of 3 \times 10^{-5} \text{ mol/L} nitro-L-arginine, the relaxations to bradykinin were inhibited partially, and the inhibition was significantly more pronounced in the rings of the denudation and DV-7028–treated groups than in the rings of the control group (Fig 2). 5-HT (10^{-4} to 10^{-5} \text{ mol/L}) caused concentration-dependent, endothelium-
1. TABLE

Table 1. Responses of Porcine Coronary Artery Rings to Prostaglandin F$_{2g}$ and KCl

<table>
<thead>
<tr>
<th>Endothelium</th>
<th>KCI, 60 mmol/L (with)</th>
<th>Prostaglandin F$_{2g}$, 2x10$^{-4}$ mol/L</th>
<th>Developed Tension, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td>9.6±1.4 (17)</td>
</tr>
<tr>
<td>Denudation group (balloon denudation plus high-cholesterol diet)</td>
<td></td>
<td></td>
<td>10.6±1.1 (18)</td>
</tr>
<tr>
<td>DV-7028–treated group (balloon denudation, high-cholesterol diet, DV-7028)</td>
<td></td>
<td></td>
<td>9.0±0.8 (18)</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. Numbers in parentheses are the number of rings tested in each group.

2. TABLE

Table 2. Relaxations of Porcine Coronary Arteries

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Control Group</th>
<th>Denudation Group</th>
<th>DV-7028–Treated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$, -log mol/L</td>
<td>Maximal Relaxation, %</td>
<td>IC$_{50}$, -log mol/L</td>
</tr>
<tr>
<td>Bradykinin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control solution</td>
<td>9.27±0.12</td>
<td>107±4</td>
<td>8.88±0.22</td>
</tr>
<tr>
<td>Nitro-L-arginine</td>
<td>8.45±0.09†</td>
<td>98±5</td>
<td>ND</td>
</tr>
<tr>
<td>Pertussis toxin</td>
<td>9.06±0.13</td>
<td>108±3</td>
<td>8.80±0.22</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>ND</td>
<td>15±5†</td>
<td>ND</td>
</tr>
<tr>
<td>5-Hydroxytryptamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control solution</td>
<td>7.34±0.14</td>
<td>79±3</td>
<td>ND</td>
</tr>
<tr>
<td>Nitro-L-arginine</td>
<td>ND</td>
<td>2±1†</td>
<td>ND</td>
</tr>
<tr>
<td>Pertussis toxin</td>
<td>ND</td>
<td>27±13†</td>
<td>ND</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>ND</td>
<td>5±2†</td>
<td>ND</td>
</tr>
<tr>
<td>Thrombin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control solution</td>
<td>2.09±0.09†</td>
<td>103±5</td>
<td>1.78±0.13§</td>
</tr>
<tr>
<td>Nitro-L-arginine</td>
<td>ND</td>
<td>38±24†</td>
<td>ND</td>
</tr>
<tr>
<td>Pertussis toxin</td>
<td>1.81±0.12</td>
<td>99±6</td>
<td>ND</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>ND</td>
<td>2±1†</td>
<td>ND</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control solution</td>
<td>2.43±0.10</td>
<td>70±12</td>
<td>ND</td>
</tr>
<tr>
<td>Nitro-L-arginine</td>
<td>ND</td>
<td>-39±5†</td>
<td>ND</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>ND</td>
<td>-82±16†</td>
<td>ND</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control solution</td>
<td>7.86±0.11</td>
<td>114±4</td>
<td>7.81±0.14</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>7.95±0.09</td>
<td>110±6</td>
<td>7.57±0.10</td>
</tr>
</tbody>
</table>

IC$_{50}$ indicates effective concentration causing 50% inhibition of the contractions to prostaglandin F$_{2a}$ (2x10$^{-6}$ mol/L); maximal relaxation, maximal relaxation in percentage of the contraction evoked by prostaglandin F$_{2a}$ (2x10$^{-6}$ mol/L). Data are expressed as mean±SEM. Number of experiments is 5 or 6.
of control, denudation, and DV-7028 groups were 104±17%, 12±12%, and 60±16%, respectively; n=5 or 6. Pertussis toxin (100 ng/mL) inhibited the relaxations to sodium fluoride in the three groups (data not shown, n=3 or 4).

Set 2: Endothelium-Independent Relaxations

The relaxations to sodium nitroprusside (10⁻⁹ to 3×10⁻⁷ mol/L) or isoproterenol (10⁻⁹ to 3×10⁻⁷ mol/L) were not different among the three groups (Fig 6).
Fig 4. Plots of relaxations to thrombin in porcine coronary rings from three groups: control, balloon denudation plus high-cholesterol diet, and balloon denudation plus high-cholesterol diet plus DV-7028. The rings with endothelium, excised 4 weeks after balloon denudation, were incubated in the control solution (control), in the presence of nitro-L-arginine (NLA: 3x10^{-5} mol/L), or in the presence of pertussis toxin (100 ng/mL). The rings without endothelium were incubated in the control solution. Then, the rings were contracted with prostaglandin (PG) F2α (2x10^{-6} mol/L), and the effects of increasing concentrations of thrombin were tested in the presence of indomethacin (10^{-5} mol/L). The data are shown as mean±SEM and expressed as percent relaxation of the contractions to PGF2α (2x10^{-6} mol/L).

Set 3: Contractions
There were no significant differences among the three groups in contractions to KCl (60 mmol/L) in coronary rings with endothelium in the control solution (Table 1). Contractions of the rings to 5-HT and histamine are shown in Table 3. The contractions evoked by 5-HT...
(10⁻⁹ to 10⁻⁵ mol/L) were augmented in the presence of nitro-L-arginine (3 x 10⁻⁵ mol/L) and in rings without endothelium compared with those in the control solution in the control and DV-7028-treated groups (Fig 7). In the denudation group, there was no difference in maximal contraction to 5-HT between rings with endothelium incubated in the control solution or with nitro-L-arginine and those without endothelium (Fig 7). Histamine (10⁻⁸ to 3 x 10⁻⁵ mol/L) induced contractions that were enhanced in rings without endothelium in the control and DV-7028 groups but not in the denudation group (Table 3).

**Morphology**

By light microscopy no intimal thickening of vessel walls was noted in all of three paraffin sections in each of three rings from the six coronary arteries of the control group (Fig 8). By contrast, apparent intimal thickening was observed at least in one or two paraffin sections in every ring of the six coronary arteries in the denudation group.

**Table 3. Contractions of Porcine Coronary Arteries**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Control Group</th>
<th>Denudation Group</th>
<th>DV-7028-Treated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC₅₀, -log mol/L</td>
<td>Maximal Contraction, %</td>
<td>EC₅₀, -log mol/L</td>
</tr>
<tr>
<td>5-Hydroxytryptamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control solution</td>
<td>6.44±0.14</td>
<td>65±10</td>
<td>6.39±0.13</td>
</tr>
<tr>
<td>Nitro-L-arginine</td>
<td>6.74±0.11</td>
<td>92±11†</td>
<td>6.71±0.07</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>6.72±0.07</td>
<td>92±5†</td>
<td>6.73±0.10</td>
</tr>
<tr>
<td>Histamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control solution</td>
<td>6.13±0.12</td>
<td>94±12</td>
<td>5.73±0.09</td>
</tr>
<tr>
<td>Nitro-L-arginine</td>
<td>6.21±0.10</td>
<td>111±9</td>
<td>6.00±0.08</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>6.10±0.16</td>
<td>149±6†</td>
<td>6.07±0.16</td>
</tr>
</tbody>
</table>

EC₅₀ indicates effective concentration inducing 50% of the maximal contractions to 5-hydroxytryptamine or histamine; maximal contraction, maximal contraction in percentage to 60 mmol/L KCl; nitro-L-arginine, in the presence of nitro-L-arginine (3 x 10⁻⁵ mol/L); and without endothelium, endothelium of the coronary rings was mechanically removed. Data are expressed as mean±SEM. Number of experiments is 5 or 6.

*P<.05 compared with control group.
†P<.05 compared with denudation group.
‡P<.05 compared with control solution in the same group.
The lumen of the artery was narrowed by an intimal plaque at the site of balloononing (Fig 8). In the DV-7028-treated group, intimal thickening was observed at least in one or two paraffin sections of every ring.

**Discussion**

The aim of the present study was to determine whether 5-HT<sub>2</sub>-serotonin receptors contribute to the progression of endothelial dysfunction after balloon denudation of the porcine coronary endothelium. In the acute phase, during or immediately after the coronary denudation, 5-HT released from aggregating platelets stimulates the formation of platelet thrombi, which can cause coronary occlusion at the site of endothelial injury. Furthermore, this may lead to chronic progression of endothelial dysfunction. To avoid an interaction with the acute effects of 5-HT on the coronary arteries, we gave DV-7028 to the pigs starting 1 day after balloon denudation. DV-7028 is a highly selective 5-HT<sub>2</sub> receptor antagonist with low affinities for 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1C</sub>, 5-HT<sub>1D</sub>, serotonin, α<sub>1</sub>-adrenergic, or D<sub>2</sub> dopamine receptors. The selection of the oral dose of DV-7028 was based on the inhibitory effects of the compound on pressor responses mediated by 5-HT<sub>2</sub> receptors. DV-7028 (10<sup>−8</sup> to 10<sup>−6</sup> mol/L) also inhibits the contraction to 5-HT in the isolated porcine coronary artery, which is mediated by 5-HT<sub>2</sub> receptors, in a competitive manner with a p<sub>A2</sub> value of 8.0 (unpublished observation). In addition, in a preliminary study, chronic treatment with DV-7028 (10 mg·kg<sup>−1</sup>·d<sup>−1</sup> PO) did not alter arterial blood pressure in the pig. Systolic and diastolic blood pressures of before and 1 and 4 weeks after starting administration of the compound were 124±3/83±3, 127±5/88±4, and 129±5/86±5 mm Hg (n=3), respectively. The animals were fed a high-cholesterol diet to enhance the impairment of the responsiveness of the regenerated endothelial cells. It is less likely that the improvement by DV-7028 of the endothelium-dependent responsiveness was due to the attenuation of hypercholesteremia, because there was no significant difference in serum cholesterol level between the denudation and DV-7028-treated groups at the end of treatments. However, the present study does not provide the actual serum cholesterol level during treatment with DV-7028.

After balloon denudation, the response of coronary arteries to 5-HT changes from dilatation to constriction. In the present study, the quantitative coronary angiography demonstrated that 5-HT decreased the luminal coronary diameter in the denudation and DV-7028-treated groups, which indicates successful denudation of the endothelium in the LAD. Even 4 weeks after the balloononing, no dilatation of the coronary arteries to 5-HT was observed, suggesting that the regenerated endothelial cells do not respond to 5-HT. These results are consistent with previous observations.

The number of endothelial cells is larger in previously denuded arteries 4 weeks after abrasion than in intact coronaries. In the present study, the regeneration of functional endothelial cells was confirmed by the fact that bradykinin induced endothelium-dependent relaxations in the previously denuded coronary arteries. The relaxations evoked by bradykinin are insensitive to pertussis toxin and the chronic treatment with DV-
These in vitro results with 5-HT are consistent with the observations obtained with quantitative coronary angiography in vivo. The relaxations to sodium fluoride, a direct stimulant of G proteins,24 were also improved by chronic treatment with DV-7028. Sodium fluoride causes endothelium-dependent, pertussis toxin-sensitive relaxations, which may be attributed to the release of nitric oxide.25-27 By contrast, the chronic treatment with DV-7028 did not affect the relaxations to thrombin, which are less sensitive to pertussis toxin,3,4 and the endothelium-independent relaxations to sodium nitroprusside and isoprotrenol. The enhancement of the contractions to 5-HT by nitro-L-arginine and the mechanical removal of the endothelium suggest that spontaneous release of nitric oxide by the coronary endothelium inhibits the contractions to the monoamine. The endothelium-dependent inhibition of the contractions to 5-HT, which was not observed in the rings previously denuded by ballooning, was preserved in the rings from animals treated with DV-7028. This preservation is explained best by the improved endothelium-dependent dilatation in response to the monoamine1,2,4 after treatment with DV-7028. Alternatively, DV-7028 remaining in the tissues after discontinuation of the treatment might affect the endothelium-dependent relaxations to 5-HT if it were to block 5-HT2 receptors in vascular smooth muscle. However, this possibility is ruled out because the relaxations to 5-HT were performed in the presence of 10−6 mol/L DV-7028 in the organ chambers to prevent the activation of 5-HT2 receptors. Furthermore, there was no difference between the denudation and DV-7028–treated groups in the contractions to 5-HT (in the absence of DV-7028) in the coronary rings without endothelium, indicating that DV-7028 no longer worked at 5-HT2 receptors at the moment of death. Thus, the present study suggests that chronic treatment with DV-7028 preserves the G protein–coupled endothelium-dependent responsiveness, which is impaired in regenerated endothelial cells.

The morphological experiments indicated that intimal thickening occurred in the coronary arteries only after balloon denudation in the presence of a high-cholesterol diet. Migration and proliferation of smooth muscle cells may be stimulated by 5-HT; however, it may suppress these activities in endothelial cells.10,13,14,28 This results in impaired regeneration of the coronary endothelium. However, the present studies do not permit a quantitative analysis of the effect of DV-7028 on intimal thickening of the coronary arteries since the coronary rings were used first for the measurement of isometric tension. Furthermore, the level of 5-HT released chronically from platelets after balloon denudation has not been determined accurately. Activated platelets may accumulate at the sites of endothelial injury and could release several substances that affect local vascular tone, platelet aggregation, and growth of vascular cells.29 Stimulation of the vascular smooth muscle cells by the platelet factors may be prolonged by their association with components of the vascular wall. Indeed, the effect of 5-HT on the migration of cultured endothelial cells is sustained for at least 6 days, and it also causes cellular hypertrophy.10 Regenerated endothelial cells are elongated and irregularly oriented 4 weeks after balloon denudation of porcine coronary arteries.1 This may be an indication that platelet factors

Fig 8. Light micrograph of an immersion fixed cross section of porcine left anterior descending coronary artery. The preparation was fixed after using it for an organ chamber study. A, Control group; B, denudation group (balloon denudation plus high-cholesterol diet); C, DV-7028–treated group (balloon denudation plus high-cholesterol diet plus chronic treatment with DV-7028). The lumen of arteries is narrowed by an intimal plaque at the site of ballooning in denudation and DV-7028–treated groups. Original magnification, ×83.
modulate extracellular matrix production by endothelial cells. Because circulating platelets are the most likely source of 5-HT at the site of endothelial injury, similar improvement of endothelium-dependent responsiveness may be achieved with other antiplatelet agents that inhibit the release of the monoamine.

In conclusion, the present study suggests that 5-HT$_2$ receptors are involved in the progression of the endothelial dysfunction, related to G protein–coupled responses, after balloon denudation of the porcine coronary artery. The impairment in endothelium-dependent responses may account for the disturbance of atherosclerotic coronary arteries after bypass graft, angioplasty, and thrombolysis. Chronic treatment with the 5-HT$_2$ receptor antagonist DV-7028 may prevent, at least in part, the dysfunction of the regenerated endothelial cells.

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