Histamine-induced coronary spasm in regions of intimal thickening in miniature pigs: roles of serum cholesterol and spontaneous or induced intimal thickening

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ABSTRACT The pathogenesis of histamine-induced coronary spasm was examined angiographically and morphometrically in Göttingen miniature pigs. In five of 36 consecutive pigs that were 4 to 5 months of age, coronary spasm was provoked by the intracoronary administration of histamine, and the left coronary arteries were examined histologically without endothelial denudation (group 1). Endothelial balloon denudation of the major branch of the left coronary artery was performed in 31 of 36 pigs and five died during the procedure. The remaining 26 pigs were randomly allotted to one of two groups, one fed a cholesterol-supplemented (group 2, n = 13) and one fed a regular low-cholesterol diet (group 3, n = 13). After 3 months, serum cholesterol increased significantly from 57 ± 6 to 222 ± 27 mg/dl (p < .01) in group 2, but remained unchanged (48 ± 5 to 55 ± 6 mg/dl) in group 3. Percent narrowing of the coronary diameter induced by 10 μg/kg iv histamine after administration of the H2 blocker cimetidine (60 mg/kg iv) was 39 ± 3% and 24 ± 2% (p < .05 between groups 2 and 3) at the nondenuded site and 78 ± 3% and 74 ± 4% at the denuded site in groups 2 and 3, respectively (p < .01 between nondenuded and denuded sites). Histamine-induced percent narrowing of the coronary diameter after cimetidine in group 1, 2, and 3 pigs correlated well with the degree of intimal thickness on an exponential curve (r = .92, p < .001). Since percent narrowing at the intact site was 27% (n = 19) in all three groups, predicted histamine-induced percent narrowing at the spastic site, applying the geometric theory, was 33 ± 3%. Accordingly, enhanced constriction of the coronary artery with intimal thickening in response to histamine can largely be explained by the acquired hyperresponsiveness of the vascular wall to autacoids. This phenomena, not related to the level of serum cholesterol, may be uniquely linked to the basic pathology of evolution of atherosclerosis.

_Circulation_ 74, No. 4, 826–837, 1986.

RECENTLY, enhanced responsiveness of the medium-sized arteries to vasoactive substances has been noted not only clinically in patients with variant angina, but also experimentally in vessels exposed to cholesterol or hypercholesterolemia, in atherosclerotic vessels, and in normal vessels after endothelial denudation. These lines of evidence strongly suggest the presence of heterogeneous vascular response to vasoactive stimuli. In a swine preparation fed a high-cholesterol diet after regional endothelial denudation, we noted augmented vasoconstriction in response to histamine on angiographic examination of the denuded coronary artery. We also noted ergonovine-induced hyperconstriction in canine coronary arteries with thickened intima after denudation and a high-cholesterol feeding. MacAlpin proposed a model of a geometric effect of coronary atherosclerosis to explain localized and transient coronary obstructions. However, the primary features of localized hyperconstriction of the denuded coronary artery have not been elucidated in detail.

We carried out angiographic studies in vivo to inves-
tigate whether hypercholesterolemia or structural and/or functional alterations of the vessel wall would potentiate vascular responses to vasospastic stimuli. We used Göttingen miniature swine and randomly assigned them to a regular laboratory chow or a cholesterol-supplemented diet after regional endotheonialization of the coronary artery. It was evident that the regionally thickened intima, whether developed spontaneously or by endothelial denudation, was hyperresponsive to histamine, irrespective of the moderate increase in serum cholesterol level.

Methods

Thirty-six disease-free male Göttingen miniature pigs that were 4 to 6 months of age (5.0 ± 0.1 months) and weighed 13 to 21 kg (18.3 ± 0.3 kg) were housed individually under conditions of controlled room temperature and were fed low-cholesterol regular swine chow (Nihon Clea Inc., Tokyo, Japan) before the experiment. These pigs were anesthetized with intramuscular ketamine hydrochloride (12.5 mg/kg) followed by the intravenous infusion of 20 mg/kg sodium pentobarbital; they were then intubated and ventilated with room air and supplemental oxygen via a positive-pressure respirator (Shinano Inc., Tokyo, Japan). Under aseptic conditions, an incision was made to expose the carotid artery. A preshaped green Kifa catheter (Kifa, Stockholm, Sweden) was inserted into the orifice of the left coronary artery under fluoroscopic guidance. Heparin, 1000 units, was infused intravenously for anticoagulation. Aortic pressure was monitored with a Statham P23Db pressure transducer. Electrocardiograms were continuously monitored in limb leads (I, II, III, and aVF) and precordial leads (V1 and V6).

Animal preparation. The intracoronary administration of histamine induced coronary spasm before endothelial denudation in five of 36 consecutive pigs. These five pigs (group 1) were killed after the histamine provocation and their coronary vessels were examined histologically. Effectiveness of the denudation technique was confirmed histologically in five pigs that died immediately after the denudation. In the remaining 26 pigs, endothelial denudation was performed as described previously13,14 after the histamine study and they were randomly assigned to a diet of laboratory chow supplemented with 2% cholesterol (group 2, n = 13) or low-cholesterol regular chow (group 3, n = 13). The concentration of total serum cholesterol in the pigs was measured enzymatically before and 3 months after the denudation procedure.

Experimental protocol. Responses of the coronary artery to vasoactive substances were examined before and 3 months after endothelial denudation in pigs under anesthesia induced with intramuscular ketamine and intravenous pentobarbital. Diameter of the coronary artery was measured by selective coronary arteriography, as described previously.14 After a control recording of the coronary diameter, 20 μg/kg nitroglycerin was administered intravenously to evaluate the resting coronary tone.16 Thirty minutes after the nitroglycerin study, the following vasoactive agents were tested at random: 3 and 10 μg/kg ic histamine and 10 μg/kg ic histamine after pretreatment with 60 mg/kg iv cimetidine and 1 and 10 μg/kg ic phenylephrine. All drugs were diluted with physiologic salt solution and the volume was 1 ml. The same amount of saline was used to flush the Kifa catheter. During intracoronary administration of drugs, the catheter position was fixed, and equal amounts were injected into the left circumflex artery and the left anterior descending artery. Coronary arteriography was performed 1 and 2 min after the intracoronary administration or 2 min after the intravenous administration of vasoactive agents. We waited at least 30 min before administering another drug. The reproducibility of the histamine-induced vasospasm was confirmed in groups 1 (n = 3), 2 (n = 10), and 3 (n = 9) by repeating provocation with 10 μg/kg ic histamine after pretreatment with cimetidine at the end of the study.

Coronary arteriography and hemodynamic recordings. During the drug studies, arterial pressure and electrocardiograms were continuously monitored on a multichannel pen recorder (NEC-Sanei, Polygraph System) and stored on tape with the use of an FM data recorder (R-280 LT, TEAC) for subsequent analysis. Selective coronary arteriograms were obtained in a left anterior oblique projection by manually injecting 5 ml of contrast medium (Urograffin 76, Nihon Schering) through the Kifa catheter. Angiograms were obtained with a TOSHIBA 0.6 mm focal spot x-ray tube on 35 mm cinefilm (CFS 746, Kodak) at 48 frames/sec by a cinecamera (Arritechno, München, Federal Republic of Germany). The posture of the swine and the distance between the swine and image intensifier were kept constant during the experiment.

Data analysis. Cinefilm was projected on a viewing screen (Tagarno 35-CX, Tagarno, Horsens, Denmark). The end-diastolic frame was determined by electrocardiographic waves recorded on cinefilm. The diameter of the coronary artery was measured with a caliper to 0.05 mm, and the absolute value of the diameter (mm) was obtained with use of a Kifa catheter as a reference. Readily identifiable branch points were used as a reference marker of the measurements. To topologically correlate the degree of coronary vasoconstriction in vivo and the intimal thickening of the postmortem sample, coronary diameters were measured every 5 mm before and after provocations. To quantitatively compare the responses of the spastic and nonspastic coronary arteries to vasoconstrictive agents, two representative sites, such as the areas in which there was maximal diameter reduction of the spastic and nonspastic coronary arteries, were monitored throughout the study. The percent narrowing of the coronary diameter induced by vasoactive agents was calculated as follows:

\[
\text{Diameter before agonist (or after nitroglycerin) - Diameter after agonist} \times 100
\]

Diameter before agonist (or after nitroglycerin)

To determine interobserver or intraobserver variability, the readings were performed by uninformed observers or by providing copied films to two of three cardiologists conducting this study (K. E., Y. Y., H. T.). We confirmed excellent correlations between repeated measurements (r = .98, p < .001) and between different observers (r = .95, p < .001). Difference in percent narrowing determined by two observers was 0 ± 1.4% (NS) and 2.3 ± 1.5% (NS) at the intact and denuded sites, respectively.

Determination of loci of coronary spasm and fixation of the coronary artery under physiologic pressure. After the termination of the angiographic study, the pigs were given a lethal dose of pentobarbital and exsanguinated. Each heart, carefully isolated so as not to damage the coronary arteries, was connected to the constant-pressure perfusion system through a polyethylene cannula inserted into the left main coronary artery. The left coronary artery was perfused with physiologic salt solution at 90 mm Hg. Left coronary angiograms were obtained in vitro and the site of coronary spasm was identified by a marker pin. As shown in figure 1, the segments of coronary trees were numbered on arteriograms obtained in vivo and in vitro. Later, a barium-gelatin mixture was infused at a perfusion
pressure of 90 mm Hg and 20% formaldehyde solution was used for fixation.

**Histologic study.** The left circumflex and left anterior descending coronary arteries were dissected serially at 5 mm intervals and were numbered according to the numbering system used on the isolated heart angiograms. Tissue samples were processed through paraffin, sectioned at 5 to 7 μm, and stained with hematoxylin and eosin or with the Weigert-van Gieson procedure for differential staining of elastic fibers and cells. The maximum thickness of the intima and tunica media was measured with an eyepiece micrometer at 250 × magnification in 19 pigs in which a barium-gelatin mixture was successfully infused at 90 mm Hg.

**Statistical analysis.** All results are expressed as mean ± SE. The statistical significance of the difference between groups was evaluated by Student’s t test. Sequential changes in mean values between periods in the same group were tested by an analysis of variance. The fit of the curve between two variables was determined by a least square method. A probability of less than 5% was considered indicative of statistical significance.

**Results**

**Mortality.** Fourteen of 36 pigs died before completion of the entire protocol, five due to ventricular fibrillation during endothelial denudation; two in group 2 and two in group 3 due to ventricular fibrillation immediately after induction of anesthesia at the third month; and two in group 1, one in group 2, and two in group 3 due to ventricular fibrillation associated with the induction of coronary spasm.

**Serum cholesterol and growth during 3 months.** Body weight and serum cholesterol at the beginning and the end of this study are summarized in table 1. There were no significant differences in body weight and serum cholesterol at the beginning of this study in the three groups. Increases in body weight over 3 months did not differ in groups 2 and 3. Serum cholesterol levels increased significantly from 57 ± 6 to 222 ± 27 mg/dl (148 ~ 388 mg/dl) after 3 months in group 2 (p < .01), but were practically unchanged (48 ± 5 to 55 ± 6 mg/dl; 26 ~ 81 mg/dl) in group 3. Coronary artery diameter was almost the same in the three groups at the beginning of the study and it increased significantly in groups 2 and 3 (20% and 24%, respectively) after 3 months due to aging. Absolute coronary diameters of the left anterior descending and left circumflex arteries after 3 months did not differ significantly in groups 2 and 3 (table 1).

**Responses of the coronary artery to vasoconstrictive agents.** Changes in coronary diameter after the administration of vasoconstrictive agents are summarized in tables 2, 3, and 4. Representative angiographic findings at control, after histamine with cimetidine pretreatment (groups 2 and 3), and after histamine alone (group 1) and nitroglycerin are shown in figures 2, 3, and 4 for groups 1, 2, and 3, respectively.

![FIGURE 1. Coronary angiograms obtained in vivo (top) and in an isolated heart (bottom). Top, Coronary angiogram obtained during coronary spasm induced by an intracoronary injection of histamine. A white arrow indicates the site of focal coronary spasm at the left circumflex coronary artery (LCX). Bottom, Coronary angiogram from an isolated heart preparation. Dye is observed to the third branch of the coronary arterial tree. Numbers beside the left anterior descending coronary artery (LAD) and LCX were determined by comparing branch points on angiograms obtained in vivo and from an isolated heart.](http://circ.ahajournals.org/)

**Responses before endothelial denudation.** In five pigs in group 1, enhanced vasoconstriction in response to histamine was noted regionally at the proximal portion of the left anterior descending coronary artery in three pigs and at that of left circumflex artery in two. The degree of vasoconstriction in these arteries was significantly larger than that in the contralateral coronary artery, as shown in figure 2. Due to the presence of enhanced vasoconstriction before denudation, these five pigs were killed before denudation was performed so their coronary vessels could be examined histologically. A thickened intima was histologically evident along the spastic site in each case.

**Responses of the nonspastic coronary artery to agents such as histamine and phenylephrine were similar in pigs in the three groups, as shown in tables 2, 3, and 4. In groups 2 and 3, changes in coronary diameter**
**TABLE 1**

<table>
<thead>
<tr>
<th>Time of measurement</th>
<th>Body weight (kg)</th>
<th>Serum cholesterol level (mg/dl)</th>
<th>CoD (mm)</th>
<th>Heart rate (beats/min)</th>
<th>sAop (mm Hg)</th>
<th>dAop (mm Hg)</th>
<th>mAop (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LCX</td>
<td>LAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1 (spontaneous intimal thickening; n = 5)</td>
<td>0</td>
<td>18 ± 1</td>
<td>51 ± 8</td>
<td>2.1 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>129 ± 11</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>Group 2 (high-cholesterol diet; n = 11)</td>
<td>0</td>
<td>18 ± 2</td>
<td>57 ± 6</td>
<td>2.1 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>119 ± 10</td>
<td>106 ± 5</td>
</tr>
<tr>
<td></td>
<td>3 mo</td>
<td>28 ± 4</td>
<td>222 ± 27</td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.2</td>
<td>122 ± 10</td>
<td>111 ± 7</td>
</tr>
<tr>
<td>Group 3 (regular diet; n = 11)</td>
<td>0</td>
<td>19 ± 1</td>
<td>48 ± 5</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>123 ± 12</td>
<td>103 ± 7</td>
</tr>
<tr>
<td></td>
<td>3 mo</td>
<td>27 ± 2</td>
<td>55 ± 6</td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>115 ± 9</td>
<td>101 ± 4</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

CoD = coronary diameter; LCX = left circumflex coronary artery; LAD = left anterior descending coronary artery; sAop = systolic arterial pressure; dAop = diastolic arterial pressure; mAop = mean arterial pressure; 0 = before endothelial denudation.

*Ap < .01 vs 0M; **p < .01 vs other group in the same experimental period.

**TABLE 2**

Vasoconstrictive responses to histamine and phenylephrine in group 1 (spontaneous intimal thickening)

<table>
<thead>
<tr>
<th>%ΔCoD from control</th>
<th>%ΔCoD from NG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spastic site</td>
<td>Intact site</td>
</tr>
<tr>
<td>Histamine, 3 µg/kg ic (n = 4)</td>
<td>56 ± 10</td>
</tr>
<tr>
<td>Histamine, 10 µg/kg ic (n = 5)</td>
<td>75 ± 4</td>
</tr>
<tr>
<td>Phenylephrine, 1 µg/kg ic (n = 4)</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Phenylephrine, 10 µg/kg ic (n = 3)</td>
<td>10 ± 4</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

%ΔCoD = % narrowing of the coronary diameter induced; NG = nitroglycerin; other abbreviations are as in table 1.

*Ap < .01 vs intact site.

**TABLE 3**

Vasoconstrictive responses to histamine and phenylephrine in group 2 (cholesterol-supplemented diet)

<table>
<thead>
<tr>
<th>Time of measurement (n)</th>
<th>%ΔCoD from control</th>
<th>%ΔCoD from NG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Denuded site</td>
<td>Intact site</td>
</tr>
<tr>
<td>Histamine, 3 µg/kg ic</td>
<td>0 (10)</td>
<td>6 ± 2</td>
</tr>
<tr>
<td></td>
<td>3 mo (7)</td>
<td>47 ± 7</td>
</tr>
<tr>
<td>Histamine, 10 µg/kg ic</td>
<td>0 (11)</td>
<td>13 ± 2</td>
</tr>
<tr>
<td></td>
<td>3 mo (11)</td>
<td>62 ± 3</td>
</tr>
<tr>
<td>Cimetidine, 60 mg/kg iv + histamine, 10 µg/kg ic</td>
<td>0 (7)</td>
<td>19 ± 3</td>
</tr>
<tr>
<td></td>
<td>3 mo (11)</td>
<td>77 ± 3</td>
</tr>
<tr>
<td>Phenylephrine, 1 µg/kg ic</td>
<td>0 (8)</td>
<td>8 ± 2</td>
</tr>
<tr>
<td></td>
<td>3 mo (10)</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>Phenylephrine, 10 µg/kg ic</td>
<td>0 (7)</td>
<td>10 ± 6</td>
</tr>
<tr>
<td></td>
<td>3 mo (8)</td>
<td>18 ± 5</td>
</tr>
</tbody>
</table>

Values are mean ± SE; abbreviations are as in tables 1 and 2.

*Ap < .05; **p < .01 vs 0 time.

*Ap < .01 vs intact site.
TABLE 4
Vasoconstrictive responses to histamine and phenylephrine in group 3 (regular diet)

<table>
<thead>
<tr>
<th>Time of measurement (n)</th>
<th>%ΔCoD from control</th>
<th>%ΔCoD from NG</th>
<th>Heart rate (beats/min)</th>
<th>sAop (mm Hg)</th>
<th>dAop (mm Hg)</th>
<th>mAop (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denuded site</td>
<td>Intact site</td>
<td>Denuded site</td>
<td>Intact site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine, 3 μg/kg ic</td>
<td>0 (7) 9 ± 3</td>
<td>12 ± 3</td>
<td>16 ± 3</td>
<td>20 ± 3</td>
<td>122 ± 13</td>
<td>109 ± 8</td>
</tr>
<tr>
<td></td>
<td>3 mo (8) 37 ± 7A,B</td>
<td>12 ± 3</td>
<td>41 ± 6A,B</td>
<td>16 ± 2</td>
<td>126 ± 11</td>
<td>117 ± 8</td>
</tr>
<tr>
<td>Histamine, 10 μg/kg ic</td>
<td>0 (9) 14 ± 3</td>
<td>19 ± 3</td>
<td>20 ± 4</td>
<td>24 ± 4</td>
<td>123 ± 10</td>
<td>108 ± 7</td>
</tr>
<tr>
<td></td>
<td>3 mo (11) 55 ± 4A,B</td>
<td>17 ± 4</td>
<td>58 ± 3A,B</td>
<td>20 ± 4</td>
<td>122 ± 10</td>
<td>117 ± 5</td>
</tr>
<tr>
<td>Cimetidine, 60 mg/kg iv + histamine 10 μg/kg ic</td>
<td>0 (8) 16 ± 3</td>
<td>15 ± 3</td>
<td>22 ± 4</td>
<td>24 ± 5</td>
<td>149 ± 10</td>
<td>110 ± 8</td>
</tr>
<tr>
<td></td>
<td>3 mo (11) 72 ± 4A,B</td>
<td>23 ± 2</td>
<td>74 ± 4A,B</td>
<td>24 ± 2</td>
<td>157 ± 11</td>
<td>110 ± 7</td>
</tr>
<tr>
<td>Phenylephrine, 1 μg/kg ic</td>
<td>0 (7) 5 ± 3</td>
<td>5 ± 4</td>
<td>12 ± 3</td>
<td>14 ± 2</td>
<td>145 ± 14</td>
<td>105 ± 6</td>
</tr>
<tr>
<td></td>
<td>3 mo (9) 15 ± 2</td>
<td>11 ± 1</td>
<td>18 ± 2</td>
<td>13 ± 1</td>
<td>147 ± 10</td>
<td>115 ± 6</td>
</tr>
<tr>
<td>Phenylephrine, 10 μg/kg ic</td>
<td>0 (7) 10 ± 5</td>
<td>11 ± 5</td>
<td>16 ± 4</td>
<td>16 ± 3</td>
<td>131 ± 10</td>
<td>134 ± 7</td>
</tr>
<tr>
<td></td>
<td>3 mo (8) 14 ± 6</td>
<td>15 ± 4</td>
<td>20 ± 4</td>
<td>22 ± 3</td>
<td>140 ± 12</td>
<td>142 ± 6</td>
</tr>
</tbody>
</table>

Values are mean ± SE; abbreviations are as in tables 1 and 2.

*P < .01 vs time.

**P < .01 vs intact site.

(% narrowing) in response to agonists did not differ in left anterior descending and left circumflex arteries. Dose-related increases in vasoconstriction induced by histamine and phenylephrine were observed in both the left anterior descending and left circumflex arteries (tables 3 and 4).

Vasoconstriction 3 months after endothelial denudation. Enhanced vasoconstriction in response to histamine and histamine with cimetidine pretreatment were repeatedly noted at the denuded site (figure 5). However, responses to phenylephrine were not augmented in pigs on a cholesterol-supplemented diet or in those on a regular low-cholesterol diet. The degree of vasoconstriction at the denuded site did not differ in groups 2 and 3. Responses of the contralateral nondenuded coronary artery to histamine after cimetidine were slightly greater in the group 2 hypercholesterolemic pigs than in group 3 (figure 5).

Time course and reproducibility of responses to histamine. Serial changes in coronary vasoconstriction in response to histamine at the intact and denuded sites along with arterial pressure and heart rate up to 120 sec

FIGURE 2. Coronary angiograms and electrocardiograms obtained at control (A) and after histamine (B) and nitroglycerin (C) from a representative pig in group 1. Tubular type of vasoconstriction is evident along the major trunk of the left circumflex coronary artery after the intracoronary administration of 10 μg/kg histamine. The angiogram obtained after nitroglycerin shows no organic lesion in either the left anterior descending or circumflex arteries. Electrocardiogram recorded at V6 shows ST depression after histamine (B).
LABORATORY INVESTIGATION—CORONARY ARTERY DISEASE

Cimetidine 60mg/kg iv
Histamine 10μg/kg ic
Nitroglycerin 20μg/kg iv

FIGURE 3. Coronary angiograms and electrocardiograms obtained at control (A) and after histamine with cimetidine pretreatment (B) and nitroglycerin (C) in a pig from group 2 (cholesterol-supplemented diet). In this pig, severe tubular stenosis, along with narrowing at the contralateral coronary artery of a lesser degree, appeared after provocation. The ST segment in lead II of the electrocardiogram was extremely elevated.

after beginning of infusion are summarized in figure 6. Arterial pressure decreased significantly by 22 ± 3 mm Hg 30 sec after intracoronary histamine and practically reverted to the control state at around 60 sec, after which the coronary arteriograms were obtained. Heart rate did not change significantly during the period of angiography. Despite such stable hemodynamics, coronary diameter of the denuded site was markedly reduced at 60 and 120 sec and the maximum level of constriction was noted at 60 sec after starting intracoronary histamine. The degree of constriction was 76 ± 3%, 78 ± 3%, and 74 ± 4% in groups 1, 2, and 3, respectively.

Reproducibility of histamine-induced vasoconstriction after pretreatment with cimetidine was tested between the first and the last provocations in each pig.

FIGURE 4. Coronary arteriograms and electrocardiograms obtained at control (A) and after histamine with cimetidine pretreatment (B) and nitroglycerin (C) in a pig from group 3 (low-cholesterol diet). Focal narrowing is evident, along with ST elevation on the electrocardiogram (arrow indicates the site of spasm).
FIGURE 5. Changes in vasoconstriction (% luminal narrowing) in response to vasospastic agents with (O = group 2) or without (● = group 3) cholesterol supplementation. The denuded portion of the coronary artery responded more than did the non-denuded site to histamine and histamine with cimetidine pretreatment and the degree of vasoconstriction did not differ in pigs receiving and those not receiving cholesterol supplementation. Note that the non-denuded arteries from the cholesterol-supplemented group (group 2) tended to constrict more in response to histamine (with cimetidine pretreatment) than did arteries from group 3 (‡ p < .05 for difference between non-denuded portions from groups 2 and 3). Effects of phenylephrine on changes in coronary diameter did not differ in denuded and non-denuded arteries or in pigs receiving and not receiving cholesterol supplementation. * p < .05 group 2 vs group 3.

There was no significant difference in degree of vasoconstriction after the two provocations in either group, as shown in table 5.

Morphologic changes in the coronary artery and the degree of vasoconstriction. In 19 pigs (four in group 1, eight in group 2, seven in group 3), moderate intimal thickening was present at the site of enhanced vasoconstriction. However, there was no difference in the thickness of the media of portions with intact and thickened intima in any of the three groups (table 6). Figure 7 shows representative histologic findings in groups 1, 2, and 3 at the intact and spastic sites. Endothelial cells were microscopically evident on the thickened intima in all groups.

Topologic correlation between sites of spasm and intimal thickening. Intimal thickening and the degree of vasoconstriction were measured along the coronary trees, as shown in figure 1. Measurements from a representative animal are illustrated in figure 8. A close topologic correlation was evident between the site of enhanced vasoconstriction and that of intimal thickening. Similar correlations were noted for pigs in all three groups. Focal and tubular vasoconstrictions were noted arteriographically in 12 and seven cases, respectively, and correlated well with the histologic distribution of focal and segmental intimal thickening. No pigs without intimal thickening had augmented vasoconstriction.

Degree of vasoconstriction and thickness of the intima. When the data on the absolute value for maximum intimal thickness (μm) and the maximum percent luminal narrowing were plotted on the x and y axis, respectively, data from all three groups showed a good fit to an exponential equation (r = .92, p < .001; figure 9). Histologic percent luminal narrowing was 3.3 ± 0.2% (n = 19). The mean observed percent narrowing in the area with thickened intima for all three groups was 79 ± 2% (n = 19), which was significantly larger than the 33 ± 3% predicted (42 ± 4% of observed narrowing; p < .01) from the intimal thickness at the denuded site and the degree of coronary constriction (27%, n = 19) of the intact artery. As shown in figure 10, observed percent narrowing was always larger than that calculated by the geometric theory. Thus, the area with intimal thickening developed hyperresponsive characteristics on exposure to histamine.

Discussion

The salient features of this study using Gottingen miniature pigs are that (1) coronary artery spasm in our experimental preparation was provoked by histamine, with some exacerbation after pretreatment with cimetidine, the H₂ blocker, (2) spontaneously occurring or mechanically induced intimal thickening was equally responsible for the augmented vasoconstriction pro-

TABLE 5
Reproducibility of vasoconstriction (%) induced by histamine (10 μg/kg ic) with (groups 2 and 3) or without (group 1) pretreatment with intravenous cimetidine

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 3)</th>
<th>Group 2 (n = 10)</th>
<th>Group 3 (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spastic site</td>
<td>Intact site</td>
<td>Spastic site</td>
</tr>
<tr>
<td>First provocation</td>
<td>78 ± 6</td>
<td>22 ± 7</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>Last provocation</td>
<td>74 ± 8</td>
<td>23 ± 6</td>
<td>74 ± 3</td>
</tr>
</tbody>
</table>

Values are mean ± SE.
voked by histamine, (3) a long-term cholesterol-supplemented diet increased the serum cholesterol level mildly to moderately and induced some increase in vasoconstriction of the non-denuded coronary artery, but did not accelerate further the degree of vascular hypercontraction along the denuded site or the degree of the intimal thickness, (4) a close topologic correlation was noted between the site of intimal thickening and the loci of coronary spasm, (5) the degree of vasoconstriction and intimal thickness correlated exponentially, irrespective of the level of serum cholesterol, and (6) observed luminal narrowing at the denuded site was consistently greater than that predicted geometrically from the thickness of the intima. The augmented constriction of the coronary artery in response to histamine can be explained, for the most part, by hyperresponsiveness of the vessel wall to autacoids and is partly the result of the geometric effects of the intimal thickness.

**Localized enhanced narrowing of the coronary diameter.** Coronary angiography is the only tool available to document regional differences in vascular responsive-

---

**TABLE 6**

<table>
<thead>
<tr>
<th>Morphometric analysis</th>
<th>Group 1 (n = 4)</th>
<th>Group 2 (n = 9)</th>
<th>Group 3 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spastic site</td>
<td>Intact site</td>
<td>Spastic site</td>
</tr>
<tr>
<td>Intima (μm)</td>
<td>83 ± 10^a</td>
<td>10 ± 4</td>
<td>80 ± 8^a</td>
</tr>
<tr>
<td>Media (μm)</td>
<td>140 ± 16</td>
<td>142 ± 36</td>
<td>154 ± 15</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

^a p < .01 vs intact site.
ness to vasoactive stimuli in situ. Due to the limited resolution of angiography in determining changes in arterial diameter,\textsuperscript{18} we obtained coronary angiograms before and after intracoronary administration of vasoactive substances in animals in a fixed position.

In a preliminary study, we noted that the locus of angiographic stenosis was the area in which distal pressure dropped with the injection of histamine proximal but not distal to the spastic site.\textsuperscript{19} The denuded portion and the portion with a spontaneously thickened intima showed enhanced vasoconstriction in response to histamine. The results suggest that the area with intimal thickening is the coronary artery responsible for coronary spasm. The ratio of wall thickness to the diameter of the vessel governs the efficacy of smooth muscle shortening when there is a change in diameter.\textsuperscript{20} Recently this theory has proved to be applicable in cases of eccentric stenosis in the artery.\textsuperscript{21} Thus, we estimated luminal narrowing from measurements obtained with the vessel caliper, microscopically determined intimal thickness, and the angiographic degree of normal vessel constriction (figures 9 and 10). Narrowing estimat-

\textbf{FIGURE 7.} Results of Weigert-van Gieson elastica staining of the spastic and intact sites of the coronary arteries of representative pigs from groups 1, 2, and 3. The intima along the spastic site is thickened considerably. Note that endothelial cells cover the intimal surface of the denuded sites.

\textbf{FIGURE 8.} Topologic correlation between intimal thickness (●), medial thickness (▲), and degree of vasoconstriction after histamine (○) in the left circumflex coronary artery (top) and left anterior descending coronary artery (bottom). Degree of regional vasoconstriction was derived by a comparison of the angiogram obtained after histamine provocation with cimetidine pretreatment with that obtained after nitroglycerin in the animal in figure 1. Hypercontraction is apparent at the place of intimal thickening on morphometric examination.

\textbf{FIGURE 9.} Relationship between the thickness of the intima and percent luminal narrowing. The former was derived from a postmortem morphometric analysis and the latter from angiograms obtained in vivo after histamine administration. A highly significant exponential relationship was noted between the two variables, irrespective of serum cholesterol. □ = group 1; ○ = group 2; ● = group 3.

\textbf{THICKNESS OF THE INTIMA (µm)}

\begin{equation}
Y=98\times(1-e^{-0.02(x+5.7)})
\end{equation}

\((r=0.92, P<0.001)\)
ed by this method was 33% (n = 19) and observed percent narrowing at the intact site in response to histamine was 27%. Thus, the contribution of geometric effect to measurements of enhanced luminal constriction was only 6%. Accordingly, the presence of enhanced luminal narrowing at the loci with intimal thickening may largely be explained by vascular hypercontraction in response to histamine. 17, 22

Agonists such as phenylephrine equally constricted both the left anterior descending and left circumflex arteries. A similar result was also noted in studies with carbocyclic thromboxane A2 and indomethacin. 22, 23

Accordingly, specific augmentation of vasoconstriction in response to histamine was evident in our preparation of coronary spasm. These results are comparable to findings of enhanced responsiveness to histamine in human atherosclerotic arterial strips 3 or of histamine provocation of clinical coronary artery spasm in patients with variant angina. 24

Mechanism of enhanced vasoconstriction. Several mechanisms of vascular hypercontraction have been proposed as a result of observation of vascular preparations in vivo 6, 14, 25, 26 and in vitro. 6, 10, 12, 27 These are (1) a role of hypercholesterolemia and/or atherosclerosis, 9, 13, 14 (2) hypercholesterolemia per se, 6, 8 and (3) injury of the vascular endothelium. 12, 25, 27, 28

Recently Faggiotto and Ross 29 documented the importance of the rate of increase, level, and duration of a given level of hypercholesterolemia in relation to type, extent, and distribution of lesion formation in nonhuman primates examined sequentially for 5 to 13 months. In the present study, after histamine with cimetidine pretreatment the nondenuded coronary arteries in group 2 (hypercholesterolemic) pigs showed enhanced vasoconstriction compared with the non-denuded arteries in group 3 (normocholesterolemic) pigs. We could not detect intimal thickening microscopically along these nondenuded coronary arteries (figure 7). This evidence suggests some amplifying effects of hypercholesterolemia per se and/or hypercholesterol-related changes in vascular properties on vascular responsiveness to agonists. This correlates well with findings of experiments in canine arterial strips done by Yokoyama and Henry 8 and those in rabbits with hereditary hypercholesterolemia by Yokoyama et al. 8

Despite such alterations in the responses of non-denuded coronary arteries to histamine after long-term feeding with a cholesterol-supplemented diet, hypercholesterolemia of 222 ± 27 mg/dl (148 ~ 388 mg/dl) did not modify the degree of vascular contraction or the thickness of the intima or tunica media along the denuded site. Heistad et al. 9 also did not find enhanced constriction of large arteries by serotonin in hypercholesterolemic monkeys, but they noted a greater than 10-fold increase in constrictor responses of large arteries to serotonin in atherosclerotic monkeys. Thus, endothelial denudation and/or resultant intimal thickening may play a major role in acquisition of enhanced responsiveness to histamine.

Several investigators have suggested that the endothelium releases a vasodilating substance in response to acetylcholine, A23184, bradykinin, α 2 -agonists, or the like. 11 It has also been reported that in the canine coronary artery, platelets and serotonin induce relaxation of a vascular strip with an intact endothelium but constrict a strip in the absence of the endothelium. 30, 31 In our preliminary studies, 14 changes in diameter in response to ergonovine were not enhanced 30 min after denudation, but they increased significantly at the denuded but not at the contralateral site 3 ~ 6 months after denudation. In addition, 3 months after denudation the animals in our study showed evidence of coronary spasm on H 1 stimulation at the denuded site, an area of the localized intimal thickening that was well
coated with a regenerated endothelium, as identified microscopically. Thus, while the functional state of the regenerated endothelium remains to be examined, the enhanced responses to autacoids may be more evident around the vessel wall with a thickened intima than around that without intimal thickening.

The coronary spasm provoked by histamine in five pigs before denudation (group 1), which was of a similar degree in groups 2 and 3, suggests the importance of structural changes in the vessel, rather than serum cholesterol levels or balloon denudation per se, as an immediate cause of vascular hypercontraction. The exponential relationship between the degree of vascular hypercontraction and the thickness of the intima suggests an important role of vascular intima in the altered responsiveness to histamine. We conclude that there is enhanced responsiveness to autacoids such as histamine during the growing process of the intima, induced spontaneously or after artificial deendothelialization. However, why there is an enhanced responsiveness to autacoids in arteries with thickened intima remains to be elucidated.

Clinical implications. Although factors promoting coronary spasm have not been evaluated quantitatively, the effects of histamine in our swine preparation were phenomenologically similar to those of an anghal attack in patients with coronary spasm: focal transient narrowing of the epicardial coronary artery and subsequent electrocardiographic ST changes were noted. The previously observed provocation of coronary spasm by histamine in patients with variant angina24 and augmented responsiveness of human atherosclerotic coronary arterial strips to histamine5 also corroborate the present findings of histamine-induced coronary spasm. However, an answer to the question of why all patients with intimal thickening do not experience coronary spasm, which is rare compared with the prevalence of intimal thickening, requires further extensive study.

Experimental studies of atherosclerosis have established endothelial injury as an initiating event in this disease.32 The site of endothelial injury in early life in humans is the site of coronary atherosclerosis in later life.33 Thus, altered responsiveness of the coronary artery along the thickened intima to autacoids such as histamine suggests a plausible step toward the development of atherosclerosis once the endothelium is injured.

We are grateful to T. Kobayashi, T. Kawasaki, R. Satoh, and K. Shozaki for technical assistance, N. Hayashi for secretarial services, and M. Ohara for comments on the manuscript.

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Circulation. 1986;74:826-837
doi: 10.1161/01.CIR.74.4.826

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

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